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Innovative nutraceutical and antibacterial proprieties of Humic extracts from composted vegetable wastes.

Ph.D dissertation by

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“Rare sono le persone che usano la mente,

poche coloro che usano il cuore,

e uniche coloro che usano entrambi.”

Rita Levi Montalcini

TABLE OF CONTENTS

Graphical abstract **7**

ABSTRACT **8**

CHAPTER 1 ..... **10**

Introduction **10**

1.1. The role of natural organic matter in sustainable agriculture. 10

1.2. Humic substances and compost teas as bioactive compounds 12

1.3. The effect of humic extracts and compost teas as biocontrol of microbial communities 14

1.4. Analytical techniques for molecular characterization of HS and CTs 15

1.5. The effect of humic extracts on plant metabolisms 17

1.6. Aromatic plants as a source of secondary metabolites with pharmacological properties 19

1.7. Metabolomic and Lipidomic approaches 21

CHAPTER 2 ..... **24**

Work objectives **24**

CHAPTER 3 ..... **26**

Chemical characterization, antioxidant and antimicrobial properties of three compost teas extracted from different vegetable biomasses. **26**

Abstract *26*

1. Introduction **27**

2. Materials and Methods **30**

2.1. Green composts and extraction of compost teas 30

2.2. Elemental analyses and FTIR–DRIFT spectroscopy 30

2.3. ^{13}C Cross-Polarization Magic-Angle-Spinning (CPMAS) NMR spectroscopy. 31

2.4. Offline pyrolysis TMAH-GC–MS of CTs 32

2.5. Germination test 33

2.6. Determination of Total Phenolic Content 33

2.7. Antioxidant activity of CTs 34

2.8. Antimicrobial activity of CTs 35

2.9. Statistical analysis 36

3. Results and Discussion **37**

3.1. Chemical composition of different CTs 37

3.2. Infrared Spectroscopy of different CTs 38

3.3. Solid-state ^{13}C NMR spectra of CTs 40

3.4. Off-line Pyr-TMAH-GC–MS of CTs *44*
3.5. Germination test with different Compost Teas (CTs) 50
3.6. Antioxidant activities and total phenolic content determination 53
3.7. Antimicrobial activity. 56
Conclusions **60**
Acknowledgments **60**
CHAPTER 4 ..... **61**
Metabolomics approach to evaluate the effect of humic derivates on secondary metabolites with nutraceutical activity in Basil plants **61**
Abstract *61*
1.Introduction **62**
2. Materials and methods **65**
2.1. Experimental design, plant growth, sampling and analyses *65*
2.2. Determination of chlorophyll content *66*
2.3. Extraction of plant leaf metabolites *67*
2.4. Composting process, extraction of humic substances (HS) *67*
2.5. Chemical characterization of HS: ^{13}C-CPMAS-NMR spectroscopy. *68*
2.6. Metabolomic approaches: *69*
2.6.1. Standard references 69
2.6.2. Liquid Chromatography High Resolution Mass Spectrometry (LC/HRMS) 77
2.6.3. Data processing LC/HRMS results 77
2.6.4. Metabolomics statistical analysis 78
2.7. Antioxidant activity: Diphenyl-1-Picrylhydrazyl (DPPH) assay *78*
2.8. Determination of total phenolic content: Folin-Ciocalteau assay *79*
2.9. Statistical analysis *79*
3. Results and Discussion. **80**
3.1. Chemical characterization of organic extracts 80
3.2. Bioactivity of different humic extracts (HS and CTs) on growth of Basil plants 84
3.3. Metabolomics approaches: metabolic profiling and PCA analyses 89
3.4. Metabolomics approaches: metabolic analysis of Basil plants treated with the application of HS and CTs from artichoke and coffee composted biomasses 94
3.5. Metabolomics approaches: implication of metabolic pathways 100
3.6. Antioxidant activity and total phenolic content of Basil leaves 105
Conclusions **107**

Acknowledgments **107**

CHAPTER 5 ..... **109**

Research in progress **109**

Lipidomics approach to evaluate the effect of Humic derivates on lipids with nutraceutical activity in Basil plants **109**

1. Introduction **109**

2. Materials and methods **110**

2.1. Experimental design, plant growth, sampling, analyses and ^{13}C-CPMAS- NMR of humic substances and compost teas 110

2.2. Lipidomic approach: extraction method, standards and solvents 111

2.3. Lipidomic approach: Liquid Chromatography High Resolution Mass Spectrometry (LC/HRMS). 112

2.4. Statistical analysis 113

3. Preliminary Results **113**

4. Further analyses to be still conducted. 125

5. Preliminary conclusions and future application. *125*

Acknowledgments **126**

CHAPTER 6 ..... **126**

Effect of humic substances on the chemical composition and antimicrobial activity of Basil Essential oils **126**

Abstract *126*

1. Introduction **127**

2. Materials and Methods **130**

2.1. Composting process and extraction of humic substances (HS) 130

2.2. Molecular characterization of HS : (^{13}C-CPMAS-NMR and TMAH-GC–MS) 130

2.3. Pots preparation and experimental design 132

2.4. Determination of chlorophyll content 133

2.5. Extraction method of Basil Essential oils (EOs) 133

2.6. Chemical composition of Basil EOs 134

2.7. Antibacterial activity of Basil EOs 134

2.8. Antioxidant activity of Basil EO 135

2.9. Statistical analysis 136

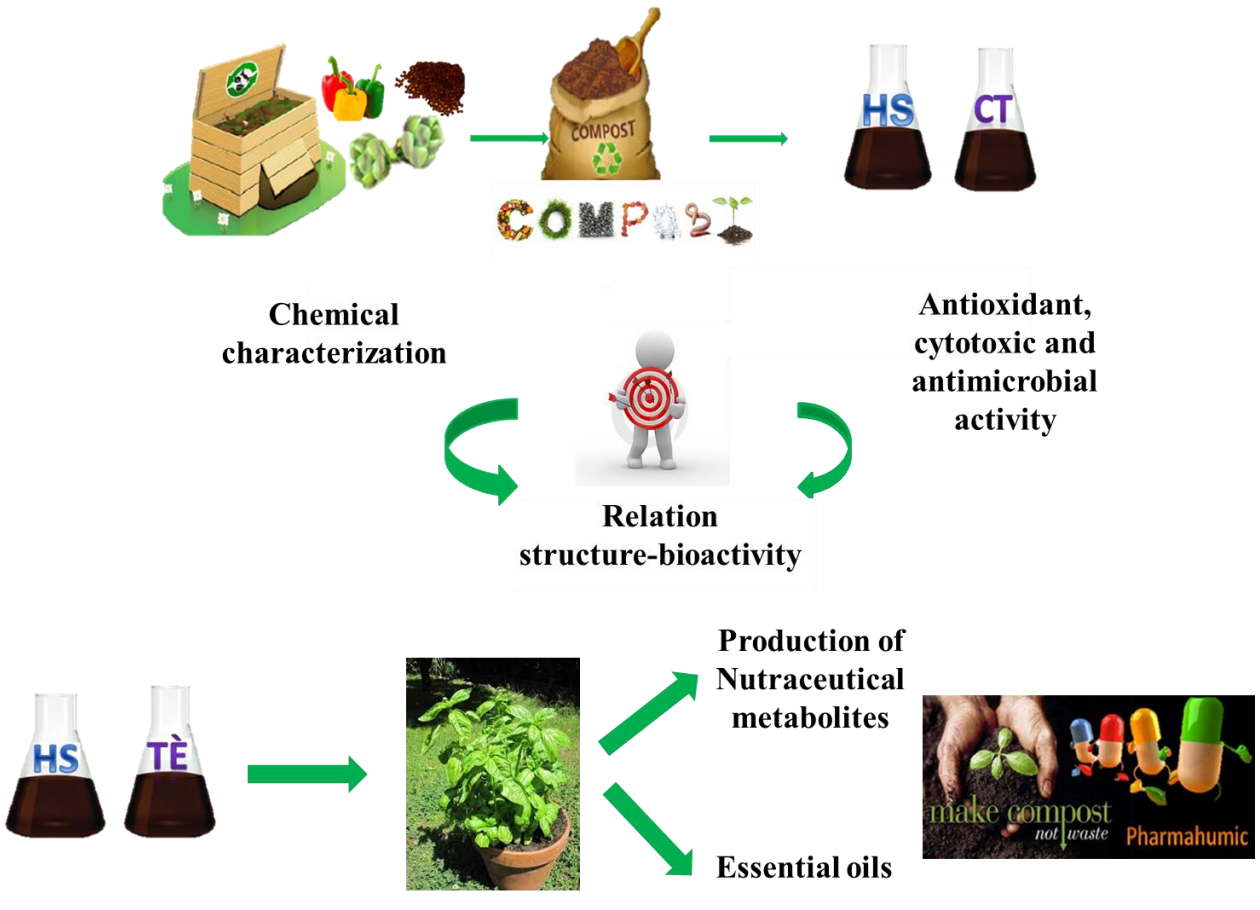
3. Results and Discussion **136**

3.1. Molecular characterization of humic substances-artichoke 136

3.2. Bioactivity of HS-artichoke on growth of Basil plants 146

3.3. Effect of HS on Basil Essential oils (EOs) production 149
3.4. Influence of Humic Substances on Basil EOs antibacterial activity 155
3.5. Effect of Humic Substances on Antioxidant proprieties of Basil EOs 158
Conclusions 159
Acknowledgments 159
CHAPTER 7 161
Potential cytotoxic effect of different compost extracts on human cell cultures 161
Abstract 161
1. Introduction 162
2. Materials and methods 164
2.1. Green composts and extraction of humic substances (HS) and compost teas (CTs) 164
2.2. ^{13}C Cross-Polarization Magic-Angle-Spinning (CPMAS) NMR spectroscopy. 164
2.3. Human cells cultures 165
2.4. Treatments and experimental design 165
2.5. Cytotoxicity assay 165
3. Results and discussion 166
Conclusions 173
GENERAL CONCLUSIONS 174
REFERENCES 177
***Ringraziamenti* 204**

Graphical Abstract



ABSTRACT

Compost is commonly used as an organic fertilizer to recover and preserve the chemical and physical properties of agricultural soils. Effectively, the use of recycled biomasses, residues and by-products, such as compost, is an effective way to improve the environmental and economic sustainability of agroecosystems and agri-food value chains. The use of green compost is increasingly acknowledged in the EU policies as one of the most sustainable approaches to implement the circular economy in agricultural sector due to an economic and environmental re-evaluation of agricultural biomass. Following the raising attention on compost as valuable organic resource for crop productivity, in the recent years the research activity was addressed on the innovative utilization of specific compost fractions such as humic substances and compost teas for crop biostimulation, acting as bio-effectors on biochemical activities, physiological processes and plant microbial interactions The research described in this thesis, is focused on the application of compost as renewable resource for the production of these potential bioactive materials such as humic substances (HS) and compost teas (CTs) as biostimulant to promote the biosynthesis of nutraceutical products in aromatic crop represented by common basil plant. These organic materials were obtained from different composted agricultural biomasses: artichoke, coffee and pepper. Compost teas and humic substances isolated from the different on-farm composts were characterized in details for their chemical properties and molecular composition. Experiments using HS and CTs obtained from artichoke, coffee and pepper composted biomasses highlighted a relationship with the capacity to increase the development of Basil plants with positive effects on phenological parameters such as fresh and dry biomasses and chlorophyll content. Moreover, the plant treatments with compost extracts promoted the synthesis of metabolites with antioxidant proprieties and antibacterial activity against some common human pathogens. In line with the most advanced research approaches, the results indicated the occurrence of structural-activity relationships based on a combination of physical and chemical features of

applied organic fractions as triggering factors for biostimulant properties. The data of molecular characterization suggested the prevalent role of hydrophobic conformation and aromatic components, lignin derivatives and peptidic cluster in the modulation of analysed metabolic pathways. The application of metabolomics and lipidomic approaches have shown an increase in the biosynthesis of secondary metabolites with nutraceutical properties such as hydroxycinnamic acids (caffeic, ferulic, trans-cinnamic and *p*-cumaric) naringenin, resveratrol and coenzyme Q-10, thus confirming the effect of compost derivatives on plant metabolism. The organic materials influenced the production of primary metabolic intermediates such as erythrosis-4-phosphate and phosphoenolpyruvate that activate the production of metabolites involved in the biochemical pathway of schikimic acid. Moreover, the treatment of Basil plants with humic materials such as HS-artichoke improved the yield, antimicrobial and antioxidant properties of Essential oils (EOs) extracted from Basil leaves by steam distillation with a clear dose-response effect on the production of most bioactive chemical components like eucalyptol, methyl-chavicol, eugenol, methyl-Eugenol, trans-α-Bergamotene, geranyl acetate. Finally, medical applications of the HS and CTs extracted from different composted biomasses were carried out using *in vitro* assays on epithelial adenocarcinoma epithelial colon cancer cells (CaCo-2) and immortalized cells of cervical tumor (HeLa). The results of the determination of cytotoxic activity showed the significant inhibition in the proliferation of these tumorigenic cell lines, thus further supporting the reliable utilization and exploitation of natural compost extracts in pharmaceutical, nutraceutical and cosmetic fields.

CHAPTER 1

INTRODUCTION

1.1. The role of natural organic matter in sustainable agriculture

Since ancient times, agriculture has provided a reference point for the sustainability of human development (*FAO, 2012*). The agri-food system plays a critical role in the global economy and it is based on the production of high quality, safe and accessible food and agricultural products for an increasing number of consumers. Indeed, according to UN assessments, the world population is projected to reach 8.9 billion by 2050 (*Cohen, 2001*). In the recent past the technological modification of productive cycles and the introduction of economy of scale, with process automation and intensification of agrochemical application have improved yield and productivity of various agri-food sectors. As a result of standardized production methods, the average crop yield was raised by over 55%, so that the 1950 decade is commonly considered the beginning of the Green Revolution (*Ray et al., 2013*). Nevertheless, this fast development of agriculture has produced on the long time span a series of negative economic and environmental impacts within agricultural areas. The intensive management of agroecosystem has promoted the progressive decrease of soil fertility associated with a decline of biodiversity, acceleration of soil erosion and an improvement of greenhouse gases emissions (*EASAC, 2018*). Pursuing the exploitation of agro- and forestry ecosystems, a depletion of soil fertility and natural resources will be inevitable in order to satisfy the increased demand for food, feed and environmental services. These practices will hence progressively increase both energy and economic inputs with a corresponding decrease of productivity and resilience capacity, further worsened by the effects of environmental changes (*Regulation (EU), 2019/1009*).This threats are

particularly important for the agro-ecosystems of the Mediterranean area, where soil and climate conditions accelerate the variations towards not reversible situation (*EC 2010, 2017*). Currently a modification of management systems is a basic requirement to face with economic and environmental concerns. Hence, the primary goal of modern agriculture is the maintenance of agroecosystem services, including long-term food security, achieved by the adoption of sustainable approaches. To meet these expectations an important role is assigned to natural organic matter. In fact, the decline of organic carbon in agricultural soils is regarded among the main causes for the loss of fertility and biodiversity of cultivated lands (*FAO, 2017*). Therefore, the re-integration of organic matter as keystone component for the development of sustainable methodologies, in terms of production and environmental impacts, is considered a priority objective of EU policies (*EC, 2011*).In this context, the use of recycled biomasses, residues and by-products-, such as compost, represent a viable way to preserve the organic resources and improve the productive capacity of agroecosystems and agro-food sectors (*EC, 2011*). The use of green compost is one of the sustained approaches in the development of the so-called circular economy in rural regions since it involves an economic and environmental re-valuation of agricultural biomasses. The recent indications of European Commission strongly support the reutilization of different organic biomasses and bio-wastes, thereby turning waste management problems into economic opportunities in order to improve the environmental and economic resilience of the agricultural sector (*EC, 2017*) For these objectives, the understanding of biological processes to refine the resources efficiency and support plant growth, crop yield and quality is also a mandatory step (*Uphoff et al., 2008*). The analyses of the interactions between organic fraction plant, soil and microorganisms is one of the main challenges to improve and integrate the main processes, such as nutrient uptakes, performance of biochemical pathways, optimization of microbial activity, physiological response to biotic and abiotic stresses, with overall plant development.

1.2. Humic substances and compost teas as bioactive compounds

Among the natural organic matter components, humic substances (HS) are regarded as a fundamental fraction for the sustainability of agro and forestry ecosystems (*Piccolo et al., 2018*). HS are ubiquitous and relatively biochemically stabilized materials, arising from the degradation of plant and animal tissues through the natural occurring chemical and biological transformation. This natural organic matter is typically found in soils, sediments, and waters (*Stevenson, 1994*). HS are essential for the maintenance of soil fertility, acting as a core intermediate in SOM dynamic with a wide influence on physical, physico-chemical, chemical and biological soil properties. HS have also a particular importance in the biogeochemistry cycle of carbon, nitrogen and phosphorous and on the fate of environmental pollutants in the global ecosystem (*Piccolo et al., 2002, 2019*). HS are made up by a collection of heterogeneous compounds and are conventionally classified according to their molecular solubility in aqueous solution at different pH, into fulvic acids (FA) soluble in all pH conditions, humic acids (HA) soluble only under alkaline conditions, and humin (HU) insoluble at any pH.

The traditional view of HS as macromolecular polymers has never been unequivocally demonstrated (*Piccolo, 1996, 2001, 2002*). Conversely, in line with the thermodynamics and kinetics properties of humic assembly a more reliable theory on the supramolecular conformation of humic fractions, has gained a larger consensus in recent years (*Piccolo et al. 2001, 2002, 2003, 2018, 2019; Simpson, 2002*). According to this new prospective, rather than being constituted by macromolecular polymers, as previously believed, HS are better described as supramolecular associations of heterogeneous molecules with average mass lower than 1000-1500 Da (*Piccolo, 2001, 2002; Piccolo & Spiteller, 2003*). The molecular components are held together by relatively weak forces (hydrogen bonds, cation bridge, π-π, CH-π, van der Waals and hydrophobic interactions) in contiguous hydrophilic and hydrophobic domains stabilized into apparently high molecular size structures (*Piccolo, 2002*). Hence, the humic associations show only apparent large molecular dimensions that can be reversibly

modified upon interaction of dissolved humic molecules with the variable ionic strength of soil circulating solutions and with the diluted concentrations of small organic compounds (organic acids, ionophores, phenols, aminoacids, etc) as those contained in root exudates (*Piccolo et al.,1992; 1999,2002, 2018, 2019*). These innovative insights in the molecular features of natural organic matter components have promoted an increasing investigation on the biological properties of HS as natural bio-active compounds able to support and stimulate the plant development (*Olaetexea et al., 2018*). In fact, the bioactivity of HS originates from both the specific molecular composition and from the conformational properties that allow the preservation, conveyance and release of bioactive components in the rhizosphere environments (*Nardi et al., 2017; Piccolo et al., 2018*). Therefore, in combination to indirect effects on crop growth (e.g. improved nutrient availabilities), the humified SOM fractions have shown direct biostimulant effects on different, physiological and biochemical processes with a significant influence on seed germination, roots and shoots development, plant differentiation, nutrient uptakes and metabolic pathways (*Nardi et al., 2007; du Jardin, 2012;Vaccaro et al., 2014*). The most studied humic materials as well as the most diffuse commercial products were mainly obtained from not renewable geochemical sources (e.g. lignite, leonardite). Currently, as previously noticed, the recent development of sustainable agriculture, strongly support the application of bioactive components derived from recycled biomasses such as on farm green compost and vermicompost, from agro-food by-products biorefinery residues etc (*Canellas et al., 2002; Canellas and Olivares, 2014; Ramos et al., 2015; Monda et al., 2018; Spaccini et al., 2019*). The composting process is a low-cost and sustainable technology to recycle organic biomasses into stabilized and sanitized products with a significant amount of humified components which act as valuable alternative to synthetic chemicals for both soil fertilization and the control treatments of plant pathogens (*Veeken and Hamelers, 2002; Pane et al., 2013; Scotti et al., 2016*).

Besides the HS, additional natural extracts from recycled biomasses, such as the water-soluble fractions from composted materials, named compost tea (CT), and the humic like components (HULIS) isolated from biorefinery residues have been applied with beneficial effects on plant

development (*Pane et al., 2014; Savy et al., 2015, 2016*). CTs are organic formulates obtained by aerobic fermentation of compost carried out on liquid phase, from few days or up to two weeks, with or without active aeration, to produce aerated or non-areated compost teas, respectively (*Ingham, 1999; Lanthier, 2007*). The HS and CTs extracted from different green composts and vermicomposts showed a marked bio-stimulating activity through a large array of direct actions on either crop development, crop productivity and plant-microbial interactions(*Pane et al., 2016; Monda et al., 2017*): stimulation of plant physiology by increasing activity of plasma membrane H+-ATPases (*Dobbs et al., 2010*), induction of plant response to biotic and abiotic stresses by the pathways of reactive oxygen species (ROS), activation of enzymatic and genetic adaptation to low nutrient availability (e.g. phosphorus) and improved efficiency of nutrients uptake (*Jindo et al., 2016; Vinci et al., 2018a, 2018b*).

1.3. The effect of humic extracts and compost teas as biocontrol of microbial communities

In addition to the indirect and direct influence on plant development, HS may act as effective substrates for the modulation of the biocontrol of microbial activities (*Canellas and Olivares, 2017; de Piedadae Melo, 2017; Huang et al., 2020*). In this respect, the humic materials have also been recently applied as starting material in the synthesis of specialized industrial products in medical preparation (*Bernstein et al., 2019; Yalman and Laçin, 2019*). HS are known to implement effective and specific pharmacological properties such as anti-inflammatory, antioxidant and antiviral activity and, in particular, may show immuno-modulatory effects and anticoagulant properties (*Klocking et al., 2005*). However, limited literature is currently available on the possible antimicrobial activity of HS; only for oxifulvic acids a clear antimicrobial activity has been demonstrated in the application against bacterial strains involved in common human diseases (*van Rensburga et al., 2000*).

The CTs obtained in the presence of discontinuous oxygenation contain bioactive molecules secreted by microorganisms, humic components and nutritional elements that play a promising role in the biological control of plant diseases. In fact, various applications revealed the ability of CTs to

suppress a wide range of both air- and soil-borne plant pathogens, when applied as foliar spray and soil drenching (*Scheuerell and Mahaffee, 2002, 2004*). In this respect, CTs are regarded as potential alternatives to the use of the common synthetic fungicides in response to the increasing needs of environmental sustainability for farming and food safety. Different factors and variables involved in the extraction process, may influence the microbiological and chemical components of compost teas, and thus affect the efficacy in plant disease suppression (*Pane et al., 2013*). The wide variety of biochemical and physiological processes modulated by green compost extracts involves alternative or complementary specific interactions, whose structural-activity relationship is still largely undefined. In this context, the molecular characterization of HS and CTs components becomes a necessary requirement to increase the comprehensive understanding of the functional mechanisms underlying the plant bio-stimulation and the biological effects on microbial communities.

1.4 Analytical techniques for molecular characterization of HS and CTs

A detailed molecular characterization of HS and CTs is an essential requirement to evaluate the biostimulant, antioxidant, and antimicrobial proprieties thus providing a comprehensive understanding of their role in agricultural and environmental processes. Non-destructive spectroscopic methods such as diffuse reflectance infrared Fourier transform (DRIFT) and ^{13}C cross-polarization magic-angle-spinning nuclear magnetic resonance (^{13}C-CPMAS-NMR) have already been applied to identify the content and distribution of organic moieties in a broad range of solid organic substrates (*Spaccini and Piccolo, 2007a, 2007b*). The main advantage of these techniques is the direct analyses of solid organic samples without any preliminary treatments, thus avoiding possible interference and bias related to the develop of artifacts The infrared spectroscopy elucidate the distribution of main functional groups hold in complex organic matrices (*Spaccini and Piccolo 2007a ; Spaccini et al., 2009*) The conventional Fourier transform IR spectroscopy (FTIR) has found widespread use for the characterization of HS (*Piccolo et al., 2000, 2001; Spaccini and Piccolo, 2008, 2009*). The application of the diffuse reflectance methodology (DRIFT spectroscopy), further

improve the feasibility of IR approach to analyze solid material preventing the technical step associated to the pellet formation thus also limiting the interference related to sample humidity (*Niemeyer et al., 1992; Piccolo and Conte,1998*). This technique is widely used in the studies on organic materials to characterize composition and reactivity of compost and HS (*Piccolo et al. 2000, 2001; Spaccini and Piccolo 2008, 2009*) as well as SOM fractions associated with soil minerals (*Spaccini et al., 2001; Dick et al., 2003*). The IR spectroscopy has been recently applied to correlate the molecular characteristics and bioactivity properties of water extractable organic matter fractions (WEOM) obtained from different composts (*Monda et al., 2017*). The ^{13}C CPMAS NMR technique allows to determine both the qualitative and reliable quantitative distribution of main C functionalities in organic materials and represents the most suitable analytical tool for the rapid investigation on heterogeneous and complex organic materials such as compost, HS and CTs (*Conte et al., 2004, Mazzei and Piccolo, 2015*). Moreover, the large informative potential and the feasible differentiation of organic components provided by CPMAS technique may be combined with the powerful statistical methodologies based on discriminant analyses to highlight the correlation of molecular properties and environmental effects of organic materials. The variation in molecular content of HS from different soil types, shown by the application of solid-state NMR technique, allowed to operate by multivariate statistical analyses a careful discrimination of SOM characteristics as related to soil properties (*Šmejkalová et al., 2008*). This approach was also applied to determine the functional structural activity relationship of composts, HS and CTs from different origin on plant development (*Monda et al., 2017, 2018; Vinci et al., 2018a, 2018b*). The ^{13}C CPMAS-NMR was successfully applied to highlight the molecular modification of HS in composting process (*Spaccini and Piccolo, 2009*), as well as to relate the molecular composition of CTs, HS and HULIS with their hormone-like bioactivity as promoter of plant root growth (*Dobbss et al., 2010; Savy et al., 2016; Monda et al., 2018; Spaccini et al., 2019*).While the IR and CPMAS NMR spectroscopy allow to determine the distribution of main classes of organic components, a more detailed molecular information on complex organic matrices is obtained by the so called thermochemolysis based on an offline pyrolysis

in the presence of tetramethylammonium hydroxide (TMAH) followed by gas chromatography-mass spectrometry (THM-GC-MS). This technique involves the thermal braking up of bound components with a simultaneous solvolysis and methylation of ester and ether bonds present in natural organic materials, thus enhancing both thermal stability and chromatographic detection of resulting methylated acidic, alcoholic, and phenolic groups. Moreover, the off-line technique allows the analysis of a large quantity of solid material, with a more effective reliable qualitative and quantitative measurement of pyrolytic products and the determination of dimensionless structural index related to the biostability of analyzed biomasses (*Spaccini and Piccolo, 2007b; Spaccini et al., 2019*). Thermochemolysis was successfully applied to characterize the molecular composition of either soil organic matter bulk compost and humic extracts (*Song et al., 2012; Spaccini and Piccolo, 2009; Spaccini et al., 2013*) and to effectively correlate the molecular features with bioactivity properties (*Martinez-Balmori et al., 2013, 2014*)

1.5 The effect of humic extracts on plant metabolisms

Recent studies highlighted an effective link between HS treatments and the increased metabolic efficiency found in plant organisms (*Olaetxea et al., 2018*). The various acknowledged interaction of HS application regards the root system development, lateral and adventitious roots formation, nutrient uptake efficiency, changes on the root exudation profile (*Canellas and Olivares, 2014*), hormone-like effects the stimulation of plasma membrane H+-ATPase activity etc. These responses involve the activation of cellular stimulation and differentiation mechanisms mediated by HS induced physiological effects. HS exert their effects on plant physiology by means of complex transcriptional networks (*Trevisan et al., 2011*). The humic molecules may elicit different biochemical activities related to primary metabolic pathways (*Nardi et al., 2007; Vaccaro et al., 2015*). Moreover, the HS are also coupled with the significant changes in the biosynthesis and concentration of secondary metabolites as those directly involved in the stress alleviation, either

abiotic (proline) or biotic (phenolic compounds, phytoalexins) (*Aguiar et al., 2016*), the activation of kinase- mediated protein phosphorylation (*Ramos et al., 2015*) as well as influence the synthesis of chlorophyll A and B and carotenoid content (*Ghasemi et al., 2015*). In particular, HS treated plants showed a biostimulating effect in metabolic pathways related to the biosynthesis of phenylpropanoids. The humic components induced a relative increase in the enzymatic activity of both phenylalanine ammonium lyase (PAL) and tyrosine ammonium lyase (TAL), which play a pivotal role in the aforementioned biosynthetic pathways (*Schiavon et al., 2010*). These two enzymes use as substrates the phenylalanine and tyrosine aminoacids derived from primary metabolism (*Schiavon et al., 2010*) thus suggesting a possible cascade effect of HS from primary to secondary plant metabolic processes. Anthocyanins and phenolic compounds (monoterpene, terpene, phenylpropanoids, flavonoids) involved in many resistance and stress responses and also in the color, taste, aroma, and scent of fruits and flowers (*Bino et al., 2004*), are the main classes of secondary metabolites influenced by the application of humic substances (*Said-Al et al., 2010; Haghighi et al., 2016*). In fruits of Capsicum annum var. Red chili the plant treatment with humic acid increased the concentration of flavonoids, capsaicin, carotenoids (lycopene and B-carotene) and antioxidant activity (*Aminifard et al., 2012*). The biochemical phenotype of an organism is the result of interactions between the genotype and the environmental inputs; but it is also modulated by intracellular physiological fluctuations that are part of homeostasis (*Weckwerth et al., 2003*). Thus, the simultaneous identification and quantification of metabolites is necessary to understand the dynamics of the metabolome analyze fluxes in metabolic pathways and understand the role of each metabolite following various inputs application (*Fiehn et al., 2003*). In this context, the biostimulants effects of products in vivo cultivation of medicinal plants, opens up the opportunity for the development of organic fertilizers for agro-ecological systems, aimed to the attainment of good quality raw material combining the absence of synthetic pesticides and increased concentration of secondary metabolites with biologically and pharmacologically interest (*Canellas et al., 2015;Olivares et al., 2017*). The innovative technologies grounded on the use of humic materials to

support crop development), can be applied in the organic cultivation of medicinal plants (*Bernstein et al., 2019*). Several studies have shown an increase of secondary metabolites levels (flavonoids, coumarins, total phenols and anthocyanins) as results of humic products application on aromatic and medicinal plants *(Khazaie et al., 2011; Aminifard et al., 2012; Ahmed et al., 2013; El-Gohary et al., 2014; Ghasemi et al., 2015; Hendawy et al., 2015; Said-Al et al., 2016*). In different methodological approaches the use of humic derivates, had a significant, influence in the synthesis of bioactive compounds with biological and pharmacological properties (*Andrade et al., 2001; Khazaie et al., 2011; Kołodziej et al., 2013*).

1.6 Aromatic plants as a source of secondary metabolites with pharmacological properties

The increasing concerns for the limitation of potentially dangerous synthetic additives in nutraceutical and pharmaceutical sectors, has promoted the use of aromatic plants as sources of natural functional constituents in the pharmaceutical, food and feed industries (*Sacchetti et al., 2005*). Medicinal plants are an essential source of secondary metabolites and bioactive compounds responsible for the biological and pharmacological activity of the plant in vivo (*Costa et al., 2012; Pereira et al., 2012*) as alkaloids, phenolic compounds and terpenes. Some of these substances may act synergistically to increase their bioactivity (*Tiwari et al., 2008*). In general, the bioactive components of aromatic plants have the ability to protect the body from damage caused by free radicals induced by oxidative stress induced by the extinction of singleton oxygen and the induction of cytochrome or other enzymes (*Couladis et al., 2003*). In addition, herbs and spices may inhibit oxidative rancidity and improve the shelf-life of the products (*Duke et al., 2002*). Many aromatic herbs and spices both in natural and cultivated systems come from the Mediterranean regions, such as rosemary, oregano, sage, thyme, peppermint and garlic (*Bampidis et al., 2005; Botsoglou et al., 2009; Kadri et al., 2011*). Researches on these species with therapeutic purposes is still recent. *O. basilicum L*. commonly known as Sweet basil is well known for its folk medicinal value and is accepted officially in many countries (*Lawrence et al., 1988*). Basil is an annual herb belonging to

the mint family (Lamiaceae). It has been utilized for millennia and is an essential ingredient in many cooking traditions and practices (*Agarwal et al., 2013*). Basil had been found to contain linalool, eugenol, methyl chavicol, methyl cinnamate, ferulate, methyl eugenol, triterpenoids and steroidal glycoside known to exhibit antioxidant activities (*Pietschmann et al., 2005; Siddiqui et al. 2007a, 2007 b; Zheljazkov et al., 2008*). The metabolic outfit of Basil includes also phenolic and flavonoids compounds such as cinnamic acid, caffeic acid, sinapic acid, rosmarinic acid and ferulic acid (*Loughrin et al., 2001*). These molecules are valuable antioxidants, free radical scavengers, and metal chelators (*Cook et al.,1996*). However, only a few studies have been devoted to assess the antioxidant activity of *O. basilicum* leaves (*Zheng et al., 2001; Stupans et al., 2002*). Pharmacological proprieties of Basil included antibacterial, antioxidant, analgesic, anti-inflammatory, hypoglycemic, hepato-protective, cardioprotective and anticancer activity. The leaves and flowers of *Basil* are used in traditional medicine as a tonic and vermifuge, and basil tea is good for treating dysentery, nausea and flatulence. However, recently the potential uses of *O. basilicum* essential oil, particularly as antimicrobial and antioxidant agents have also been investigated (*Suppakul et al., 2003;Sartoratotto et al., 2004;Lee et al., 2005 ;Wannissorn et al., 2005; Politeo et al., 2007*). Basil Essential oils (EOs) exhibited a wide and varying array of chemical compounds, depending on variations in chemotypes, leaf and flower colors, aroma and origin of the plants (*Da-Silva et al., 2003, Sajjadi, 2006*). The essential oils of *O. basilicum* is beneficial for the alleviation of spasm, rhinitis mental fatigue, cold, and as a first aid treatment for wasp stings and snakebites (*Khair-ul-Bariyah et al., 2012*). Moreover, higher content and concentration of active ingredients of the essential oils of *Hyssopus officinalis L., Mentha piperita L, Mintha piperita var. Citrate, Anethum graveolens L.* was also observed, showing a positive correlation between the increase of dry or fresh biomass with the essential oil content (*El-Gohary et al., 2014; Hendawy et al., 2015*). In this context, aromatic plants can be considered as a source of potential bioactive compounds with pharmaceutical proprieties.

1.7 Metabolomic and Lipidomic Approaches

Metabolomics represents a relatively new "OMIC" science that is able to determine the effects of different environmental inputs on plant metabolism. Metabolomics has proven to be a valuable tool for the detailed comprehensive profiling of plant derived samples for the study of plant systems and natural products research, especially when combined with chemometric data analysis approaches (*William Allwood et al., 2010*).The term metabolome was firstly described by *Oliver et al. (1998)*, as the range of low molecular weight compounds required for the maintenance, growth and normal function of a biological cell, in a particular physiological or developmental stage (*Fiehn, 2002*). It has been estimated, for example, that the plant kingdom as a whole may develop more than 200,000 metabolites and phytochemicals (*Maloney et al., 2010*). Several protocols of analysis and a wide range of analytical platforms have been developed, to help the identification of all metabolite extracted from biological samples (*Dunn et al., 2005*). However, due to the complexity of the metabolome, a unique stand-alone methodology does not exhaust the different molecular variations of all metabolites, and the use of multiple protocols and analytical instruments is required (*Zhang et al., 2012*). The high precision of mass spectrometry (MS) and the reproducibility of nuclear magnetic resonance (NMR) spectroscopy combined with their ability to elucidate chemical structures (*Kim et al., 2010*) represent the most applied analytical technologies in metabolomics studies. Quantitative metabolomic analysis applied to plants is a tool that helps to improve our understanding of plant biochemistry and metabolism by providing an accurate measurement, prior to statistical and bioinformatics analysis, of known metabolite concentrations occurring at different levels in plant samples (*Jorge et al., 2016*). Mass spectrometry, in particular when combined with powerful chromatographic techniques (e.g. liquid chromatography - mass spectrometry (LC-MS) and gas chromatography - mass spectrometry (GC-MS), allows the separation and characterization of the very high diversity of compounds present in plant metabolism (*Saito et al, 2010; Fernie et al., 2011*). Furthermore, Q Exactive HF system combines a state-of-the-art segmented quadrupole for the

selection of high-performance precursor ions with an Orbitrap ultra-high field high resolution (HR / AM) mass analyzer to give a superior combination of scanning speed, resolving power, mass accuracy, spectral quality and sensitivity. Effectively, Q Exactive HF uses a quadrupole segmented mass filter to achieve more precise precursor isolation for better discrimination between analyses and co-elution interference. In addition, instrument has greater ion transmission than combined with previous features leads to higher sensitivity and greater quantitative dynamic range, especially when it comes to complex mixtures such as plant metabolites (*Glauser et al., 2013*). Conversely, gas chromatography coupled with mass spectrometry (GC-MS) facilitates the identification and quantification of a few hundred of metabolites in a single plant extract, thus resulting in comprehensive coverage of the central pathways of the primary metabolism. The main advantage of this technology is that it has long been used for metabolite profiling and thus there are stable protocols for instrumental settings, and chromatographic evaluation, and mass spectra interpretation (*t'Kindt et al., 2009*). Conversely, NMR is a powerful method because it allows the simultaneous detection of diverse groups of secondary metabolites (flavonoids, alkaloids, terpenoids and so on), besides abundantly identifying the primary metabolites (sugars, organic acids, aminoacids and so on). Moreover, NMR signals are proportional to their molar concentration and is very useful for the elucidation of molecular structures. In fact, various two-dimensional NMR techniques can be used to identify structures in complex metabolic mixtures without the need of any further fractionation of extracts (*Kim et al., 2010*). Nevertheless, the application of NMR spectroscopy in the metabolomic analysis has several limitations, the major of which is perhaps its low sensitivity. In the light of the above, the best metabolomic approach is the one that can employ LC-MS, GC-MS and NMR techniques with the aim to evaluate the influence exerted by different treatments on plants. The challenge of metabolomics is to find changes in biochemical pathways, and metabolic networks that might correlate with the physiological and developmental type of a cell, tissue, or organism. Using metabolomics, it is possible to identify pathways responsible for the production of important food metabolites that could be important in improving human health. There are several examples where

the modification of certain metabolic pathways led to the production of plants with an increased nutritional value (*Mehta et al., 2002; Paine et al., 2005*). Additionally, Lipidomics can be considered as branch of metabolomics focused on the analysis of the non-water-soluble metabolites. Lipidomics has emerged as a powerful technique complementing global approaches that are focused on simultaneous analysis of all or large number of cellular metabolites. Lipids are defined as hydrophobic or amphipathic small molecules which consist of a number of structurally and functionally distinct molecules that span from nonpolar to neutral and up to polar compounds. Changes in lipid metabolism and composition, as well as of distinct lipid species have been linked with altered plant growth, development, and responses to environmental stresses including salinity. Recently, improved liquid chromatography mass spectrometry (LC-MS)-based techniques have provided the rapid expansion of lipidomics research (*Rupasinghe et al., 2018*). General expectations from lipidomics studies by the novice in the field is that it will: (1) identify all lipid classes in the sample, (2) identify all lipids within a particular class, (3) identify isobaric species and structural isomers, (4) quantify individual components (using either absolute or relative quantitation methods) and identify differentially expressed lipids using multivariate statistics, (5) identify metabolic networks, (6) identify spatial distribution of the lipids within tissues, and (7) help to formulate hypotheses based on global lipidomics studies or test hypotheses using targeted analysis. Lipid molecules are functioning as metabolic intermediates, structural membrane lipids, pigments and electron carriers for energy transduction, or signaling molecules to activate an array of biological responses, these molecules mostly function in a heterogeneous and complex lipophilic environment. Collecting lipid profiles from extracts of plant tissues most certainly has its place in modern plant biochemistry (*Shulaev et al., 2011*).

CHAPTER 2

WORK OBJECTIVES

The main objective of this work was to evaluate the effects of new agricultural sustainable technologies, such as composting, to increase the production of nutraceutical products from aromatic plants, and concomitantly improve our understanding on chemical features, antimicrobial and antioxidant proprieties of organic components obtained from composted vegetable wastes. Composting is the biological decomposition of organic waste by bacteria, fungi, worms and other organisms under controlled aerobic (occurring in the presence of oxygen) conditions. The humic extracts and compost teas isolated from recycled biomasses are a valuable resources obtained with environment-friendly methods that may find application not only in agricultural practices as biostimulant but also in medical-nutraceutical fields due to their anti-inflammatory, antioxidant and antiviral features. The integration of several approaches is a necessary requirement to provide a powerful solution to characterize new materials, which are applied in several industrial contexts without compromising environmental sustainability. To this purposes, **Chapter 3** reports chemical characterization and evaluation of biological activities of compost teas extracted from different vegetable biomass composted such as artichoke, coffee and pepper. In these chapters, the scavenger activity as well as antibacterial proprieties against common human bacterial pathogens were investigated to understand a possible correlation between chemical proprieties and biological activities of water extracts to highlight their possible innovative application in nutraceutical field. In the subsequent **Chapter 4** and **Chapter 5,** the effects of humic materials extracted from composted artichoke and coffee biomasses were tested on a very common Mediterranean aromatic plant as

Ocinum basilicum (Napoletano green variety). In these sections, the application of metabolomic and lipidomic approaches have been used to investigate the biostimulant activity of humic materials on the production of secondary metabolites with nutraceutical properties in Basil plants. The molecules selected as targets in metabolomic and lipidomic analysis included hydroxycinnamic acids such as caffeic acid and derivatives, but considered also other compounds such as narigenin, resveratrol, or coenzyme Q10 that find an extensive application in nutraceutical industry. The results obtained in this works could be suggest a possible application of humic materials from recycled biomasses in cosmetics or nutraceuticals fields to increase the production of bioactive molecules in aromatic plants. Metabolomic and lipidomic target analysis conducted in this works, was performed by LC-MS/MS technique using a Q Exactive HF system that combines a segmented quadrupole for the selection of high-performance precursor ions with an Orbitrap ultra-high field high resolution (HR / AM) mass analyzer to give a superior combination of scanning speed, resolving power, mass accuracy, spectral quality and sensitivity. The characteristics of this instrument are used to obtain optimal performance in the case of identification and quantification of secondary metabolites and phyto-hormones in complex mixtures such as plant extracts.

Another objective of this thesis was to estimate the influence of humic substances on yield and antimicrobial activity of Essential oils (EOs) obtained from Basil plants comparing different extraction methods (steam-distillation/sohxlet) and analyzing volatile chemical compounds by Gas Chromatography-Mass Spectrometetry (GC-MS). The preliminary results of this activity are shown in **Chapter 6**.

Finally, an additional goal of this doctoral research was to understand the possible medical application of humic derivates testing their preliminary cytotoxic activity on two different eukaryotic cell lines such as Hela (immortal line culture derived from cervical cancer cells) and Ca-Co2 (continuous line culture of heterogeneous human epithelial colorectal adenocarcinoma) as reported in **Chapter 7**.

CHAPTER 3

Chemical characterization, antioxidant and antimicrobial properties of three compost teas extracted from different vegetable biomasses

Abstract

Environmental valorization of agro-industrial wastes and residues by composting process plays a key role in sustainable development of agro-food field. Moreover, in recent years, practical opportunities of use of compost derivatives including compost teas (CTs) are developing rapidly in the agricultural sector. CTs are well known in sustainable agricultural approaches for their bio-stimulating effects on plant growth and productivity and for suppressive properties against fungal pathogens of different agricultural crops. The goals of this work were: i) to obtain CTs using on-farm composting materials as artichoke, coffee and pepper agro-food wastes; ii) to evaluate the molecular and chemical composition of CTs and, iii) to test the potential antioxidant properties and antibacterial effects of CTs on common human bacterial strains diseases such as *Staphylococcus aureus* and *Pseudomonas Aeruginosa*. CTs extraction was obtained in the presence of discontinuous oxygenation for 7 days from high quality dry mature compost. The characterization of CTs materials was performed by solid state Nuclear Magnetic Resonance Spectroscopy (^{13}C-CPMAS-NMR) and Gas Chromatography and Mass Spectrometry (THM-GC-MS), while the relative content of phenolic compounds was determined by Folin-Ciocalteau. Antioxidant properties were evaluated using ABTS and DPPH assays while antibacterial activity was determined by scalar dilutions in liquid medium (MIC) on strains of *Staphylococcus aureus* and *Pseudomonas Aeruginosa*. The results show that all

CTs have antioxidant properties with higher TEAC values (mmol of Trolox kg^{-1} of sample) in the case of CT-coffee. In addition, all CTs show significant antibacterial activity against both strains tested in a range of concentrations ranging from 1 to 1000 g L^{-1}. In detail, CT from coffee showed high antimicrobial activity at the lowest inhibitory concentration of 1 g L^{-1}. The different performance of CTs may be due to their different chemical composition, particularly to the higher relative content of aromatic compounds and phenols in CT-coffee. These results may open new horizons towards a potential application of CTs as natural bio-active compounds, not only in the agro-food field as a replacement for common synthetic fungicides and mineral fertilizers, but also in the nutraceutical industry as a source of bioactive molecules.

Keywords: *on-farm composting, compost tea, chemical characterization, antioxidant, antibacterial.*

1. Introduction

Composting is one of the most ecological technologies for the management of bio-waste and organic biomasses through controlled microbial processing based on an initial intensive degradative thermophilic active step followed by a mesophilic phase with slow biochemical modification combined to final stabilization (humification) of organic products (*Spaccini et al., 2019*). The green wastes are recognized as valuable substrates for composting process compared with other organic materials since they present a lower risk of toxicity due to the lower content of inorganic and organic pollutants (*Moretti et al., 2015; Morales et al., 2018*). The horticultural vegetables produce a significant amount of crops residues, by-products of agro-industrial transformations and additional unmarketable products that represent an important potential source of organic matter to reuse in the productive cycle as compost (*Pane et al., 2015*). In this respect, the recycling of these agricultural residues within the farms (*On-farm* composting) could represent an economic and technical efficient

biological method for the recycling of residual agricultural biomasses (*Maniadakis et al., 2004*). In fact, the on farm recycling allow the careful check of both materials and energy inputs, fluxes and outputs, thereby resulting in a high qualitative humified final organic matrices (*Pergola et al., 2018*). *On farm* compost resolve the problem of the disposal of plant residues providing, at the same time, the farmer with a self-sufficiency and effective strategy for the improvement of soil quality (*Scotti et al., 2016*). Besides the conventional use of compost as soil amendment and fertilizer (*Regulation (EU) 2019/1009*), there are noticeable evidence on the effectiveness of water soluble compost derivates such as compost teas (CTs) to act as either active biostimulants or suppressive compounds (*Vaccaro et al., 2009; Shaheen et al., 2013; Pane et al., 2014,2016*). Since specific or univocal mechanisms of action have not yet been clearly elucidated, an increasing attention is devoted to the comprehensive understanding of the structural activity relationship of water dissolved compost isolates (*Monda et al., 2017, 2018*). The scientific term "compost tea", refers to a variety of aqueous solutions and/or suspensions obtained by fermentation of compost in a liquid phase for a few days, with or without forced aeration (*Gaius Eudoxie et al., 2019*). Compost teas (CTs), are hence classified as a suspension of dissolved organic and inorganic bioactive molecules and useful microorganisms that, potentially, produce health benefits for various plants (*Pane et al., 2014*). Therefore, the use of CTs may relieve the factors that limit the productivity in intensive agricultural systems and may contribute at reducing the unsustainable use of chemical-based pesticides and fertilizers in the ecosystems (*Kim et al., 2015*). Both, no-aerated and aerated CTs may alternatively promote the growth and health of plants (*Naidu et al., 2010; Zaccardelli et al., 2018*), and show a large suppressive ability (*Scheuerell et al., 2002; Dionne et al., 2012*). Effectively, CTs are applied as potential alternatives to the application of synthetic fungicides for the preservation of farming environmental sustainability and food safety (*Pane et al., 2012*). Some experimental evidences highlighted the CTs suppressiveness of plant diseases such as *Phytium ultimum*, *Choanephero cucurbitarum*, *Rhizoctonia solani*, *Fusarium oxysporum*, *Verticillium dahlia*, and *Phytophthora capsici* (*Pane et al., 2012; Mohd Din et al., 2018*). Notwithstanding with the wide use of CTs in sustainable agriculture, limited

information are available about the relationship between chemical properties of compost derivates and their suppressive or biological activity (*Loffredo et al., 2008*). Both molecular characteristics of dissolved organic compounds in CTs an antagonist suppression mechanism related to microbiological component of this water extracts have been associated with the biological effects (*Xu et al., 2012; Pane et al., 2014, 2016*). A more detailed chemical characterization of organic components involved in the bioactivity of CTs is required to increase the knowledge of their efficiency, bio-stimulation and suppression mechanisms (*Pane et al., 2016*). Furthermore, no information are currently present in literature about suppressive effects of CTs against common human bacterial strain. Only for general not characterized humic extracts, specific studies showed particular pharmacological proprieties such as anti-inflammatory, antioxidant and anti-viral activity associated with immune-modulating, anticoagulant, and desmutagenic effects on animal tissues (*Klocking, 2005*). Oxifulvic acids were previously indicated to exert an antimicrobial activity against bacterial strain involved in common human diseases (*van Rensburga, et al., 2000*).

Therefore, the investigation of new potential source of natural antimicrobials is an advanced promising research field also to improve the antibiotic susceptibility of drug resistant bacteria (*Palaniappan et al., 2010*). The innovative objectives of this work was to provide a detailed chemical characterization of CTs extract from different agriculture vegetable wastes (artichoke, coffee, and pepper) to evaluate their potential antioxidant and antimicrobial activity against human common bacterial strains such as *Pseudomonas Aeuruginosa* and *Stafiloccocus aureus*. The correlation between chemical properties and the suppressive effects suggests new potential applications in the biomedical and phytopharmaceutical field as natural bio-active compounds.

2. Materials and Methods

2.1. Green composts and extraction of compost teas (CTs)

The green composts used in this work were obtained in the on-farm composting facility of the Experimental Farm of University of Napoli Federico II at Castel Volturno (CE). Three agricultural biomasses from vegetable crop residues, artichoke and pepper, and from industrial transformation, coffee pellets, were mixed with maize straw and woodchips from poplar trimming at 70/30 w/w ratio. The on-farm composting processes were performed on static piles spread on an air insufflation systems formed by perforated rubber tubes and a rotative pump. The composting processes lasted 100 days with an initial 45 days of active phase. At the end of composting process, the piles were randomly sampled to obtain a final amount of 1 kg for each compost. The organic materials were air dried, sieved at 2 mm and stored a 4 °C for the subsequent analyses. For the attainment of compost teas 200 grams of each on-farm compost were weighted within a gauze bag and placed in a plastic becker containing 1 liter of distilled water (proportion 1/10). The extractions of compost teas were hence carried out with actively aerated solution, oxygenated at regular intervals (5 min every 3 hours) with an automatic aeration device coupled with an air pump. The final solution of CTs were then freeze-dried and stored a 4 °C for the subsequent analytical characterization and biological experiments.

2.2. Elemental analyses and FTIR–DRIFT spectroscopy

Dried CTs samples were characterized for their elemental content (C, N and H) with a Fison EA 1108 Elemental Analyzer. The DRIFT-IR spectra were recorded with a Perkin Elmer 1720-X FT-IR spectrometer (Waltham, MA, USA), equipped with a Perkin-Elmer Diffuse Reflectance accessory, by accumulating 32 scans with a resolution of 4 cm^{-1} from 4000 to 400 cm^{-1} wavenumber interval.

2.3. ^{13}C Cross-Polarization Magic-Angle-Spinning (CPMAS) NMR spectroscopy

The solid state ^{13}C NMR CPMAS spectra of CT samples were recorded on a Bruker AV-300 equipped with a 4 mm wide-bore MAS probe, with the following acquisition parameters: 13,000 Hz of rotor spin rate; 2 s of recycle time; 1H-power for CP 92.16 W: 1H 90° pulse 2.85 μs; 13C power for CP 150, 4 W; 1 ms of contact time; 30 ms of acquisition time; 4000 scans. Samples were packed in 4 mm zirconium rotors with Kel-F caps. The cross-polarization pulse sequence was applied with a composite shaped "ramp" pulse on the 1H channel to account for the inhomogeneity of Hartmann-Hann condition at high rotor spin frequency. The Free Induction Decay (FID) was transformed by applying a 4 k zero filling and an exponential filter function with a line broadening of 100 Hz. For the interpretation of ^{13}C-CPMAS-NMR spectra, the overall chemical shift range was divided into the following six regions assigned to the main organic functional groups (*Monda et al., 2018*): 0–45 ppm (aliphatic-C), 45–60 ppm (methoxyl-C and N-alkyl-C), 60–110 ppm (O-alkyl-C), 110– 145 ppm (aromatic-C), 145–160 ppm (O-aryl-C), 160– 190 ppm (carboxyl-C). To estimate the contribution of specific functional group, the area of each spectral region (Riabs) was divided by the sum of all spectral areas, to obtain a relative amount (MestreNova 6.2.0 software, Mestre-lab Research, 2010): Ri % = (Riabs / ΣRiabs) × 100. In order to summarize the structural composition of CT samples, four dimensionless indices were calculated from the relative areas of NMR spectra (*Spaccini and Piccolo 2007b, Cozzolino et al., 2016; Monda et al., 2018*): the O-Alkyl ratio is derived from the relation between Alkyl-C and O-Alkyl-C:A/OA= [(0–45)/(60–110)]; Aromaticity index, is calculated as the percent of Aromatic signals over the sum of alkyl-C groups: ARM = [(110–160)/(0–45)+(60–110)]; Hydrophobic index, correspond to the comparison of namely hydrophobic C signals versus potentially more hydrophilic functional groups: Σ[(0–45)+(45–60)/2+(110–160)]/ Σ[(45–60)/2+(60–110)+(160–190)]; Lignin ratio is based on the relation between Methoxyl-C/C-N and O-Aryl-C: LigR= [(45–60)/(140–160)]. Although the A/OA, ARM, and HB/HI indexes are mainly used as references to determine the biochemical stability of organic matrices, it has been shown that the

molecular features and conformational properties correlated with these structural parameters are closely related with the bioactivity of compost extracts (*Canellas et al., 2010; Aguiar et al., 2013; Pane et al., 2016*). The LigR ratio is a useful indicator to discriminate between signals owing to lignin and other phenolic compounds (lower LigR) with respect to the prevalent inclusion of peptidic moieties (larger LigR) in the 45–60 ppm range (*Monda et al., 2017; Spaccini et al., 2019*).

2.4. Offline pyrolysis TMAH-GC–MS of CTs

For the termochemolysis about 500 mg of dried CT samples were placed in a quartz boat and moistened with 1 ml of TMAH (25% in methanol) solution. After drying the mixture under a stream of nitrogen, the quartz boat was introduced into a Pyrex tubular reactor (50 cm × 3.5 cm i.d.) and heated at 400 °C for 30 min in a round furnace (Barnstead Thermolyne 21,100). The products released by thermochemolysis were continuously transferred by a helium flow (20 ml min^{-1}) into a series of two chloroform (50 ml) traps kept in ice/salt baths. The chloroform solutions were combined and concentrated by roto-evaporation. The residue was dissolved in 1 ml of chloroform and transferred in a glass vial for GC–MS analysis. The GC–MS analyses were conducted with a Perkin- Elmer Autosystem XL by using a RTX-5MS WCOT capillary column (Restek, 30 m × 0.25 mm; film thickness, 0.25 μm), that was coupled, through a heated transfer line (250 °C), to a PE Turbomass-Gold quadrupole mass spectrometer. The chromatographic separation was achieved with the following temperature program: 60 °C (1 min isothermal), rate 7 ° min^{-1} to 320 °C (10 min isothermal). Helium was used as carrier gas at 1.90 ml min^{-1}, the injector temperature was set at 250 °C, the split-injection mode had a 30 ml min^{-1} of split flow. Mass spectra were obtained in EI mode (70 eV), scanning in the range 45–650 m/z, with a cycle time of 1 s. Compound identification was based on comparison of mass spectra with the NIST library database, published spectra, and real standards. For quantitative analysis, external calibration curves were built by mixing methyl-esters and/or methyl-ethers of the following standards: heptadecane, octadecanoic acid, cinnamic acid,

octadecanol, 16-hydroxy hexadecanoic acid, docosandioic acid, and beta-sitosterol. Increasing amount of standards mixture was placed in the quartz boat and moistened with 0.5 ml of TMAH (25% in methanol) solution. The same thermochemolysis conditions as for compost samples preparation were applied to the standards (*Monda et al., 2017, 2018*).

2.5. Germination test

The germination assay was performed in accordance to *Elias et al., (2012)*. The test was conducted in a growth-chamber at 25 °C in the dark by setting relative humidity at 85%. Twenty Basil seeds (*Ocinum Basilicum Italian Large Leaf ILL*) were placed on a filter paper in Petri dishes (9 cm diameter) and moistened with 10 ml of either distilled water (control) or various Compost teas extracts at different concentrations (10-50-100 gC L^{-1}). All treatments were carried out in 5 replicates. After 5 days of incubation, germination rate, length of roots and epycotile were digitally measured by the Win-Rhizo software (*Cozzolino et al., 2016*). The percentages of relative seed germination (RSG) was calculated in reference to control as follows: RSG (%) = (n° seeds germinated COMPOST TEA / n° seeds germinated CTRL) * 100.

2.6. Determination of Total Phenolic Content

Total phenolic content of CTs was measured using a modified Folin-Ciocalteu colorimetric method (*Singelton et al., 1999; Gajula et al., 2009*). Briefly, 4 mg of each extract were dissolved in 2 ml of metanolic/water solution (50/50). Then, 12.5 µL of each sample was added to 50 µL of Milli-Q water and 12.5 µL of Folin-Ciocalteu's phenol reagent was added to the mixture. After 5 min, 125 µL of 7% Na_2CO_3 solution was added to the mixture and the samples were incubated for 90 min at 25°C. For spectrometric analysis the sample absorbance at 760 nm was evaluated using Perkin Elmer Lambda 25 UV/Vis Spectrometer. The blank reference was made up with all the reaction agents

excluding the extracts. A calibration curve (R^2 = 0.998) was prepared using increasing amounts of gallic acid (0.1-100 mg L^{-1}) with the same analytical conditions. The results are expressed as milligrams of gallic acid equivalents (GAE) per gram of dry sample.

2.7. Antioxidant activity of CTs

The evaluation of antioxidant activity of CTs was performed by ABTS and DPPH assays. The ABTS test was performed in accordance to *Re et al. (1999)*. This method is founded on the oxidation of 2, 20-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS) by potassium persulphate to form a radical cation (ABTS•+). The presence of the ABTS•+ allow the measurement of the total antioxidant activity of different solutions by spectrophotometric methods. The ABTS reagent was dissolved in distilled water up to a 7mM concentration to obtain the ABTS stock solution. The ABTS radical cation (ABTS•+) was produced by reacting ABTS stock solution with 2.45mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. Then, working solution of ABTS•+ was prepared by diluting the 10 ml of radical cation (ABTS•+) solution with 800 ml of water/ethanol (50:50, v/v) mixture with an absorbance between 0.75-0.80 at 734 nm using UV/vis spectrophotometer. For the preparation of samples 4 mg of each extract were dissolved in 2 ml of water. About 100µl of each CTs was hence added to 1.9 ml of ABTS•+ working solution. The mixture was shaken for 2 minutes at dark to promote the reaction between sample and radical solution. Then, the absorbance was measured at 734 nm. The results were expressed as Trolox Equivalent Antioxidant Capacity (TEAC) by means of a linear calibration curve of Trolox in the range 0.1-100 mg·L^{-1} (R^2 = 0.991). The, DPPH-free radical scavenging assay was performed in accordance to *Sulaiman et al., (2012)*. DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable and color-free radical that has been widely employed to determine antioxidant capacity. Briefly, a 0.3 mM solution of DPPH in ethanol was prepared. An aliquot (50µL) of aqueous extract (1 mg ml^{-1}) was added to 150µL of the DPPH solution. For the blank, only 50µL

of Milli Q water was added to the DPPH solution. The mixture was vortexed for 2 minutes and incubated in dark condition for 20 min at room temperature. The absorbance was measured using Perkin Elmer Lambda 25 UV/Vis Spectrometer at λ max = 517 nm. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The radical scavenging activity (RSA) was calculated as a percentage of DPPH inhibition using the equation followed:

$\% RSA = \{[(A_0 - A_i)/ A_0] \times 100\}$

A_0 = absorbance of CTRL

A_i = absorbance of compost tea samples.

2.8. Antimicrobial activity of CTs

Antibacterial activity assays were carried out by i) agar dilution plate viable-count method (*Pane et al. 2016, 2018*) and ii) broth microdilution method (*Wiegand et al., 2008*). The bacterial strains used in this study included *Escherichia coli ATCC35218, Staphylococcus aureus ATCC6538P, Pseudomonas aeruginosa ATCC27355, Enterococcus Faecalis ATCC29212* and *Klebisella pneumonie ATCC700503* provided by Department of Biology (University of Naples Federico II). Briefly, for the agar dilution plate viable-count method a single colony of *Pseudomonas aeruginosa (ATCC27355)* or *Staphylococcus Aureus (ATCC6538P)* was suspended in 5 ml of Luria-Bertani (LB) medium (Becton, Dickinson) and overnight incubated at 37 °C. When the cultures reached an OD600nm of 1 unit, they were diluted 1:100 in 20 mM sodium phosphate buffer (NaP), pH 7.5. Samples were performed adding 40 µL of bacterial cells to several CTs at 10, 25, 50, 100, 200 and 1000 µg ml $^{-1}$ final concentrations, in 500 µL of 20 mM NaP buffer, pH 7.5. Cells incubated with a common antibiotic (ampicillin 0.05 mg ml $^{-1}$) were used as positive control, whereas cells incubated without any antibiotics or CTs were employed as negative control. Particularly, in this case, a substances that no shown antibacterial activity as Bovine Serum Albumin (BSA) was tested at same

concentration of CTs to validate the procedure. All samples were incubated at 37 °C for 4 hours and dilutions (1:100 and 1:1000) of all the samples were placed on LB/agar medium and incubated overnight at 37 °C. The next day, survived cells were estimated by colonies counting on each plate and compared with all controls. Furthermore, all humic materials were tested in triplicate experiments. Standard deviations were always less than 5%. Conversely, the test for the determination of Minimal Inhibitory Concentration (MIC) was performed by broth microdilution method in accordance to *Wiegand et al., (2008)* with some modifications. The test was carried out in Mueller-Hinton Broth medium using sterile 96-well polypropylene microtiter plates. Twofold serial dilutions of different CTs were carried out in the test wells to obtain concentrations ranging from 10 to 1000 µg ml $^{-1}$. Then, bacterial cells were inoculated from an overnight culture at a final concentration of ~ $5x10^5$ CFU ml $^{-1}$ per well and incubated with a different CTs overnight at 37 °C. Finally, the MIC values were estimated measuring the absorbance of microtiter plates at 570 nm. Moreover, the lowest concentration at which no turbidity was observed was considered the MIC value. Three independent experiments were performed for each MIC value. MIC values were measured on *Escherichia coli DH5α, Escherichia coli ATCC35218, Staphylococcus aureus ATCC6538P, Pseudomonas aeruginosa ATCC27355, Enterococcus Faecalis ATCC29212* and *Klebisella pneumonie ATCC700503.*

2.9. Statistical analysis

All results are expressed as means ± SE (standard error). Data obtained from this study were processed using XLSTAT software. The effects of the treatments were tested by one-way analysis of variance (ANOVA) and the means (n=3) were tested appling the least-test difference (LSD) test at the 0.05 significance level.

3. Results and Discussion

3.1. Chemical composition of different CTs

The elemental composition, C/N ratio and H/C ratio of green Compost Tea extracts are shown in **Table 1**. The total carbon and hydrogen content were higher in CT-artichoke compared to other materials tested. Additionally, C/N ratio was found larger in the case of CT-coffee, whereas CT-pepper and CT-artichoke exhibited lower values concerning this parameter. Commonly, a lower C/N ratios may be related to the preferential incorporation of low molecular-weight N-rich materials compared to C-rich component such as polysaccharides present in the residual vegetable biomasses whereas the smaller H/C values, founded for CT-pepper, suggests an accumulation of aromatic compounds and a decrease of aliphatic structures (*Monda et al., 2018*).

Table 1: Total C, N and H (%) of Compost Teas. All results are expressed as means (n=3) ± SE (standard error).

	Total C	**Total H**	**Total N**	**C/N[a]**	**H/C[a]**
CT-coffee	19.7 ± 1	2.5 ± 0.03	1.68 ± 0.1	16.5	1.52
CT-artichoke	30.7 ± 1	5.6 ± 0.2	2.9 ± 0.2	11.7	2.17
CT-pepper	26.2 ± 0.5	2.8 ±0.2	1.6 ± 0.03	10.3	1.28

a: atomic ratio

3.2. Infrared Spectroscopy of different CTs

The DRIFT spectra of CTs samples (**Figure 1**) revealed a similar distribution of main functional groups. The broad absorption band around 3000–3500 cm^{-1}, is attributed to the intense-OH stretching vibrations in alcohols, phenolic and carboxylic acids (*Monda et al., 2018*) while the symmetric and asymmetric C-H bond stretching at 2960 and 2850 cm^{-1} indicated the inclusion of methylene groups in aliphatic chains. In the central region, the sharp shoulders around 1660 cm^{-1} may be associate to the presence of deprotonated or salt form of carboxylic functional groups. (*Carballo et al., 2008*). The bands around 1620 cm^{-1} and 1590 cm^{-1} may be related to either the amide I and amide II bonds of peptidic material (*Monda et al., 2017*) as well as to ring vibrations of aromatic moieties The peak at 1514 cm^{-1}, in DRIFT spectra of CT-pepper sample may be also assigned to the ring stretching vibrations in lignin aromatic fractions. The presence of alkyl chains and of carboxylates anions in aliphatic acids derivatives were also highlighted by the corresponding bending vibrations of C-H and C-O groups at 1400 $cm^{-1}$and 1370 cm^{-1}(*Brunow, 2001; Monda et al., 2017*). The incorporation of carbohydrates derivatives was revealed by peaks at 1111 and 1045 cm^{-1}, assigned to C-O stretching bonds in both poly-alcoholic and glycosidic functional groups. No significant differences in the composition of CT samples were shown by the DRIFT spectra. Apart from the slight larger abundance of alkyl groups in the CT from pepper (**Figure 1**), the main signals indicated the preferential solubilization from bulk composts of polar components formed by aliphatic acids, carbohydrates and peptide derivatives. However, the broad bands in the 1660-1510 wave number regions may also include the usually less intense vibrations of aromatic rings pertaining to soluble fractions such as polyphenolic and lignin units. This finding is also supported by the slight shoulders found around 1300-1200 cm^{-1} (**Figure 1**), partly masked by the intense signals of carbohydrates, that may be derived from the C-O bending of phenolic moieties and aryl ether groups (*Spaccini and Piccolo, 2007a*).

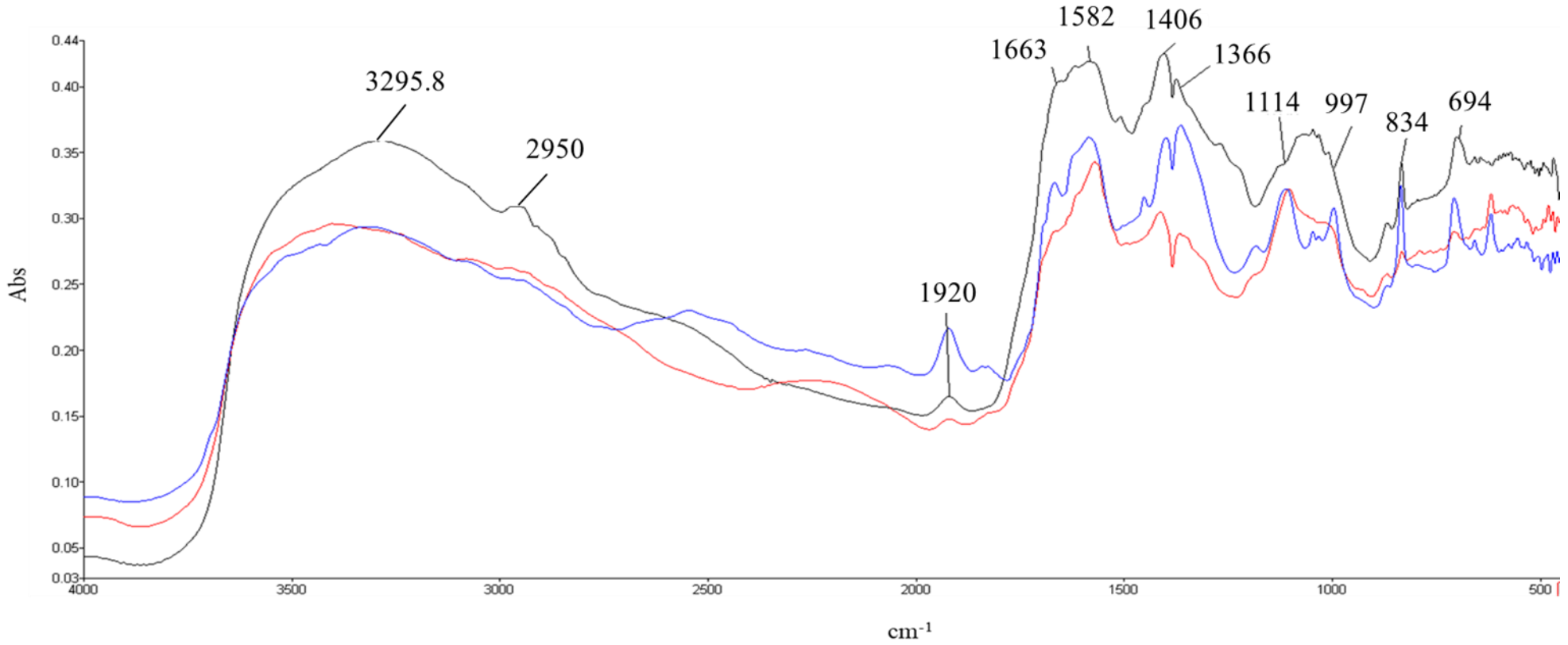


Figure 1: Diffuse Reflectance Infrared Fourier Transform spectroscopy (DRIFT-IR) spectra of CT-pepper (black) , CT-coffee (red) and CT-artichoke (blue) .

3.3. Solid-state ^{13}C NMR spectra of CTs

The ^{13}C-CPMAS-NMR spectra of CTs are shown in **Figure 2**, while the **Table 2** reports the relative carbon distribution of main functional groups over chemical shift regions (*De Marco et al., 2012; Spaccini et al., 2019*). The ^{13}C-CPMAS-NMR spectra of all water extracts indicated a predominance of O-alkyl carbons (60–110 ppm) due to the inclusion of mono- oligo and polysaccharides (*De Marco et al., 2012; Spaccini et al., 2019*). The relative amounts of carbohydrates in CT-coffee, CT-artichoke and CT-pepper spectra accounted for the 26.4, 27.3 and 31.1 % of total signal area, respectively (**Table 2**). The intense signal at 72 ppm is attributed to the overlapping resonances of C-2, C-3, and C-5 carbons in the pyranosidic structures of cellulose and several hemicelluloses (*Monda et al., 2017*), while the broad peak around 105 ppm indicated the di-O alkyl C-1 involved in the glycosidic linkage. The lower evidence of the shoulders of both C-6 at 64-66 ppm and of C-4 around 82-85 ppm (**Figure 2**), suggested the solubilization of either pentose components of hemicellulose as well as that of oligomers and simple carbohydrates derived from the breakdown of the 1→4 bonds. This finding is further supported by the broadening of anomeric C signals combined with their upfield shift at 100 ppm which revealed the incorporation of unbound C1 nuclei (*de Aquino et al., 2019; Spaccini et al., 2019*). The large NMR resonances between 0 and 45 ppm are assigned to the presence

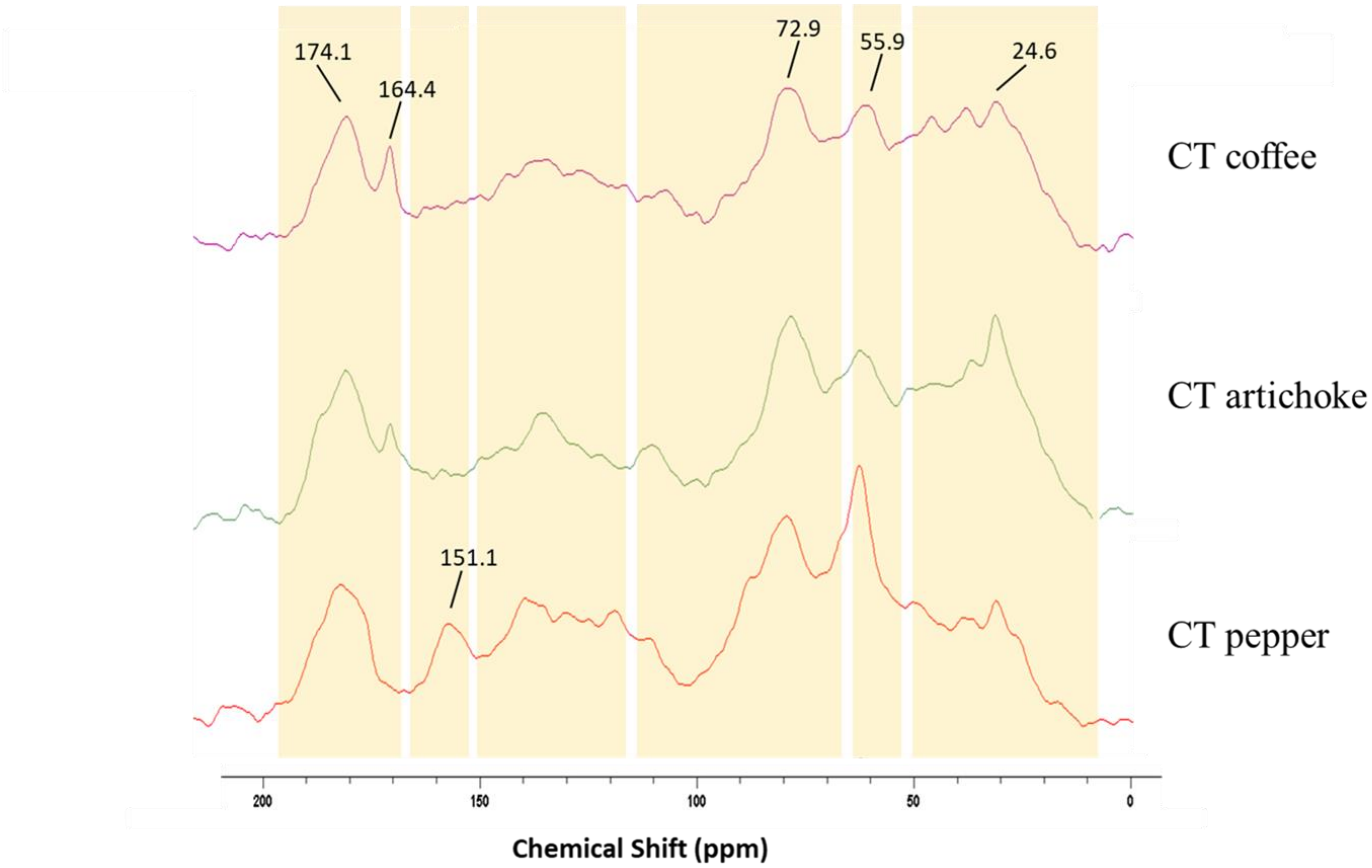


Figure 2 : ^{13}C CPMAS NMR spectra of different compost teas.

Table 2: Relative distribution (%) of main C structures over chemical shift regions (ppm) and structural indexes of different green compost teas as measured using ^{13}C CPMAS-NMR spectra of compost teas.

	^{13}C NMR regions						**^{13}C NMR structural indexes**			
Compost Teas	Carboxyl-C 190–160	Phenol-C 160–140	Aryl-C 140–110	O-Alkyl-C 110–60	C-O/C-N 60–45	Alkyl-C 45–0	HB/HI[a]	A/OA[b]	ARM[c]	LigR[d]
CT-coffee	14.0	4.3	16.6	26.4	11.9	26.9	1.2	1.02	0.39	2.79
CT-artichoke	13.9	4.2	15.0	27.3	12.1	27.6	1.1	1.01	0.35	2.88
CT-pepper	12.3	5.8	19.2	31.1	14.3	17.4	1.0	0.56	0.52	2.46

[a] HB/HI=hydrophobicity index=[Σ(0–45) +(45–60)/2+ (110–160)/ Σ(45–60)/2 + (60–110) + (160–190)]

[b] A/OA=alkyl/O-alkylratio (0–45)/(60–110)

[c] ARM = aromaticity index [(110–160)/Σ(0–45) + (60–110)]

[d] LigR = Lignin ratio (45–60)/(145–160)

of methyl and methylenic groups in alkyl chains from different lipid molecules (*Pane et al., 2016; Monda et al., 2018; Spaccini et al., 2019*). The Alkyl-C region represented the 27.6 % of the total area in CT-artichoke followed by CT-coffee with a 26.9% whereas a lower value was observed for CT-pepper (17.4%). The wide extension of multiple alkyl signals in the 15 to 45 ppm range, suggested the inclusion of various methyl and methylene carbons in both linear and branched short chain (<C10) alkyl components, such as amino acids, oligopeptides, carboxylic acids etc. (*Monda et al., 2017; Spaccini et al., 2019*). The broad band in the 45–60 ppm region is associated to either methoxyl substituents in lignin structures such as guaiacyl and syringyl units as well to the presence of C-N functions in aminoacids or peptides (*De Marco et al., 2012; Monda et al., 2017; Spaccini et al., 2019*). A significant amount of aryl-C pertaining to both lignin residues and other dissolved aromatic biomolecules were found in the three CT samples in the 110–160 ppm region of the spectra (**Figure 2**). The larger relative C distribution of total aromatic units was found in the CT-pepper sample with the 25.0% of the total area followed by the CT-coffee and CT-artichoke with the 20.9 % and 19.2 % in the order (**Table 2**). The first part of aromatic region between 110–140 ppm is related to the contributions of non-substituted and C-substituted phenyl carbons. The signals in the subsequent 140–160 ppm chemical shift interval are assigned to O-substituted C in aromatic structures of hydroxy and methoxy carrying ring C in polyphenol compounds and lignin molecules, which carbon in position 3 and 5 are coupled to the signal related to methoxyl substituents (*Monda et al., 2017, 2018*. Finally, the sharp intense signal centered at 174 ppm in all the CT samples (**Figure 2**) corresponding to carbonyl carbons, confirmed the preferential solubilization of polar functions of low molecular weight components of short chain aliphatic acids, amide functional groups and carboxylated derivatives of pectin components (*De Marco et al., 2012; Spaccini et al., 2019*). The main differences among CT samples were related to the different contribution of specific organic components. These specificities can be highlighted by the calculation of the dimensionless structural parameters, such as aromaticity (ARM) and hydrophobic (HB/HI) indexes, and alkyl (A/OA), and lignin (LigR) ratios (**Table 2**). All compost extracts revealed a significant hydrophobic composition

with a slight lower value of HB/HI index from CT-pepper, in respect with CT-artichoke and CT-coffee (**Table 2**). However, differential contributions of aliphatic and aromatic fractions were shown by the additional structural parameters. The values of ARM and A/OA found for CT-pepper indicated the preferential incorporation of aromatic components and carbohydrates (**Table 2**). Conversely, a larger contribution of alkyl functionalities was revealed by the ARM index of CT-artichoke and CT-Coffee, with a prevalence of Alkyl-C in respect to O-alkyl derivatives (**Table 2**). The values of LigR found in all the three CT extracts (**Table 2**) suggested the prevalent incorporation of lignin units in the spectral regions associated with the methoxyl groups and O-aryl-C molecules with a possible slight larger contribution of nitrogen moieties for CT-coffee and CT-artichoke (*Monda et al., 2017; Spaccini et al., 2019*).

3.4. Off-line Pyr-TMAH-GC–MS of CTs

The total ion chromatograms (TIC) derived from the thermochemolysis of CTs are shown in **Figure 3** while the list of most relevant detected compounds and their quantitative estimation are reported in Tables 3 and 4, respectively. The thermochemolysis of CTs samples released a large range of alkyl and aryl molecules which were primarily identified as methyl ethers and esters of natural compounds of main plant and microbial origin (**Table 3**). As compared to the results of NMR analyses, lower amounts of carbohydrates were found among the pyrolysis products of CTs. The reduced content of mono, oligo and polysaccharides components was already observed in the pyrolytic products of plant woody tissues, soil organic matter and organic biomasses (*Spaccini et al., 2013; Martinez-Balmori et al., 2013, 2014*). This finding is attributed to the poor efficiency of the thermochemolysis in detecting saccharide components in complex matrices. In fact, the thermal behavior and pyrolitic rearrangement of poly-hydroxy compounds combined with the TMAH reaction conditions are believed to negatively interfere in carbohydrates detection (*Martinex-Balmori et al., 2014; Spaccini et al., 2019*). The most

abundant compounds were represented by lignin components, followed by straight chain fatty acids, and lipid metabolites of microbial cellules and by-products (**Tables 3, 4**).

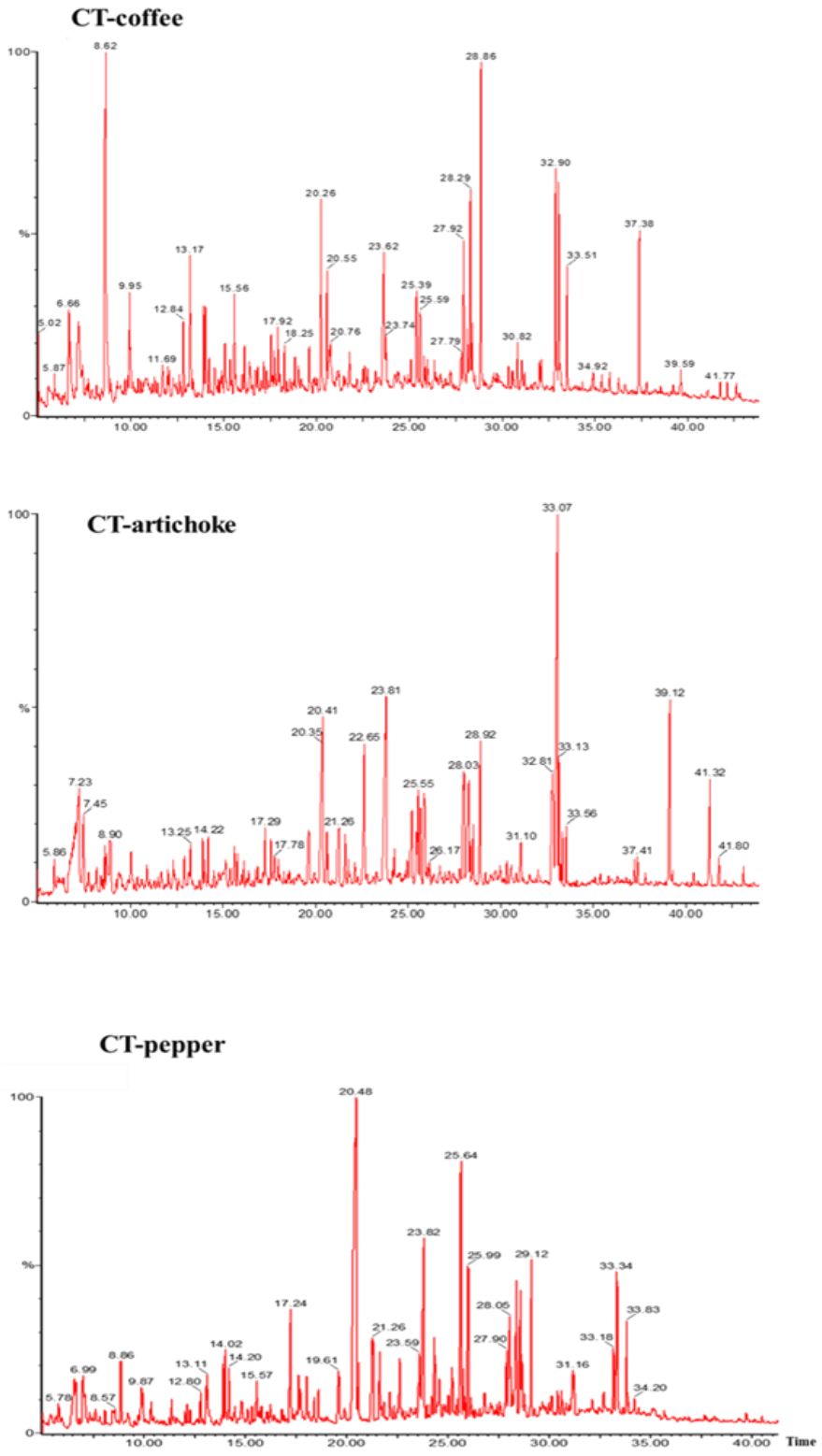


Figure 3: Total ion chromatograms of thermochemolysis products respectively of CT-pepper, CT-coffee and CT-artichoke.

Table 3. List of the main products released by CTs thermochemolysis

RT	Assignment	Origin
5.87	Butanedioic Acid, Dimethyl Ester	lip
6.74	Phenol 2 Methyl	phenol
8.14	1,2 di-CH3O benzene Lig G1	Lig G1
8.62	Phenol, 2,4-imethyl	phenol
9.68	m/z 128	N
9.93	N derivative	N
10.38	3-4 di Methoxy Toulene	Lig G2
11.29	Indole-1 methyl	N
11.46	Carbohydrate derivative	carbohydrate
11.69	Carbohydrate derivative	carbohydrate
11.98	Carbohydrate derivative	carbohydrate
12.29	1,2,3-Tri-CH 3 O benzene	aromatic
12.83	m/z 128	N
13.16	m/z 98	N
13.33	Phenol 2-Dimethoxy	phenol
13.89	Benzene, 4-Ethenyl-1,2-Dimethoxy-	Lig G3
14.01	1,2,3-Tri-CH 3 O benzene	Lig S1
14.21	benzoic acid 4-CH3O,me	Lig P6
14.52	2-Propenoic Acid, 3-Phenyl-, Methyl Ester, (E)	lip (biop)
14.9	N derivative	N
15.33	N derivative	N
15.56	N derivative	N
16.01	2hindol-2one,1-3-Dihydro-1-Methyl	N
16.09	1h-Indole, 5-Methoxy-2-Methyl	N
16.38	1h-Indole,1-2-3 Trymethyl	N
17.32	Benzaldehyde, 3,4-Dimethoxy	Lig G4
17.53	Carbohydrate derivative	carbohydrate
17.72	N derivative	N
17.92	Carbohydrate derivative	carbohydrate
18.25	C12 FAME	lip
19.62	Ethanone, 1-(3,4-Dimethoxyphenyl)-	Lig G5
20.26	benzoic acid 3,4-DiMethoxy, Methyl ester	Lig G6
20.55	Benzaldehyde, 3,4,5-Trimethoxy-	Lig S4
20.74	m/z 98	N
21.21	cis-2-(3,4-Di-CH3O phenyl)-1-CH3Oethylene	Lig G7
21.58	trans-2-(3,4-Di-CH3O phenyl)-1-CH3Oethylene	Lig G8
22.15	cis-1-(3,4-Di-CH3O phenyl)-1-CH3O 1-propene	Lig G11
22.53	Ethanone, 1-(3,4-Dimethoxyphenyl)-	Lig S5
22.62	trans-3-(4-CH3O phenyl)-3-propenoic acid ME	Lig P18
22.74	C14 fame	lip
23.18	C16 alcohol	alcohol
23.61	Benzoic Acid, 3,4,5-Trimethoxy-, Methyl Ester	Lig S6
23.73	C14 fame	mic
24.24	trans-1-(3,4-Di-CH3OPhenyl)-3-CH3O-1-propene	Lig G13

24.44	cis-1-(3,4,5-Trimethoxyphenyl)-2-methoxyethylene	Lig S7
25.09	trans-1-(3,4,5-tri-CH3Ophenyl)-2-CH3Oethylene	Lig S8
25.39	C15 iso fame mic PLFA	mic
25.59	c15 anteiso Fame mic -PLFA	mic
25.77	N derivative	N
25.85	2,5-Piperazinedione, 3-Methyl-6-(Phenylmethyl)-	N
27.79	C18 alcohol	alcohol
27.9	C16 fame	lip
27.9	trans-3-(3,4-Di-CH3O phenyl)-3-propenoic acid ME	Lig G18
28.13	3-(4'-Methoxy-1,1'-Biphenyl-4-Yl)Butanenitrile	N
28.29	C16:1 Fame Mic PLFA	mic
28.86	C16iso fame mic PLFA	mic
29.84	C 17 iso fame mic PLFA	mic
31.03	cis-1-(3,4,5-Tri-CH3O phenyl)-1,3 di CH3O-Prop1-ene	Lig S16
31.2	C17 anteiso FAME mic plfa	mic
32.16	C18 iso FAME mic PLFA	mic
32.88	C18:1 fame mic	mic
33.01	C18:1 fame	lip
33.5	C18 fame	lip
34.33	C19 FAME	lip
34.91	Carbohydrate derivative	carbohydrate
35.81	Carbohydrate derivative	carbohydrate
37.38	Carbohydrate derivative	carbohydrate
37.78	C18:1, 18CH3O, Fame	lip
38.24	Carbohydrate derivative	carbohydrate
39.21	N derivative	N
39.59	Carbohydrate derivative	carbohydrate
41.07	Carbohydrate derivative	carbohydrate
41.77	C22 FAME	lip
42.78	Carbohydrate derivative	carbohydrate
43.94	C22,2-CH3O, Fame Mic	mic
45.45	C24 Fame	lip
46.8	Squalene	sterol
47.18	Sterol	sterol
48.33	C24 2-Ch3O , fame mic	mic
48.88	C26 fame	lip
49.99	C31 alkane	alkane
50.66	Sterol	sterol
51.33	Sterol	sterol
52.75	Sterol	sterol

FAME fatty acid methyl ester, Lg lignin, P phydroxyphenyl, G guayacil, S syringyl, ME methyl ester, Mic microbial origin, RT retention time (min).

Table 4: Composition (%) of main thermochemolysis products released from the three compost teas.

%	CT-coffee	CT-artichoke	CT-pepper
FAME	9.2	15.2	13.2
Lignin	61.3	72.8	30.6
Mic	6.4	3.0	23.7
Carbohydrates	0.7	5.1	9.8
N derivates	22.4	3.9	22.6
Ad/Al$_G$	18.3	0.5	6.5
Ad/Al$_S$	7.6	0.3	1.4

FAME fatty acid methyl ester, Mic microbial origin fatty acids, AD/AL$_G$ [G6/G4], AD/AL$_S$ [S6/S4].

Besides carbohydrates minor but noticeable yields were found for N containing molecules and finally for alicyclic compounds (sterols).The larger relative yield of identified molecules released by thermochemolysis of CTs consisted in a wide range of methyl ethers and esters of lignin monomers (**Tables 3, 4**). The lignin products listed in **Table 4** were classified with the current symbolisms used in scientific literature (*Martinez-Balmori et al., 2014; Spaccini et al.,2019*): P for *p*-hydroxyphenyl, G for guaiacyl (3-methoxy, 4-hydroxyphenyl), and S for syringyl (3,5-dimethoxy, 4-hydroxyphenyl). The various methylated forms of *p*-hydroxyphenyl, guaiacyl, and syringyl structural units found in CTs confirms the multiple origin of lignified tissues. In detail, the soft wood of gymnosperms consists nearly exclusively of guaiacyl-based compounds, whereas both guaiacyl and syringyl units are the main components of the hardwood of perennial angiosperm, and all three structure are the building blocks of lignin network in herbaceous plants, the *p*-hydroxyphenyl unit being the major constituent (*Martinez-Balmori et al., 2013, 2014; Spaccini et al., 2019*). The most abundant lignin monomers were the oxidized forms of di- and tri-methoxyphenyl-propane molecules such as benzaldehydes (G4, S4) and benzoic acids (G6, S6) derivatives (**Table 4**). Other important and conventional products found in the thermochemolysis analyses of lignin were the cis and trans isomers of 1-(3,4-dimethoxyphenyl)-2-methoxyethylene (G7, G8) and 1-(3,4,5-trimethoxyphenyl)-2-methoxyethylene

(S7, S8), as well as the enantiomers of 1-(3,4-dimethoxyphenyl)-1,2,3-trimethoxypropane (G14 and G15), and 1-(3,4,5-trimethoxyphenyl)-1,2,3-trimethoxypropane (S14 and S15). The 3-(4-methoxyphenyl)-2-propenoic acid (P18) was the most abundant P product, which may have resulted from both the oxidation of *p*-hydroxyphenyl units in lignin as well as from the transesterification of aromatic domains of suberin biopolymers in plant woody tissues. The presence of oxidized lignin units allows to determine dimensionless structural indices currently associated with the decomposition of lignin residues (*Spaccini et al., 2013*). In fact, the aldehyde and acid forms of guaiacyl and syringyl structures are related to the progressive oxidation of corresponding original monomers. Therefore, the ratios of acid structures to the corresponding aldehydes (G6/G4, S6/S4) are useful indicators of the degree of lignin bioxidative transformation during composting process (*Monda et al., 2017; Spaccini et al., 2019).*

In CT samples the larger ratios found for the water extracts from coffee and pepper derived composts indicated the solubilisation of decomposed and mainly unbound molecules, whereas the lower values shown by CT-artichoke suggested the prevalent inclusion of less or partially modified linked structural units in lignocellulose intact fragments (*Monda et al., 2017; de Aquino et al., 2019; Spaccini et al., 2019; Bento et al., 2020).*The linear fatty acids were mainly represented by the hexadecanoic and octadecanoic homologues, with a predominance of even carbon chains that indicated their prevalent plant origin. The CT samples revealed a significant content of methyl branched short chain fatty acids, which are currently assigned to the phospholipid components of microbial cells (*Cozzolino et al., 2016*). The relative abundance of thermochemolysis products confirmed the prevalent incorporation in the compost extracts of dissolved lignin derivatives (**Table 4**). In contrast to the NMR results a larger amount of lignin molecules was found in the CT-artichoke and CT-coffee, while the lower yield was shown by the CT-pepper sample (**Table 4**). This finding may be related to the irregular detection and uneven release of thermally labile compounds such as carbohydrates and peptidic moieties that may bias the correct relative estimation of aromatic and alkyl apolar compounds. In fact, a larger content of nitrogen derivatives was found in CT-coffee and CT-

pepper which was also characterized by a significant contribution of microbial components (**Table 4**). Moreover, in the comparison of quantitative outputs it has to be recall that the cross-polarization NMR technique provide a semiquantitative estimation of C distribution. The estimation of various functional groups strongly depends on the proton density of complex matrices and on the consequent efficiency of spin diffusion that determine the magnetization transfer (*Conte et al., 2004*). In this respect, the low-protonated organic components such as the aromatic fractions may undergo a potential underrated quantification.

3.5. Germination test with different compost teas (CTs)

Biological activity of different CTs was evaluated with a germination test on Basil seeds performed with increasing concentration (10, 50 and 100 g L^{-1}) of dissolved CTs samples. The analyzed parameters included the phenological evaluation of root and epicotyl elongation and the percentage of relative seeds germination (RSG). The results showed the absence of phytotoxic effect and a significant increase of both root and epicotyl lengths following the application of CT-coffee and CT-pepper, while the application of CT-artichoke had an additional biostimulation effect only for roots development (**Table 5**). Moreover, a progressive dose-response trend was also found for the improvement of root and epicotyl length by all treatments with water extracts from composted biomasses. With respect to biostimulant activity of CTs, previous works have already pointed out the positive role exerted on the physiological activities of various crop species in both laboratory experiment and open field trials by the specific structural properties and chemical components of compost extracts (*Pane et al., 2016; Monda et al., 2017, Spaccini et al., 2019*). Although the biological activity of natural organic fractions was thoroughly investigated in the recent years, no univocal structural activity relationships have been highlighted to explain the main mechanisms of action (*Nardi et al., 2017; Olaetxea et al., 2018*). The most acknowledged hypotheses identify in the integration of structural properties and specific chemical components the main variables that trigger

the potential bio-effector properties of various organic extracts. The hormone-like functions and the modulation of biochemical pathways were related to either the presence of dissolved aromatic and lignin fragments as well as to the release of polar bio-labile compounds such as oligo and monosaccharides and peptide clusters and/or aminoacids (*Nardi et al.,2016; Scaglia et al., 2016; Monda et al., 2017; de Aquino et al., 2019*). Moreover, it is the structural arrangement and the conformational behavior of dissolved organic fraction that determine the effective release of bioactive molecules in the rhizosphere environments (*Canellas and Olivares, 2017; Nardi et al., 2017; Olaetxea et al., 2018*). In particular, the flexible wrapping envelope formed by the hydrophobic components may create a microemulsion carrying system that allow a feasible physical and biochemical preservation of bioactive fragments thereby promoting a favorable adhesion to cell. membrane and suitable release of retained molecules (*Muscolo et al., 2007; Dobbss et al., 2010; Aguiar et al., 2013; Canellas and Olivares, 2014; Martinez-Balmori et al., 2014*).

In this work the structural properties and molecular composition of tested CTs, stressed by both NMR and thermochemolyses analyses, seems hence support an effective reliable biostimulation activities shown on the root and shoot development of maize plantlets. In fact, all the water dissolved extracts were characterized by a defined hydropohobic conformation, with the incorporation of potential bioactive molecules represented by lignin units, nitrogen derivatives and carbohydrates. The comparable but differential contribution of involved components did not allow to identify a unique correlation between a specific chemical feature of CT samples with the stimulation activities. As previously indicated, also in this experiment the data underline the occurrence of joint multifactor bio-activity interactions. The slight larger influence displayed by CT-coffee and CT-pepper on plantlets development may be also related to the different molecular characteristics. The larger content in CT-artichoke of undecomposed lignocellulose fragments may have reduced the conformational flexibility with a decrease of both content and release of bioavailable and bioactive lignin monomers (*Monda et al., 2018; Spaccini et al., 2019; Bento et al., 2020*).

Table 5: Roots and Epicotyls length (cm) and percent germination of Basil seedlings under compost teas (CTs) isolated from different vegetable biomasses composted (standard deviation in brackets).

Treatment	Roots (cm)	Epicotyls (cm)	Germination (%)
H_2O	1.5 ±0.06	1.9±0.16	95
CT-coffee 10	2.5±0.13	1.9±0.04	100
CT-coffee 50	2.9±0.10	2.3±0.10	98
CT-coffee 100	3.2±0.07	2.7±0.13	96
CT-artichoke 10	2.1±0.12	1.7±0.07	95
CT-artichoke 50	2.0±0.06	1.8±0.07	96
CT-artichoke 100	2.3±0.06	1.9±0.07	96
CT-pepper 10	2.6±0.21	1.9±0.06	97
CT-pepper 50	2.8±0.11	2.1±0.12	97
CT-pepper100	3.0±0.06	2.5±0.03	95

3.6. Antioxidant activities and total phenolic content determination

Antioxidants are usually involved in several mechanisms of action, including inhibition of free radical generation, enhancement of scavenging capacity against free radicals, and activation of reducing power. In this respect, no single or univocal assay can accurately reflect the wide range of antioxidant properties in a mixed or complex system. Therefore, it is necessary to use at least two complementary different methods to evaluate the antioxidant capacities of organic products (*Antolovich et al., 2002*). In this work, two antioxidant assays, based on 2,2-diphenyl-1-picrylhydrazyl assay (DPPH) and 2,20 -azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS) were applied to evaluate the antioxidant properties of the compost teas (CTs) extracted from different vegetable composted biomasses. Currently, various humic substances and plant extracts have been classified as powerful antioxidants showing reactive oxygen species (ROS) with scavenging properties (*Wang et al., 1996; Avvakumova, et al., 2011; Zykova et al., 2018*), while minor information are available on the antioxidant properties of compost teas. A correlation between the presence of phenolic or quinoid moieties and polyphenolic compounds with the antioxidant and scavenger activities was claimed in natural fruit extracts and humic material based on the behavior of organic fractions as electron donors or acceptors, depending on the redox state of these complex systems (*Jayaprakasha and Patil 2007*; *Aeschbache et al., 2012*). It has been conceived that the supramolecular humic assembly may include many different antioxidant sites, which makes them very attractive for environmental and biomedical applications (*Shang et al. 2017*). In this work, we have also determined the total phenolic content (TPC) of all CTs based on the role of phenolic compounds as key indicators correlated to antioxidant activity (*Muhammed Akif Açıkgöz, 2019*). The antioxidant activity of CTs evaluated by ABTS and DPPH assays and the determination of total phenolic content are shown in **Figure 4**. The results of ABTS assay reported a higher inhibition percentage for the CT-coffee followed by CT-pepper (70.5 % and 40.7 respectively), while lower antioxidant activity was found for the CT-artichoke whit a percentage of inhibition of 19.2 %.

Additionally, referring to the values found for TEAC (mmol Trolox equivalent Kg sample $^{-1}$) parameter, the best antioxidant materials were confirmed the Compost Teas extracted from coffee residues with a TEAC values equal to 40.7 while lower value was observed for the CT-pepper (22.44 TEAC). This trend was also confirmed by results obtained using the DPPH test where the scavenger inhibition percentages was more effective for CT-coffee followed by CT-pepper as reported in **Figure 4**. The total phenol content determined using the Folin Ciocalteau reagent followed the same trend of antioxidant activities with progressive decreasing measured amounts for CT-coffee, CT-pepper and CT-artichoke (**Figure 4**). Although it has been reported that Folin-Ciocalteu reagent is not specific to determine the polyphenols but could also react with any other reducing substances that could be oxidized by the Folin reagent (*Escarpa et al., 2001*; *Chunhe Gu et al., 2019*), the present data clearly revealed a strict correlation between total phenolic content of Compost teas ant antioxidant assays in agreement with previous studies (*Samaniego Sanchez et al., 2007; Dudonné et al., 2009*). Currently the determination of the effective antioxidant activity of natural organic compounds is a problem of considerable concern for the development of new ecofriendly antioxidant systems such as humic acid (*Efimova et al., 2012*). Conversely, notwithstanding the easier extraction procedure and the consequent potential larger amounts of effective compounds, no studies have been carried out to evaluate the antioxidant properties of CTs obtained from composted vegetable residues. In this work, the positive correlation between antioxidant activity and total phenolic contents suggest a connection of antioxidant activity and aromatic composition of all tested materials. In fact, besides the questionable measurements with Folin reagent, the antioxidant activities of CT-coffee and CT-pepper may be related to the amount of lignin derivatives shown by the IR spectroscopy and by the data of thermochemolysis (**Tables 3 and 4**). With respect to the contrasting response obtained for the CT from artichoke residues, the lower decomposition of lignin fragments highlighted by the structural Ad/Al indexes may have limited both the antioxidant activity and the detection of active unbound phenolic components. This result seems in agreement with previous studies on phenolic moieties, in

which mono- and oligohydroxylated benzene units, exhibited antioxidant properties (*Rice-Evans et al., 1996; Bravo et al., 1998; Kaurinovic et al., 2018*)

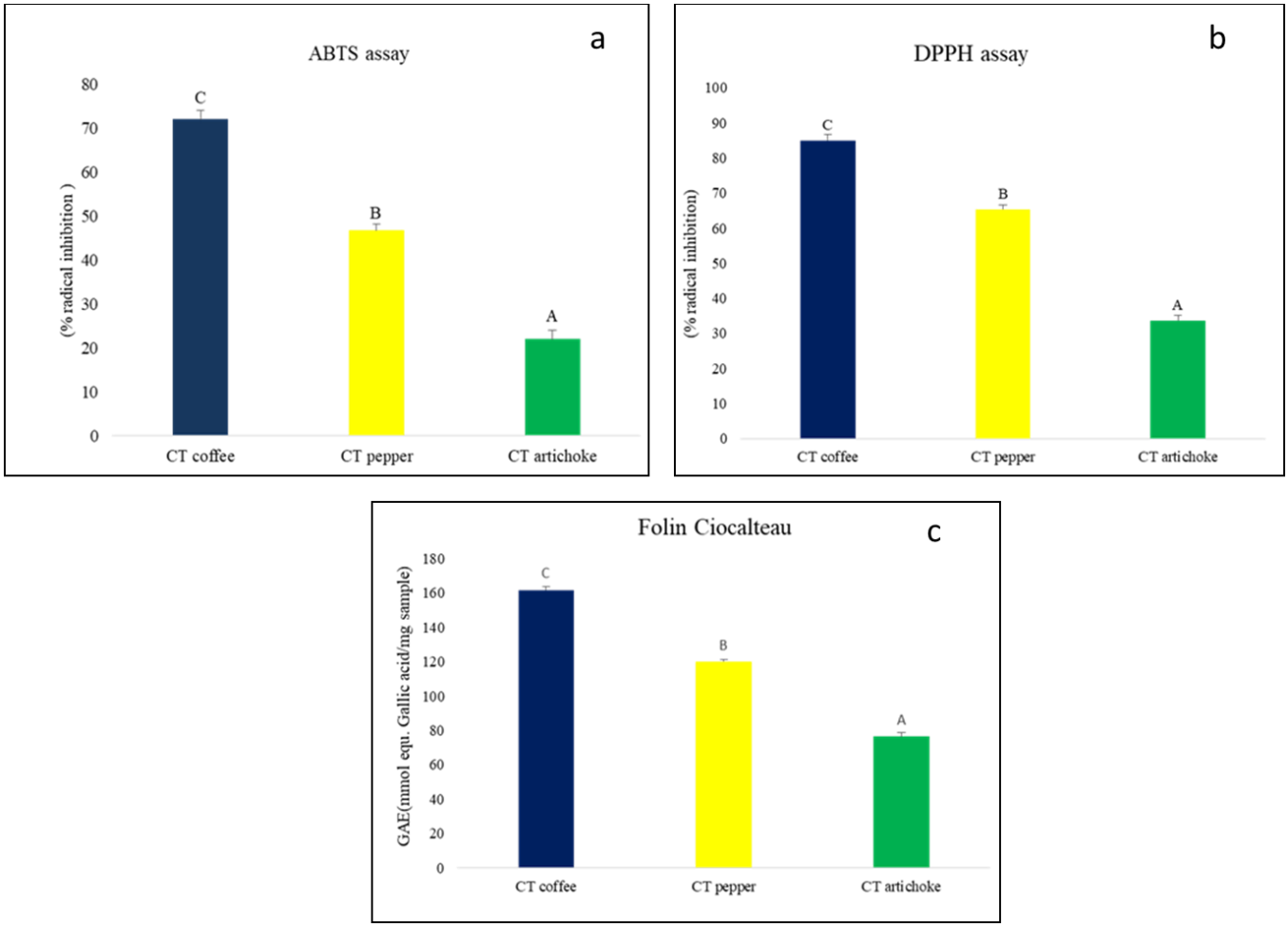


Figure 4: Determination of Antioxidant activity and Total phenolic content of different Compost Teas: panel (a) ABTS assay; panel (b) DPPH assay; panel (c) Folin Ciocalteau assay. Vertical bars represent the standard deviation of the mean. Statistical analysis are performed using LSD test ($p \leq 0.05$).Different letters indicate significant differences between groups.

3.7. Antimicrobial activity

An increasing attention is devoted to the possible exploitation of the antimicrobial activities of compost teas to control plant pathogens as sustainable ecofriendly alternative to the use of synthetic agents for the control of plant diseases (*Siddiqui et al., 2009*). In scientific literature, the application of compost teas in organic agriculture to reduce the effect of micro-organisms responsible for plant diseases is extensively described (*Yogen et al., 2006; Xu et al., 2012 ; Mohd Din et al., 2016 ;Pane et al., 2016 ; Elsaiid et al., 2018*). Non-aerated compost teas (NCT) have shown antibacterial activity against tomato root pathogens and on the development of damping-off diseases caused by *Pythium ultimum* and *Rhizoctonia solani* (*Dionne et al., 2012*). Additionally, suppressive compost tea showed inhibitory effects against bacteria from and mycelial growth of *Alternaria solania* and *Botrytis cinerea* on tomato fruit (*On et al., 2015*). Conversely, no available studies have attempted to evaluate the antimicrobial effect of CTs against bacterial strains involved in common human diseases. In this work, we have tested the possible antibacterial activity of different CTs from green composts against some bacterial strains involved in common human diseases. A preliminary test to study the antibacterial activity of this compost extract was performed by agar dilution plate with viable-count method. The measurements of cells survival revealed that the treatments with compost extracts have an inhibition effect on *Staphylococcus aureus* and *Pseudomonas aeruginosa* (**Figure 5**). The cells growth inhibition was directly proportional to the CTs concentration with a clear dose- effect curve for all tested bacterial cells (**Figure 5**). A stronger antimicrobial effect was observed against *Pseudomonas Aeruginosa* (Gram negative bacterial strain) whereas for *Staphylococcus aureus*, the antibacterial efficacy was reduced for extracts (**Figure 5**). The antibacterial activity was larger for the treatment with CT-coffee followed by CT-pepper and CT-artichoke. In agar dilution plate viable-count method at applied concentration of 50 µg/ml the inhibition of cells proliferation for *Pseudomonas aeruginosa* was equal to 25% for CT-coffee and 37% for CT-pepper, while at maximum concentration (1000 µg/ml) the surviving bacterial cells decreased to 2% , 6% and 12% for

CT-coffee, CT-pepper and CT-artichoke, respectively (**Figure 5**). Similar trend was obtained by the analyses of minimum inhibitory concentration (MIC) as reported in **Table 6**. The comparison of the of minimum suitable antimicrobial concentration of different compost tea extracts, confirmed that the CTs were more effective against *Escherichia coli ATCC 35218*, *Pseudomonas aeruginosa ATCC27355* and *Klebisella pneumonie ATCC700503* whereas low susceptibility to the tested treatments was found for gram positive bacterial strains such as *Staphylococcus aureus ATCC 5538P* and *Enterococcus faecalis ATCC 29212* which showed generally larger MIC values. In detail, CT-coffee has shown the best antibacterial performance with the smallest MIC value against and *Escherichia coli* and *Pseudomonas aeruginosa* (1 µg ml $^{-1}$ and 1.50 µg ml $^{-1}$ respectively). Moreover, CT-coffee exhibited a lower antibacterial effect against *Enterococcus faecalis* (MIC value 2 µg ml $^{-1}$). An average MIC value was found for *Klebisella pneumonie* (2.5 µg ml $^{-1}$). Additionally, CT-pepper displayed relatively low MIC values with an antibacterial activity against *Escherichia coli* (MIC=1.5 µg ml $^{-1}$). These results suggest a potential antimicrobial effect of water compost extracts in accordance to *Van Rensburg et al (2010)* that have investigated the possible antibacterial effect of humic material (oxifulvic acid and humic acid) on the growth of microbial pathogens involved in common human diseases. Furthermore, it was observed that peat and coal HA showed a complete growth inhibition against *Staphylococcus aureus* and *Candida albicans*, and a decrease in the number of colonies from 78–80% in *Escherichia coli* and from 58–70% in *Salmonella enteritidis* (*Yarkova, 2011*). The antimicrobial efficacy of different CTs could be influenced by their chemical composition. All Compost teas have shown an effective antimicrobial activity which may be associated to either the abundance of aromatic and phenolic fractions as well as to the concomitant presence of competitive microbial communities for CT-pepper In agreement with our work, a relation between the phenolic content and antimicrobial activities against human pathogens was previously reported (*Cueva, et al., 2012*), while the competitive and bioactive effects exerted by natural microbial communities is a promising application for the sustainable beneficial control against pathogens (*Naidu et al., 2010; Panappian et al., 2010; Pane et al., 2012*)

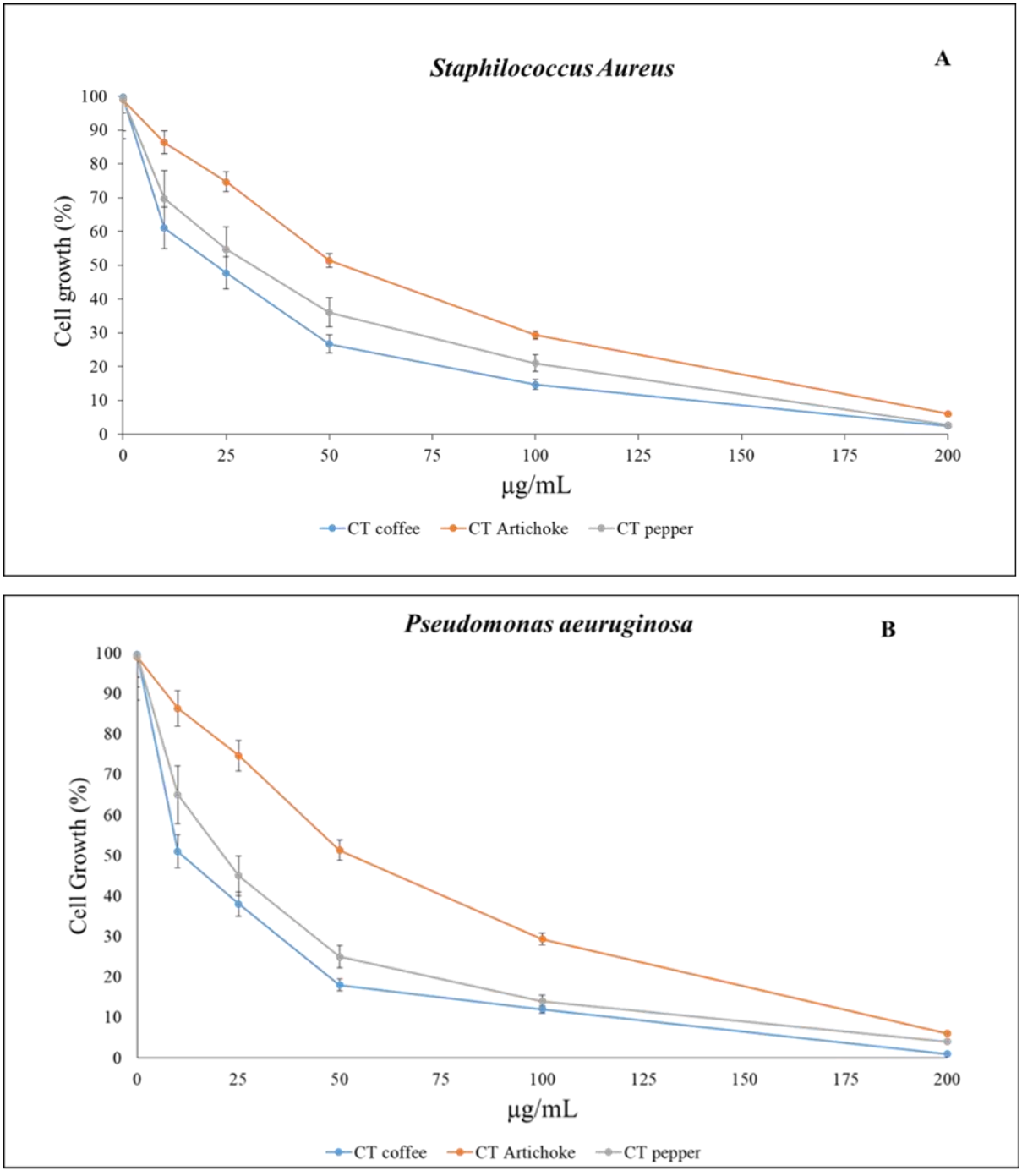


Figure 5: Antibacterial activity of Compost tea extracted from different composted biomasses from artichoke, coffee and pepper at increasing concentration (10-25-50-100-1000 µg ml $^{-1}$).Assays were carried out by viable-count method. (A) *Staphylococcus aureus ATCC 35281*; (B) *Pseudomonas aeruginosa ATCC 27355*. Dose-effect curves: final concentrations were tested.

Table 6: Antibacterial activity of Compost tea extracted from different biomasses composted: coffee, artichoke, pepper and determination of MIC (Minimal Inhibitory Concentration) against some Gram positive and Gram negative bacterial strains. a Assays were carried out by broth diluition method in Nutrient Broth. Replicates were from three independent experiments.

MIC (µg ml $^{-1}$)[a]

	CT-Coffee			**CT-artichoke**			**CT-pepper**		
Bacterial strain	Replicates[b]			Replicates[b]			Replicates[b]		
Escherichia coli ATCC35218	1	1.5	1	5.5	5.5	5.5	1.5	1.5	1
Pseudomonas aeruginosa ATCC27355	1.5	1.5	2	6.5	6	6.5	2.5	2.5	3
Klebisella pneumoniae ATCC700503	2.5	2.5	2.5	8.5	8.5	8	2.5	2.5	2.5
Staphylococcus aureus ATCC5538P	3	3	3	10.5	10	10	3.5	3.5	3
Enterococcus faecalis ATCC29212	2	2	2	14	14.5	14.5	4.5	4.5	5.5

Conclusions

Studies on growth stimulation of the crops induced by compost teas, are receiving higher attention in the last years. Our work suggests that the chemical composition and, in this case, the content of phenols and aromatic compounds have an influence on the properties of compost teas and especially on the antioxidant and antimicrobial activities. Moreover, the efficacy of aromatic plants seeds germination confirmed a strong relationship between the structure and the activities of compost extracts. Thus, identification of the overall chemical structure of compost teas is important to understand and elucidate the potential application of this bioactive molecules source not only in agricultural field but also in nutraceutical industry. In fact, the analysis of CTs in vitro evidence efficiency against human pathogenic microorganisms, gives a rational basis of a potential therapeutic use of compost extracts.

Acknowledgments

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CHAPTER 4

Metabolomics approach to evaluate the effect of humic derivates on secondary metabolites with nutraceutical activity in Basil plants

Abstract

Natural active molecules extracted from aromatic plants are extensively used in nutraceutical, pharmaceutical and cosmetic industries. Eco-friendly methodologies to increase the production of secondary metabolites with pharmacological properties in aromatic plants are currently investigated. Humic materials such as humic substances (HS) and compost teas (CTs) extracted from composted vegetable wastes biomass may influence plant metabolism. In this work, HS and CTs extracted from artichoke and coffee composted biomasses were employed as bioactive treatments on *Ocinum Basilicum* plants. The application of HS and CT affects some phenological parameters such as weight of fresh or dry biomass and chlorophyll content. Additionally, 147 metabolites of different organic compounds classes such as amino acids, organic acids, carbohydrates and phenolic compounds were identified using a Metabolomic approaches by Liquid Chromatography High Resolution Mass Spectrometry (LC/HRMS). The application of HS-artichoke and CT-coffee influences the content of hydroxicinnamic acids with nutraceutical properties such as caffeic acid, ferulic acid, rosmarinic acid but also narigenin and resveratrol. These bioactive humic materials are characterized by a larger percentage of aromatic compounds shown by ^{13}C-CPMAS-NMR analysis. Furthermore, antioxidant activity and total phenol content of Basil leaves treated with humic materials were positively influenced compared to control. These results validate a potential application of humic materials in

nutraceutical and cosmetic fields to increase the production of bioactive compounds in medicinal aromatic plants.

Keywords: *Humic substances, compost tea, metabolomic approach, phenol compounds, pharmacological activity*.

1. Introduction

Medical aromatic plants include a large range of species that produce a diversity of valuable bioactive molecules with a great interest for different pharmaceutical, nutraceutical, cosmetic and agri-food application (*Abdul Rasool Hassan, 2012*). Natural products or secondary metabolites can be considered as a heterogeneous group of metabolic products, which although not being essential for the plants growth, play a major role in the adaptation of plants to their environment with different functionalities such as resistance to botic and abiotic stresses or acting as signaling molecules in ecological interactions (*Thirumurugan et al., 2018*). These plants secondary metabolites can be used for the production of drugs, aromas, perfumes, insecticides or dyes and for this reason, they are defined as products with a potential great economic value (*Verpoorte et al., 2002*). The large variety of secondary metabolites found in plants can be summarized in five main classes: tannins, terpenoids, alkaloids, flavonoids, and phenolic compounds according to their bio-synthetical pathways. (*Bernards, 2010*). Particularly, phenolic compounds are identified as biologically active molecules with a wide range of applicability as antioxidants, antimicrobial and inhibitors of carcinogenesis (*Tanase et al., 2014, 2019*). The majority of known phenolic compounds are synthesized either through the shikimic acid pathway or by the malonate/acetate pathway (*Croteau et al., 2000*). Generally, these bioactive phytochemical compounds are produced in low abundance representing about the 1% of the total carbon and are stored in specific cells or cell organelles (*Ramakrishna et*

al., 2011; Chomel et al., 2016; Bourgaud et al., 2001). Basil (*Ocimum basilicum L.*) is an aromatic annual plant of the Lamiaceae family that can be considered as a good source of bioactive compounds, such as phenolic acids (rosmarinic, chicoric, caffeic, and caftaric) or flavonol (quercetin, kaempferol) involved in antioxidant and antibacterial properties (*Flanigan et al., 2014;; Ghasemzadeh et al., 2016; Złotek et al., 2016*). The World Health Organization has estimated that more than 80% of the world's population in developing countries depends primarily on herbal medicine for basic healthcare needs (*Viens et al., 2004)*. The world market for herbal medicines including herbal products and raw materials is actually growing at an annual rate of 5–15%. This fast raising rate may indicate a reliable growth of demand for natural products in the next years (*Kumar et al., 2008*). The production of natural substances for industrial use requires a preliminary extraction of these compounds from aromatic plants. Since the usual low concentration and the striking sensitivity to isolation condition of these metabolites, there is a steady attention to develop novel methodologies aimed to improve the synthesis of these substances in plants tissues and thus facilitate the subsequent isolation steps and increase the extraction yield (*Malik et al., 2011*). In order to increase crop productivity, intensive agriculture techniques have been indiscriminately used not only in traditional agricultural crops, but also in medicinal plants (*Pereira and Raimunda, 2016*). Inorganic fertilizers provide readily available nitrogen but frequently they have been linked to environmental contamination, soil acidification and salinity (*Nchu et al., 2018*). Moreover, the application of chemical fertilizers and agrochemicals can quantitatively influence plant metabolic processes and trigging quality changes in the production of secondary metabolites with pharmacological properties (*Ncube et al., 2012*). Residues of pesticides or mycotoxins could be found in herbal medicines and phyto-therapies that alter the quality of products with therapeutic proprieties in the treatment of various diseases (*Pereira et al., 2019*). In addition, an increasing focus is devoted on sustainable approaches to raise the synthesis of secondary metabolites in aromatic plants without altering the quality of the final products. In this context, the natural organic fraction obtained from recycled biomasses, such as humic substances (HS) and compost teas (CTs) can found an extensive application due to their ability to influence directly and

indirectly the plant metabolism (*Canellas et al., 2014; Vaccaro et al., 2015; Pane et al., 2016; Olaetxea et al., 2018*). These molecules may indeed and act as bioactive compounds or bioeffectors in various physiological processes such as, nutrient up-take, ion transport, enzyme activities, with a large effect on biochemical pathways connected to primary and secondary metabolites (*Pereira et al., 2019*). Omics-science are defined as analytical approaches to study the molecular responses induced in plants by different treatments or specific environmental conditions (*Welti et al., 2007; Bundy et al., 2009*). The large qualitative variability and quantitative of available metabolites in the plant kingdom, are the useful basis of the new active pharmacological substances discovery. However, this wide starting line, increase the difficulty for the careful isolation, detection and characterization processes of this bioactive molecules (*Wolfender et al., 2010*). Metabolomics approach focuses on the complete analysis of all the metabolites present in a biological system (*Fiehn et al., 2001*). This advanced technique represents one of the rapidly evolving technologies of systems biology that studies the interactions between the different functional levels of biological organisms (*Glauser et al., 2013*). In recent years, liquid chromatography (LC) and, in particular, ultra-high-performance chromatography LC (UHPLC) coupled with high resolution mass spectrometry (HRMS) techniques has become a reference standard method for multi-omics studies due to its ability to quickly isolate and identify a large range of low abundant molecules, such as secondary plant metabolites (*Theodoridis et al., 2012*). The Q Exactive HF quadrupole-Orbitrap integrates ultra-high performance rapid separation of liquid chromatography (UHPLC), diode array detection (PDA or DAD) and HRMS efficiency of an orbital trap with the assistance of a quadrupole combined with excellent diagnostic capabilities using a high-energy collision dissociation cell (HCD), which provides high-resolution MS_n fragments (*Simirgiotis et al., 2017*). This approach is already applied for the rapid analysis of small low concentrated compounds, such as toxins, pesticides, terpenes and phenols (*Simirgiotis et al., 2017*).

The objective of this study is to evaluate the activity of different natural organic derivates such as HS and CTs obtained from on–farm composting treatment of artichoke and coffee biomasses on the

production of secondary metabolites with pharmacological application in *Basil* plants. The metabolomic approach was applied for the careful qualitative and quantitative detection of plant metabolites to investigate the structural activity relationship between molecular features of applied compost derivates and activated metabolic processes.

2. Materials and methods

2.1. Experimental design, plant growth, sampling and analyses

To set up the pot experiment, we used the surface layer (0–15 cm) of soil collected at the Long-Term Experimental field site of the University Farm of Agricultural Dept. of University of Naples "Federico II", located in Castel-Volturno (CE). The pot experiment was conducted from March to June 2018, under greenhouse conditions (25–33 °C, daily temperature range). The pH and electrical conductivity (EC) of soil were measured in a 1:5 soil/water suspension (w/v) after 1 h end-over-end shaking at 25 °C. Soil organic carbon (C) was determined by the Walkley–Black procedure (*Nelson and Sommers, 1982*) and total nitrogen (N) by the Kjeldahl digestion method (*Bremner and Mulvaney, 1982*). Available soil phosphorus (P) was extracted with sodium bicarbonate (Olsen method) and then determined by the molybdenum-blue method (*Murphy and Riley, 1962*). Soil was defined as a clay loam (44.6%, 28% and 27.4% for sand, silt and clay in the order), alkaline (pH 8.6) and classified as a Vertic Xerofluvent, containing 1.11 g kg^{-1} total N, 10.5 g kg^{-1} organic carbon, 11 mg kg−1 of $NaHCO_3$-extractable P. The soil was sieved to 5 mm, mixed with quartz sand at the ratio of 2:1 (w/w) and thoroughly homogenized. Basil plants were grown in pots filled with 1 kg of soil/sand substrate. A basal nutrients supply was performed adding (N) nitrogen as ammonium nitrate (NH_4NO_3) at the rate of 150 mg N kg^{-1} dry substrate, (K) potassium as triple superphosphate (TSP) at the rate of 75 mg K kg^{-1} dry substrate and (P) phosphorus as potassium sulphate (K_2SO_4) at the rate of 160 mg P kg^{-1} dry substrate. The fertilizers were added in powder form to the substrate (soil/sand

mixture) of each individual pot and mixed thoroughly. Then, HS and CTs extracted from artichoke and coffee composted biomasses were tested at three different concentration (10-50-100 g L^{-1}) and applied to the soil one time a week for 4 weeks to obtain a final concentration of applied organic material of 0.10, 0.20 and 0.40 g Kg^{-1} respectively.

The pot trial was designed as follows:

CTRL: Control (H_2O) +mineral fertilizers

Soil treatments as control plus organic extracts:

A: HS artichoke 10 g L^{-1}; ***B***: HS artichoke 50 g L^{-1}; ***C***: HS artichoke 100 g L^{-1}

D: CT artichoke 10 g L^{-1}; ***E***: CT artichoke 50 g L^{-1}; ***F***: CT Artichoke 100 g L^{-1}

G: HS Coffee 10 g L^{-1}; ***H***: HS Coffee 50 g L^{-1}; ***I***: HS Coffee 100 g/L

L: CT coffee 10 g L^{-1}; ***M***: CT coffee 50 g L^{-1}; ***N***: CT coffee 100 g L^{-1}.

Each treatment was replicated four times for a total of 52 pots. Harvested leaves were weighed, immediately frozen in liquid nitrogen, in order to quench the metabolism, and stored at -80 °C.

2.2. Determination of chlorophyll content

The chlorophyll content was determined as follows: 9 mm of fresh foliar tissue from each plant were ground in liquid nitrogen, homogenized in 5 ml of pure acetone, and then transferred into 15 ml glass centrifuge tubes to be centrifuged at 3000 x g for 5 min at 8°C. The supernatant absorbance at 645 and 663 nm were measured using a spectrophotometer and the concentrations of Chl a, Chl b, total Chl (Chl a + b) were calculated according the method of *Lichtenthaler* (1987) and expressed in mg of pigment per g of leaf fresh weight.

2.3. Extraction of plant leaf metabolites

Basil leaves stored at -80 °C were homogenized by using a mortar and pestle under liquid nitrogen. Then, 60±0.5mg of pooled homogenized plant material was weighed into pre-chilled 2 ml Eppendorf tube. The metabolites extraction was conducted by adding 1 ml of water/methanol/chloroform mixture (1:3:1 ratio) pre-cooled at -20 °C. The samples were mixed for 60 s and incubated for 30 min at 70 °C in order to inhibit the activity of possible enzymes present in the extract. The mixtures were then centrifuged for 10 min at 10000 rpm and 4 °C, and the supernatants were recovered and transferred into 2 ml Eppendorf tubes. Milli Q water (400 µL) was added to allow the separation of polar and apolar phases corresponding to the methanol/water (upper) and chloroform (lower) phases, respectively. The chloroform phase was used to Lipidomic identification (see **Chapter 7**). All extracts were finally stirred for 30 s and centrifuged for 10 min at 4 °C at 10000 rpm. A volume of 400 µL was collected from the upper phase, transferred into 1.5 ml glass tubes for LC–MS analyses, dried using speed-vacuum and stored at -80 °C.

2.4. Composting process, extraction of humic substances (HS) and compost teas (CTs)

The composts used in this study were obtained through a 45-days on-farm composting process (active or thermophilic phase) of static piles of chipped artichoke plant residues under forced aeration, followed by a two months-curing period. Humic substances was obtained through the following extraction procedure: an aliquot of 100 g of each air-dried compost (2 mm sieved) was suspended in 500 ml 0.1 mol L^{-1} KOH and mechanically shaken for 24 h. The suspension was then centrifuged at 7000 rpm for 20 min and glass wool filtered. The extraction was repeated 2 times (1h agitation step). The suspension was acidified to pH 7 with 6 mol $L^{-}1$ HCl. Then, humic substances was collected and dialyzed against deionized water using 1-kD cutoff spectrapore membrane until the electrical conductivity resulted lower than 0.5 dS m^{-1}. Humic substances were then freeze-dried for further

analytical characterization. The isolation of CTs was performed as reported in the previous **Chapter 3.**

2.5. Chemical characterization of CT and HS samples: ^{13}C-CPMAS-NMR spectroscopy

The solid state NMR spectra of humic substances and compost teas were recorded on a Bruker AV-300 equipped with a 4 mm wide-bore MAS probe, with the following acquisition parameters: 13,000 Hz of rotor spin rate; 2 s of recycle time; 1H-power for CP 92.16 W: 1H 90° pulse 2.85 μs; 13C power for CP 150,4 W; 1 ms of contact time; 30 ms of acquisition time; 4000 scans. Samples were packed in 4 mm zirconium rotors with Kel-F caps. For the interpretation of ^{13}C-CPMAS-NMR spectra, the overall chemical shift range was divided into the following main resonance regions: alkyl-C (0–45 ppm); methoxyl-C and N-alkyl-C (45–60 ppm); O-alkyl-C (60–110 ppm); unsubstituted and alkyl-substituted aromatic-C (110–145 ppm); O- substituted aromatic-C (145-160 ppm); carboxyl- and carbonyl- C (160–200 ppm). In order to summarize the molecular characteristics of organic extracts, the following dimensionless structural indexes were calculated from the relative amount of C distribution in the NMR spectra (*de Aquino et al., 2019; Spaccini et al., 2019*):

- the Hydrophobic index is the ratio of signal intensities found in chemical shift intervals for apolar alkyl and aromatic C components over those of hydrophilic C molecules

  $HB/HI = \Sigma[(0–45) + (45–60)/2+(110–160)] / \Sigma[(45–60)/2+(60–110)+ (160–190)]$;
- the Aromaticity index compare the amount of aromatic compounds to that of alkyl fraction

  $ARM = [(110–160)/\Sigma(0–45) + (60–110)]$
- the Alkyl ratio estimate the relative abundance of apolar over that of polar-alkyl fraction

  $A/OA= (0–45)/(60–110)$
- the Lignin ratio, relates the area of Methoxyl-C+N-alkyl groups to that of O-aryl-C

  $LigR = (45–60) / (145–160)$

The HB, ARM and A/OA parameters have been extensively applied to estimate the either biochemical stability of NOM, as well as the relation of structural properties with biostimulant activities of organic

extracts, while the LR is a useful indicator to discriminate between signals owing to lignin and other phenolic moieties (lower LigR) with respect to the prevalent inclusion of peptidic clusters (larger LigR) in the 45–60 ppm interval (*Martinez-Balmori et al., 2014; de Aquino et al., 2019; Spaccini et al., 2019*).

2.6. Metabolomic approaches:

2.6.1 Standard references

For the metabolomic analyses metabolite standards were purchased from Sigma, Carbosynth (Compton Berkshire, England) Avanti Polar Lipids, Inc. (Alabaster, Alabama, USA), Fluka and Merck (Vienna, Austria). A multi-metabolite mix called MoMo Mix (309 metabolites) were prepared (a comprehensive list of the standards can be found in **Table S1**. Standard stock solutions of 1 or 10 mM were prepared in water and used for the preparation of a multicomponent standard called MoMoMix of 0.5, 1, 5, 10, 50, 100, 500 nM and 1, 5, 10, 50 µM. Additionally, a quality control (QC) of 1 µM was prepared. Then, a dry mix including rosmarinic acid, caffeic acid, epicatechin, kaempferol, salvianolic acid B, trans-cinnamic acid, resveratrol, p-coumaric acid, tocopherol, 4-hydroxybenzoic acid, chicoric acid and quercetine-ß-D-glucoside was dissolved in 1 ml methanol to obtain a 100 µM solution of phenolic compounds. This solution was diluted 1:10 with 5% methanol to be injected as a quality control. Furthermore, the 100 µM phenolic mixture was mixed 1:1 with a 100 µM MoMoMix solution. This total 50 µM was used as a stock solution and further diluted to 0.1, 0.3, 0.5, 1, 3, 5, 7.5, 10 µM solutions used for external calibration.

Table S1: Composition of multi-metabolite standard mix called MoMo Mix used to metabolomic analysis.

	Compound Name	m/z	Adduct	Charge
1	1-Methylhydantoin	113.03565	M-H	-1
2	1-Methylnicotinamide	135.05639	M-H	-1
3	2'-Deoxyuridine	227.06734	M-H	-1
4	2-(Carbamoylamino)butanedioic acid	175.03604	M-H	-1
5	2-Deoxycytidine	226.08333	M-H	-1
6	3-Methyl-2-oxovaleric acid	129.05572	M-H	-1
7	3-Methylcytidine	256.09389	M-H	-1
8	3-Phosphoglycerate	184.98566	M-H	-1
9	3AMP	346.05581	M-H	-1
10	4-Hydroxy-proline	130.05097	M-H	-1
11	5'-Deoxy-5'-Methylthioadenosine	296.08228	M-H	-1
12	5-Methyluridine	257.07791	M-H	-1
13	6-Phosphogluconate	275.01736	M-H	-1
14	Adenine	134.04722	M-H	-1
15	Adenosine	266.08948	M-H	-1
16	ADP	426.02214	M-H	-1
17	Alanine	88.0404	M-H	-1
18	alpha-Aminoadipic acid	160.06153	M-H	-1
19	alpha-Ketoglutarate	145.01425	M-H	-1
20	AMP	346.05581	M-H	-1
21	Arginine	173.1044	M-H	-1
22	Argininosuccinic acid	289.11536	M-H	-1
23	Asparagine	131.04622	M-H	-1
24	Aspartate	132.03023	M-H	-1
25	ATP	505.98847	M-H	-1
26	Betaine	116.0717	M-H	-1
27	Biotin	243.08089	M-H	-1
28	cAMP	328.04524	M-H	-1
29	Carnitine	160.09792	M-H	-1
30	cGMP	344.04016	M-H	-1
31	Choline chloride	102.09244	M-H	-1
32	cis-Aconitate	173.00916	M-H	-1
33	Citrate	191.01973	M-H	-1
34	Citrulline	174.08841	M-H	-1
35	CMP	322.04457	M-H	-1
36	CTP	481.97723	M-H	-1
37	Cystathionine	221.06015	M-H	-1
38	Cysteic acid	167.99722	M-H	-1

39	Cysteine	120.01247	M-H	-1
40	Cysteinyl-glycine	177.03394	M-H	-1
41	Cystine	239.01657	M-H	-1
42	Cytidine	242.07824	M-H	-1
43	Cytosine	110.03599	M-H	-1
44	dAMP	330.06089	M-H	-1
45	dATP	489.99355	M-H	-1
46	dCMP	306.04966	M-H	-1
47	dCTP	465.98232	M-H	-1
48	dGTP	505.98847	M-H	-1
49	Dihydroxyacetonephosphate	168.99075	M-H	-1
50	Dihydroxyisovalerate	133.05063	M-H	-1
51	Erythrol	121.05063	M-H	-1
52	Erythrose-4-phosphate	199.00131	M-H	-1
53	Flavinadenin dinucleotide	784.14986	M-H	-1
54	Fructose	179.05611	M-H	-1
55	Fructose-1,6-bisphosphate	338.98877	M-H	-1
56	Fructose-6-phosphate	259.02244	M-H	-1
57	Fumarate	115.00368	M-H	-1
58	Galactose	179.05611	M-H	-1
59	GDP	442.01705	M-H	-1
60	Gluconate	195.05103	M-H	-1
61	Glucose	179.05611	M-H	-1
62	Glucose-1-phosphate	259.02244	M-H	-1
63	Glucose-6-phosphate	259.02244	M-H	-1
64	Glutamate	146.04588	M-H	-1
65	Glutamine	145.06187	M-H	-1
66	Glutamyl-cysteine	249.05507	M-H	-1
67	Glutathione, oxidized	611.14468	M-H	-1
68	Glutathione, reduced	306.07653	M-H	-1
69	Glycine	74.02475	M-H	-1
70	Glyoxylic acid	72.99312	M-H	-1
71	GMP	362.05072	M-H	-1
72	GTP	521.98338	M-H	-1
73	Guanidineacetic acid	116.04655	M-H	-1
74	Guanine	150.04213	M-H	-1
75	Guanosine	282.08439	M-H	-1
76	Histidine	154.0622	M-H	-1
77	Homocysteine	134.02812	M-H	-1
78	Homoserine	118.05097	M-H	-1
79	Hydroxyglutaric acid	147.0299	M-H	-1
80	IMP	347.03982	M-H	-1
81	Inosine	267.07349	M-H	-1
82	Inositol	179.05611	M-H	-1
83	Isocitrate	191.01973	M-H	-1

84	Isoguanosine	282.08439	M-H	-1
85	Isoleucine	130.08735	M-H	-1
86	Ketoisovalerate	115.04007	M-H	-1
87	Kynurenine	207.07752	M-H	-1
88	Lactate	89.02442	M-H	-1
89	Leucine	130.08735	M-H	-1
90	Lysine	145.09825	M-H	-1
91	Malate	133.01425	M-H	-1
92	Mannitol	181.07176	M-H	-1
93	Mannitol 1-phosphate	261.03809	M-H	-1
94	Mannose	179.05611	M-H	-1
95	Melatonine	231.1139	M-H	-1
96	Methionine	148.04377	M-H	-1
97	Methionine sulfone	180.0336	M-H	-1
98	Mevalonic acid	147.06628	M-H	-1
99	N-Acetyl-Asp-Glu	303.08339	M-H	-1
100	N-Acetyl-L-aspartic acid	174.0408	M-H	-1
101	N-Acetyl-serine	146.04588	M-H	-1
102	N4-Acetylcytidine	284.08881	M-H	-1
103	NAD+	662.10184	M-H	-1
104	NADH	664.11749	M-H	-1
105	NADP+	742.06817	M-H	-1
106	NADPH	744.08382	M-H	-1
107	Nicotinamide	121.04074	M-H	-1
108	Octopamine	152.0717	M-H	-1
109	Ornithine	131.0826	M-H	-1
110	Oxaloacetic acid	130.9986	M-H	-1
111	Palmitic acid	255.23295	M-H	-1
112	Phenylalanine	164.0717	M-H	-1
113	Phosphocreatine	210.02853	M-H	-1
114	Proline	114.05605	M-H	-1
115	Propionyl-L-carnitine	216.12413	M-H	-1
116	Pseudouridine	243.06226	M-H	-1
117	Pyruvate	87.00877	M-H	-1
118	Ribose	149.04555	M-H	-1
119	Ribose-5-phosphate	229.01188	M-H	-1
120	Ribulose-5-phosphate	229.01188	M-H	-1
121	S-(Adenosyl)-methionine	397.12996	M-H	-1
122	S-Adenosyl-homocysteine	383.11431	M-H	-1
123	Sarcosine	88.0404	M-H	-1
124	Sedoheptulose-7-phosphate	289.03301	M-H	-1
125	Seleno-methionine	195.98822	M-H	-1
126	Serine	104.03532	M-H	-1
127	Serotonine	175.08769	M-H	-1
128	Spermidine	144.15062	M-H	-1

129	Spermine	201.20847	M-H	-1
130	Succinate	117.01933	M-H	-1
131	Thiamine	264.10503	M-H	-1
132	Threonine	118.05097	M-H	-1
133	Thymidine	241.08299	M-H	-1
134	Thymine	125.03565	M-H	-1
135	TMP	321.04933	M-H	-1
136	Trehalose	341.10893	M-H	-1
137	Tryptophan	203.0826	M-H	-1
138	TTP	480.98199	M-H	-1
139	Tyrosine	180.06662	M-H	-1
140	Methylhydantoin	117.04907	M-H	-1
141	Methylnicotinamide	142.07987	M-H	-1
142	Deoxyuridine	236.09754	M-H	-1
143	(Carbamoylamino)butanedioic acid	180.05282	M-H	-1
144	Deoxycytidine	235.11352	M-H	-1
145	3-Methyl-2-oxovaleric acid	135.07585	M-H	-1
146	Methylcytidine	266.12744	M-H	-1
147	3-Phosphoglycerate	187.99573	M-H	-1
148	AMP	356.08936	M-H	-1
149	4-Hydroxy-proline	135.06774	M-H	-1
150	5'-Deoxy-5'-Methylthioadenosine	307.11919	M-H	-1
151	Methyluridine	267.11146	M-H	-1
152	Phosphogluconate	281.03749	M-H	-1
153	Adenine	139.06399	M-H	-1
154	Adenosine	276.12303	M-H	-1
155	ADP	436.05569	M-H	-1
156	Alanine	91.05047	M-H	-1
157	alpha-Aminoadipic acid	166.08166	M-H	-1
158	alpha-Ketoglutarate	150.03102	M-H	-1
159	Arginine	179.12453	M-H	-1
160	Argininosuccinic acid	299.14891	M-H	-1
161	Asparagine	135.05963	M-H	-1
162	Aspartate	136.04365	M-H	-1
163	ATP	516.02202	M-H	-1
164	Betaine	121.08848	M-H	-1
165	Biotin	253.11443	M-H	-1
166	cAMP	338.07879	M-H	-1
167	Carnitine	167.1214	M-H	-1
168	cGMP	354.07371	M-H	-1
169	Choline chloride	107.10921	M-H	-1
170	cis-Aconitate	179.02929	M-H	-1
171	Citrate	197.03985	M-H	-1
172	Citrulline	180.10854	M-H	-1
173	CMP	331.07477	M-H	-1

174	CTP	491.00743	M-H	-1
175	Cystathionine	228.08363	M-H	-1
176	Cysteic acid	171.00728	M-H	-1
177	Cysteine	123.02254	M-H	-1
178	Cysteinyl-glycine	182.05071	M-H	-1
179	Cystine	245.0367	M-H	-1
180	Cytidine	251.10844	M-H	-1
181	Cytosine	114.0494	M-H	-1
182	dAMP	340.09444	M-H	-1
183	dATP	500.0271	M-H	-1
184	dCMP	315.07985	M-H	-1
185	dCTP	475.01251	M-H	-1
186	dGTP	516.02202	M-H	-1
187	Dihydroxyacetonephosphate	172.00081	M-H	-1
188	Dihydroxyisovalerate	138.06741	M-H	-1
189	Erythrol	125.06405	M-H	-1
190	Erythrose-4-phosphate	203.01473	M-H	-1
191	Flavinadenin dinucleotide	811.24044	M-H	-1
192	Fructose	185.07624	M-H	-1
193	Fructose-1,6-bisphosphate	345.0089	M-H	-1
194	Fructose-6-phosphate	265.04257	M-H	-1
195	Fumarate	119.0171	M-H	-1
196	Galactose	185.07624	M-H	-1
197	GDP	452.0506	M-H	-1
198	Gluconate	201.07115	M-H	-1
199	Glucose	185.07624	M-H	-1
200	Glucose-1-phosphate	265.04257	M-H	-1
201	Glucose-6-phosphate	265.04257	M-H	-1
202	Glutamate	151.06266	M-H	-1
203	Glutamine	150.07864	M-H	-1
204	Glutamyl-cysteine	257.0819	M-H	-1
205	Glutathione, oxidized	631.21178	M-H	-1
206	Glutathione, reduced	316.11008	M-H	-1
207	Glycine	76.03146	M-H	-1
208	Glyoxylic acid	74.99983	M-H	-1
209	GMP	372.08427	M-H	-1
210	GTP	532.01693	M-H	-1
211	Guanidineacetic acid	119.05661	M-H	-1
212	Guanine	155.05891	M-H	-1
213	Guanosine	292.11794	M-H	-1
214	Histidine	160.08233	M-H	-1
215	Homocysteine	138.04154	M-H	-1
216	Homoserine	122.06439	M-H	-1
217	Hydroxyglutaric acid	152.04667	M-H	-1
218	IMP	357.07337	M-H	-1

219	Inosine	277.10704	M-H	-1
220	Inositol	185.07624	M-H	-1
221	Isocitrate	197.03985	M-H	-1
222	Isoguanosine	292.11794	M-H	-1
223	Isoleucine	136.10748	M-H	-1
224	Ketoisovalerate	120.05684	M-H	-1
225	Kynurenine	217.11106	M-H	-1
226	Lactate	92.03448	M-H	-1
227	Leucine	136.10748	M-H	-1
228	Lysine	151.11838	M-H	-1
229	Malate	137.02767	M-H	-1
230	Mannitol	187.09189	M-H	-1
231	Mannitol 1-phosphate	267.05822	M-H	-1
232	Mannose	185.07624	M-H	-1
233	Melatonine	244.15751	M-H	-1
234	Methionine	153.06055	M-H	-1
235	Methionine sulfone	185.05038	M-H	-1
236	Mevalonic acid	153.08641	M-H	-1
237	N-Acetyl-Asp-Glu	314.12029	M-H	-1
238	N-Acetyl-L-aspartic acid	180.06092	M-H	-1
239	N-Acetyl-serine	151.06266	M-H	-1
240	N4-Acetylcytidine	295.12571	M-H	-1
241	NAD+	683.1723	M-H	-1
242	NADH	685.18795	M-H	-1
243	NADP+	763.13863	M-H	-1
244	NADPH	765.15428	M-H	-1
245	Nicotinamide	127.06086	M-H	-1
246	Octopamine	160.09854	M-H	-1
247	Ornithine	136.09937	M-H	-1
248	Oxaloacetic acid	135.01202	M-H	-1
249	Palmitic acid	271.28663	M-H	-1
250	Phenylalanine	173.1019	M-H	-1
251	2-phosphoenolpyruvate, PEP	167.042	M-H	-1
252	Phosphocreatine	214.04195	M-H	-1
253	Proline	119.07283	M-H	-1
254	Propionyl-L-carnitine	226.15768	M-H	-1
255	Pseudouridine	252.09245	M-H	-1
256	Pyruvate	90.01883	M-H	-1
257	Ribose	154.06232	M-H	-1
258	Ribose-5-phosphate	234.02865	M-H	-1
259	Ribulose-5-phosphate	234.02865	M-H	-1
260	S-(Adenosyl)-methionine	412.18028	M-H	-1
261	S-Adenosyl-homocysteine	397.16128	M-H	-1
262	Sarcosine	91.05047	M-H	-1
263	Sedoheptulose-7-phosphate	296.05649	M-H	-1

264	Seleno-methionine	201.005	M-H	-1
265	Serine	107.04538	M-H	-1
266	Serotonine	185.12123	M-H	-1
267	Spermidine	151.1741	M-H	-1
268	Spermine	211.24202	M-H	-1
269	Succinate	121.03275	M-H	-1
270	Thiamine	276.14529	M-H	-1
271	Threonine	122.06439	M-H	-1
272	Thymidine	251.11654	M-H	-1
273	Thymine	130.05242	M-H	-1
274	TMP	331.08287	M-H	-1
275	Trehalose	353.14919	M-H	-1
276	Tryptophan	214.1195	M-H	-1
277	TTP	491.01553	M-H	-1
278	Tyrosine	189.09681	M-H	-1
279	UDP	412.02511	M-H	-1
280	UMP	332.05878	M-H	-1
281	Uracil	115.03342	M-H	-1
282	Urea	60.02844	M-H	-1
283	Uridine	252.09245	M-H	-1
284	UTP	491.99144	M-H	-1
285	Valine	121.08848	M-H	-1
286	Xanthine	156.04292	M-H	-1
287	Xylose	154.06232	M-H	-1
288	UDP	402.99492	M-H	-1
289	UMP	323.02859	M-H	-1
290	Uracil	111.02	M-H	-1
291	Urea	59.02509	M-H	-1
292	Uridine	243.06226	M-H	-1
293	UTP	482.96125	M-H	-1
294	Valine	116.0717	M-H	-1
295	Xanthine	151.02615	M-H	-1
296	Xylose	149.04555	M-H	-1
297	Rosmarinic Acid	359.0845	M-H	-1
298	Caffeic acid	179.042252	M-H	-1
299	Epicatechin	289.079041	M-H	-1
300	Kaempferol	285.047729	M-H	-1
301	Salvianolic acid B	717.153381	M-H	-1
302	Trans Cinnamic Acid	149.052429	M-H	-1
303	Resveratrol	227.078644	M-H	-1
304	p-Coumaric acid	163.047348	M-H	-1
305	Tocopherol	429.381073	M-H	-1
306	4-Hydroxy benzoic acid	137.031693	M-H	-1
307	Chicoric Acid	473.079834	M-H	-1
308	Quercetin-glucoside	319.053223	M-H	-1

309	Naringenine	271.25	M-H	-1

2.6.2 Liquid Chromatography High Resolution Mass Spectrometry (LC/HRMS)

High-resolution mass spectrometry was conducted on a high field Thermo Scientific Q Exactive HF quadrupole-Orbitrap mass spectrometer equipped with an electrospray source. For metabolomic analysis different gradients were applied all using MS-grade solvents. In detail, for metabolomics approach, HPLC system was equipped with an Aquity UPLC HSS T3 column (150 × 2.01 mm, particle size 1.8 µm; Waters). Eluent A was 0.1% formic acid and eluent B was 0.1% formic acid in acetonitrile. The gradient was adapted in accordance to Farag et al., 2017 and was as follows: 0-1 min isocratic 95% A, 1-19 min linear gradient starting from 95% A to 5% A, 19-22 min isocratic at 5% A and final re-equilibration from 22 to 25 min at 95% A. The flow rate was 200 µl per minute. All mass spectrometric measurements were performed at the Department of Analytical Chemistry at the University of Vienna. The analyses were carried out on a Q Exactive HF mass spectrometer (Thermo Fisher Scientific) using electrospray ionization (ESI). For metabolomics approach, full mass scan (full MS, 100−1500 m/z) in negative mode was used at a resolution of 120 000 for all calibration standards and samples. The automatic gain control (AGC) target was set to 1 × 106 ions and the maximum injection time (IT) was 200 ms. The ESI source parameters were the following: sheath gas 50, auxiliary gas 14, sweep gas 3, spray voltage 2.75 kV, capillary temperature 230 °C, S-Lens RFlevel 45, and auxiliary gas heater 380 °C.

2.6.3 Data processing LC/HRMS results

Targeted data evaluation for the quantification of metabolites (organic acids, sugar, sugar phosphates, and nucleotides) was performed using Skyline software with an external calibration. All

calibration curves were linear and weighted 1/x. Then, for metabolomics non-targeted data processing, Thermo Scientific Compound Discoverer 2.0 software was applied. This software combines feature detection with statistical data evaluation. Retention time alignment within 0.15 min and 5 g L^{-1} mass tolerance was performed. For the detection of unknown features on the MS^1 level, a minimum peak intensity of 10 000, 3 g L^{-1} mass tolerance. Unknown compounds were grouped according to a mass tolerance of 3 g L^{-1} and 0.15 min. The "Fill Gaps" node was used with 3 g L^{-1} and 0.1 min mz-Cloud search was performed with 5 g L^{-1} mass tolerance and an assignment threshold for compound annotation of 70.

2.6.4 Metabolomics statistical analysis

The evaluation of LC-MS chromatograms was performed by normalizing the area of each peak by the area of the control quality and further modulating it as a function of sample fresh weight (mg). The total metabolomics data set composed of LC-MS (147 variables) data was subjected to Principal Component Analysis (PCA) using XLStat software v.9.0 (Addinsoft). PCA represents an unsupervised classification method requiring no a priori knowledge of the data set and reduces the dimensionality of multivariate data while preserving most of information, expressed in terms of variable variance. Significant differences in metabolome amounts as a function of the studied treatments were tested by one-way ANOVA, followed by Tukey's test ($p< 0.05$).

2.7. Antioxidant activity: Diphenyl-1-Picrylhydrazyl (DPPH) assay

Antioxidant capacities were determined in accordance to *Kim et al., (2011)* with some modifications. Briefly, 40 µl of methanolic basil extract at the concentration of 20 mg/mL, was combined with 60 µl of 80% methanol, 400 µl of 0.1 M tris–HCl buffer (pH=7.5), and 500 µl of a 0.3 mM solution of DPPH dissolved in methanol 80%. The mixture was vortexed then incubated in the dark at room temperature for 20 min. The absorbance of the sample solution (A_{sample}) was measured

and compared to the absorbance of a control ($A_{control}$) containing 100 µl of 80% methanol, 400 µl of tris–HCl buffer, and 500 µl of a 0.3 mM solution of DPPH dissolved in methanol 80%. The free-radical scavenging was then calculated by the equation: % DPPH scavenging = $(1 - A_{sample}/A_{control}) \times 100$. The % DPPH free-radical scavenging was then compared to a Trolox standard curve, and antioxidant capacities were expressed as Trolox equivalent antioxidant capacities (TEAC, mmol of trolox equivalents/100g DW). All the measurements were carried out in three replicates.

2.8. Determination of total phenolic content: Folin-Ciocalteau assay

The total phenolic concentrations in basil leaves were obtained using the Folin-Ciocalteau colorimetric assay (*Kim et al., 2011*). Briefly, 37.5 µl of methanolic basil extract (20 mg/mL), 337.5 µl of water, 187.5 µl of Folin-Ciocalteu reagent and 937.5 µl of 2% aqueous sodium carbonate were mixed and incubated for 20 minutes at room temperature in darkness. After this step, samples were centrifuged for 10 minutes at 10000 rpm. The absorbance was measured at 735 nm against a blank composed of 0.75 ml of water and 1.25 ml of 2% aqueous sodium carbonate. The concentration of the total phenolic compounds was expressed as a milligram of gallic acid equivalents (GAE) per gram of a dried extract (d.e.), using the standard curve of gallic acid. All the measurements were carried out in three replicates.

2.9. Statistical analysis

All results are expressed as means ± SE (standard error). Data obtained from this study were processed using XLStat software v.9.0 (Addinsoft). The effects of the treatments were tested by one-way analysis of variance (ANOVA) and the means (n=3) were tested appling LSD's test at the 0.05 significance level.

3. Results and Discussion

3.1. Chemical characterization of organic extracts

The NMR spectra of water extracts (CTs samples) were presented in **Chapter 3** whereas the ^{13}C-CPMAS-NMR spectra of humic substances obtained from artichoke and coffee wastes composted biomasses are shown in **Figure 1.** The data in **Table 1** indicates the relative carbon distribution of main functional groups over chemical shift intervals (*Martinez-Balmori et al., 2014; Spaccini et al., 2019*). The former region in NMR spectra of HS includes the alkyl-C resonances (0-45 ppm), associated to the presence of aliphatic chains (-CH_2- groups) pertaining to various lipid compounds, such as fatty acids, plant waxes and bio-polyesters. Furthermore, the less intense broader shoulders within the 40-55 ppm range, more evident in HS-coffee (**Figure 1**), are mainly attributable to the inclusion in the humic fraction of tertiary (CH) and quaternary (C–R) carbons in assembled rings of sterol derivatives as well as to CH and CH_2groups in α and β position of peptidic moieties (*Pane et al., 2016; de Aquino et al., 2019)*. The sharp signals centred around 56 ppm combine either the methoxyl substituent on the aromatic rings of guaiacyl and syringyl units in lignin components, as well as the C-N bonds in amino acid moieties. The different peaks in the O-alkyl-C chemical shift (60-110 ppm) are conventionally assigned to monomeric units of polysaccharide chains such as cellulose and hemicellulose of plant tissue. The signals at 61/62 ppm represent the carbon nucleus in position 6, followed by the intense coalescence around 73 ppm formed by the overlapping resonances of carbon 2, 3, and 5, in pyranoside structure. The shoulders at 82 ppm derive from the carbon 4 involved in the glycosidic bond with the most deshielded di-O-alkyl anomeric carbon at 103/5 ppm in glucose units. The broad bands extended along the aryl-C interval (116-140 ppm) involve the un-substituted and C-substituted phenyl units of different aromatic components, while the signals shown in the phenolic region (140-160 ppm) are indicative of O-bearing carbon 3, 4, and 5 in the aromatic ring of lignin derivatives, carbon 3 and 5 being coupled to methoxyl substituent.

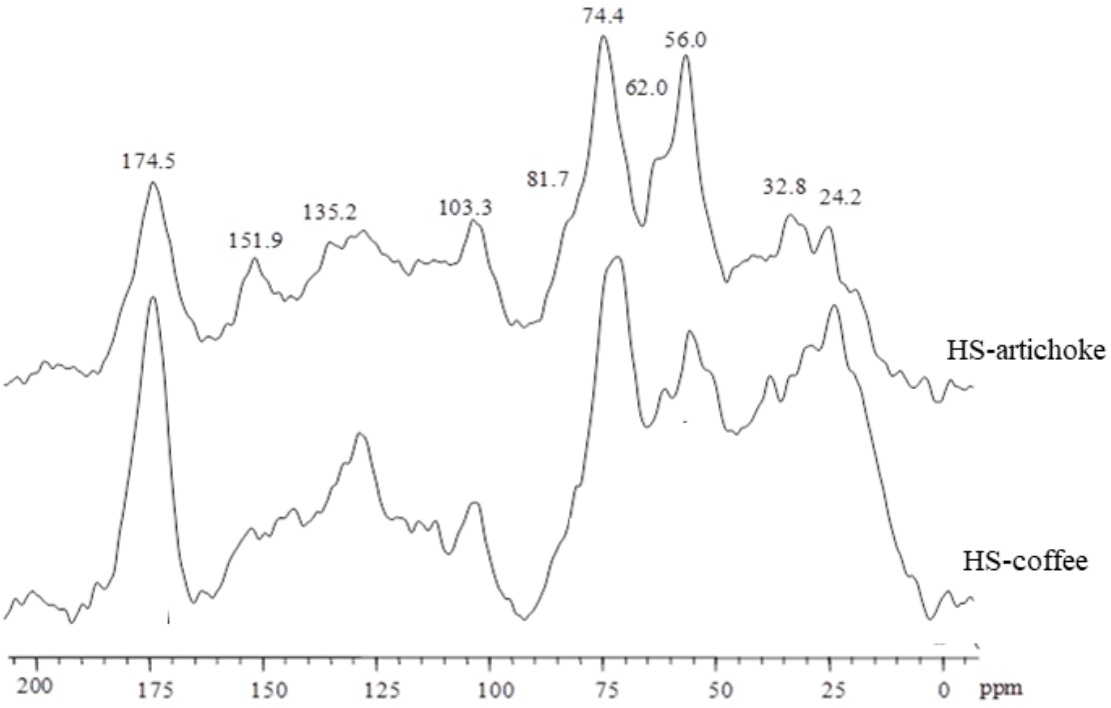


Figure 1: ^{13}C-CPMAS-NMR spectrum of humic substances from artichoke and coffee composted biomasses.

Finally, the sharp signal at 174 ppm include all carbonyl and carboxyl groups of different components such as aliphatic acids or amino acid moieties).

The molecular characteristics of compost extracts can be highlighted by the calculation of the dimensionless structural parameters, such as aromaticity (ARM) and hydrophobic (HB/HI) indexes and alkyl (A/OA), and lignin (LigR) ratios (**Table 1**). Although all compost extracts showed a shared significant amount of carbohydrates and polysaccharides, which relative abundance ranged from 24.7 to 27.3%, the values of HB/HI index revealed for all HS and CTs samples a significant and comparable hydrophobic composition (**Table 1**). However, the A/OA and ARM parameters clearly

highlighted a differential contribution of apolar alkyl-C and aryl-C groups. In fact, the humic extracts from coffee wastes and both CT fractions showed a prevalence of aliphatic compounds, while the humic substances extracted from the artichoke-compost were characterized by the larger amount of aromatic materials, which represented about the 35 % of total carbon distribution (**Table 1**). The calculation of LigR ratio further suggested a differential incorporation of specific derivatives in humic and water fractions (**Table 1**). The lower value in HS-artichoke seemed to confirm the prevalent incorporation of aromatic lignin units in the spectral regions associated with the methoxyl groups (45-60 ppm) combined with the larger level found in the O-aryl-C molecules in the 145-160 ppm region.

Conversely the larger ratios shown by the CT samples and HS-coffee may be related with a possible slight larger contribution of nitrogen moieties (*Monda et al., 2017; Spaccini et al., 2019)* as also suggested by the broad bands in the 40-55 ppm region in NMR spectra of HS-coffee (**Figure 1**) and of CTs samples (**Chapter 3**).

Table 1: Relative distribution (%) of main C structures over chemical shift regions (g/L) and structural indexes of different humic substances (HS) as measured using ^{13}C CPMAS-NMR spectroscopy.

	^{13}C NMR regions						^{13}C NMR structural indexes			
Humic substances	Carbonyl-C (190-160)	O-Aryl-C (160-145)	Aromatic-C (145-110)	O-Alkyl-C (110-60)	CH_3O/CN (60-45)	Alkyl-C (45-0)	HB/HI[a]	A/OA[b]	ARM[c]	LigR[d]
HS-artichoke	10.6	5.6	28.9	24.7	13.8	16.3	1.4	0.7	0.84	2.5
HS-coffee	11.8	4.0	14.9	25.3	13.4	30.6	1.3	1.2	0.34	3.4
CT-artichoke	13.9	4.2	15.0	27.3	12.1	27.6	1.1	1.0	0.35	2.9
CT-coffee	14.0	4.3	16.6	26.4	11.9	26.9	1.2	1.0	0.39	2.8

a HB/HI=hydrophobicity index=[Σ(0–45) +(45-60)/2+ (110–160)/ Σ(45–60) ++(45-60)/2+ (60–110) + (160–190)]

b A/OA=alkyl/O-alkyl ratio (0–45)/(60–110)

c ARM = aromaticity index [(110–160)/Σ(0–45) + (60–110)]

d LigR = Lignin ratio (45–60)/(145–160)

3.2. Bioactivity of different Humic extracts (HS and CTs) on growth of Basil plants

The impact of humic substances and compost teas on plant growth was determined through the evaluation of phenological parameters represented by fresh and dry biomass (**Figure 2**) and chlorophyll content of *Basil* plants (**Figure 3**). All the analyzed parameters were positively influenced by the soil application of different organic extracts in respect to the control samples (**Figures 2, 3**). Moreover, the different tested concentrations revealed a shared general improvement of plant development, without a direct dose-response effect. A steady larger performance was found for the application rate of 50 g L^{-1} followed by the treatment at 10 g L^{-1} which showed slight larger but uneven responses compared to the higher applied dose of 100 g L^{-1}, except for the total chlorophyll content found in the CT-coffee samples (**Figures 2, 3.**). In particular, the most significant biostimulant effect on the measured phenological variables was obtained with the application of HS-artichoke that showed the larger increase for plant fresh (+25%) and dry (+27.7%) biomass, and for the total chlorophyll content (+17.0%) respectively.

As previously noted, a part for the no significant changes produced in the fresh biomass for the soil addition with CT-artichoke (**Figure 2**), all the other treatments revealed potential bioactive properties on basil development, highlighting a not linear or simple relation with specific organic materials and applied rates. The positive effects of Humic derivates as bioeffectors on plant growth, root and shoot biomass development and biosynthesis of photosynthetic pigments, have been extensively described in previous studies (*Nardi et al., 2002; Ameri and Tehranifar, 2012; El-Bassiouny et al., 2014*; *Monda et al., 2017*). However, these effects were shown to depend on either concentration and source of the applied substances, as well as on species and physiological stages of the plant and the specific growing conditions of the experiment (*Trevisan et al., 2010; Olaetxea et al., 2018*). Also for water extracts and compost teas the corresponding literature indicated a biostimulant activities on biomass development, chlorophyll content and plant nutrition on different crops species and medical herbs (*Hargreaves et al. 2009; Siddiqui et al., 2011; Pane et al., 2014*).This

effect may be explained by the excessive synthesis of carbohydrates that are used to store energy for vegetative development. The wide range of biostimulant effects of humic and water organic extracts have been associated to either direct stimulation of enzymatic and biochemical pathways as hormone-like molecules (*Nardi et al., 2002; Vaccaro et al., 2009; Zaccardelli et al., 2012; Ertani et al., 2013*), as well to the activation of stress induced response (*Garcia et al., 2016; Olaetxea et al., 2015, 2018*). The investigation on the possible occurrence of a "cause and effect" mechanism about the structural-activities correlation between organic fraction and plat development have pointed out the concomitant influence of different conformational properties and molecular/chemical features on biochemical and physiological plant processes. The structural arrangement of dissolved organic fractions based on the dynamic interaction of hydrophobic and hydrophilic domains was found to determine the bioactive properties of bioactive molecules pertaining to either SOM pools and recycled biomasses (*Dobbss et al., 2010; Canellas et al., 2009, 2015; Canellas and Olivares 2014; Martinez-Balmori et al., 2014*). The hydrophobic core of humic superstructures may act as effective carrying-sink able to incorporate, preserve and transport the active molecules, mainly represented by low-molecular weight compounds including both apolar molecules and bioavailable polar components, such as aromatic derivatives, lignin/phenol fragments, carbohydrates, peptides, aromatic acids etc. (*Nardi et al., 2007*; *Aguiar et al., 2013*; *Muscolo et al., 2013; de Aquino et al., 2019*). In the rhizosphere environments, the large concentration of root exudates and microbial products greatly increase the chance of physical-chemical interactions with the weakly bound supramolecular structures. The dynamic and flexible conformation of dissolved organic fractions may hence undergo both adhesion to cell membranes of plant roots and favorable thermodynamic rearrangement followed by subsequent possible release of retained molecules that can unfold the bio- active properties in the close proximity of root cells membranes (**Figure 4**).

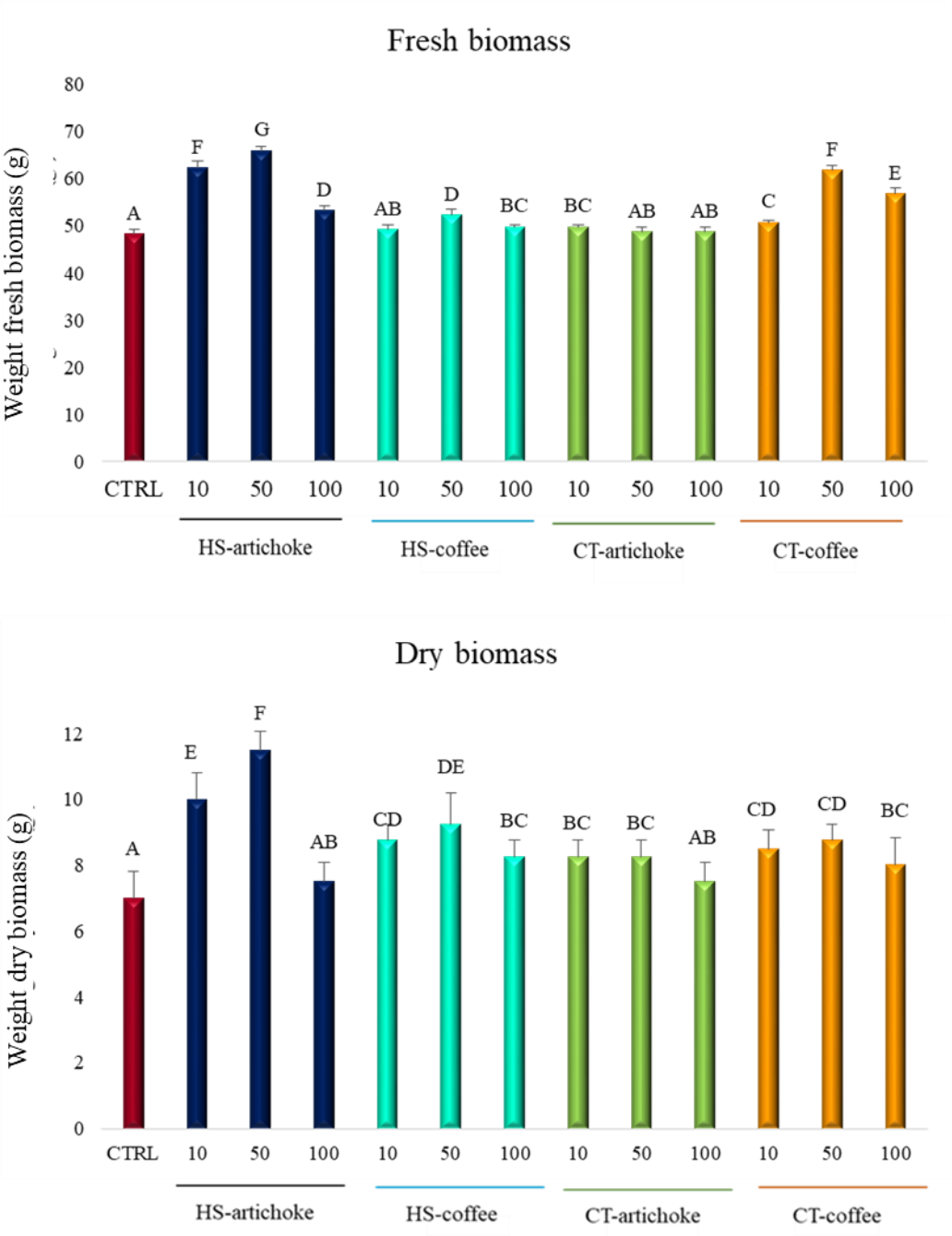


Figure 2: Effect of HS (artichoke and coffee) at different concentration (g L^{-1}) on weight freshand dry biomass of *Basil* plants. Vertical bars represent the standard deviation of the mean. Columns (mean ± S.D.) followed by different letters indicate significant difference according to LSD test ($p \leq 0.05$).

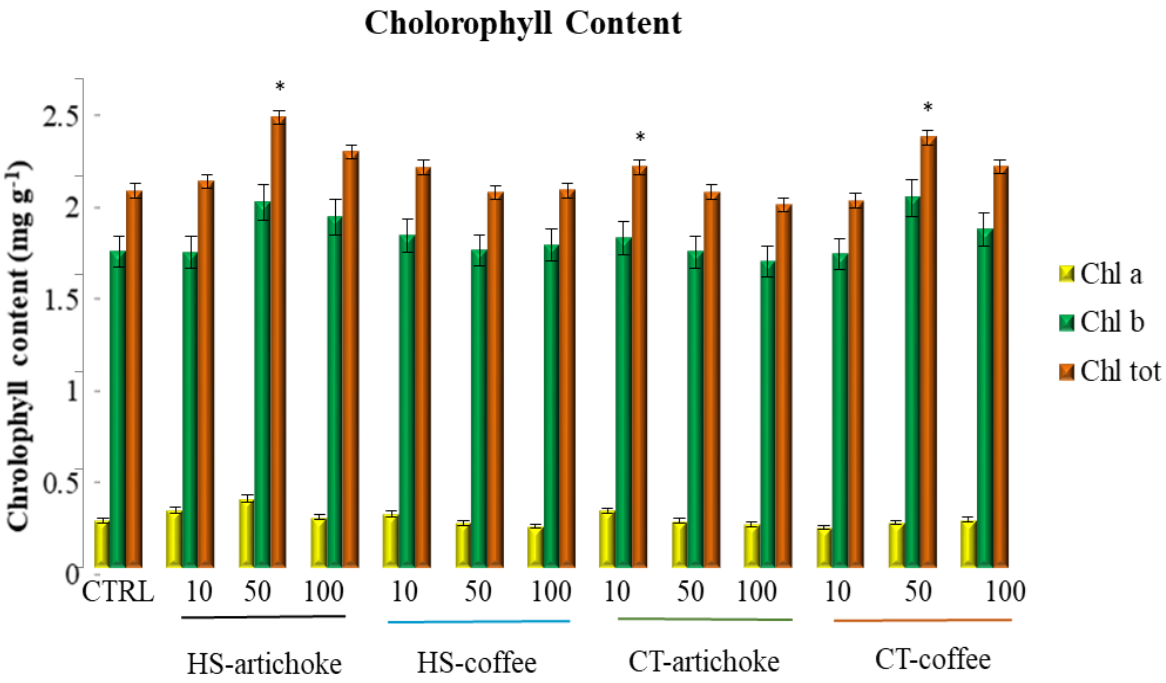


Figure 3: Effects of HS and CT (artichoke and coffee) at different concentration on chlorophyll content of *Basil* plants. Columns (mean ± S.D.) followed by * indicate significant difference in the case of total cholorophill content in according to LSD test ($p \leq 0.05$).

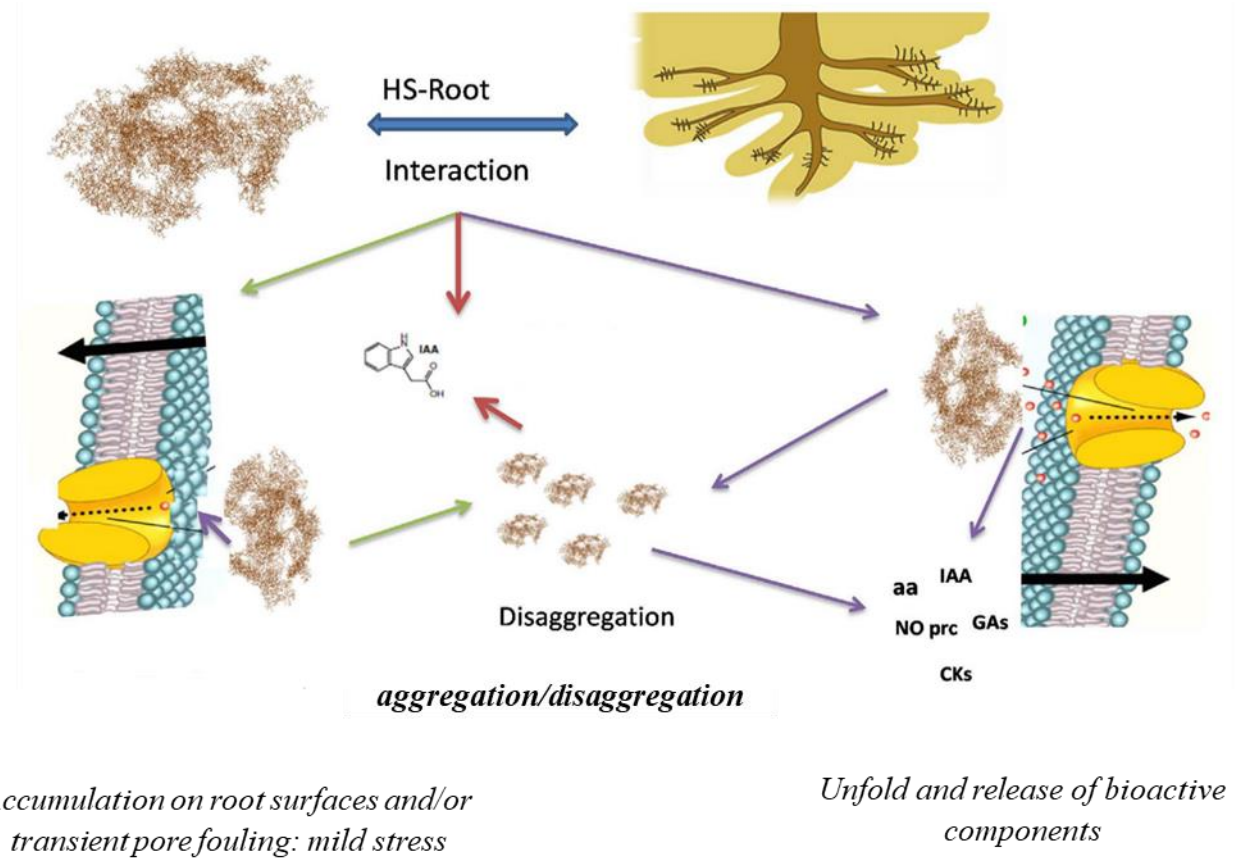


Figure 4: Hypotheses for the primary event arising from the interaction of humic substances at root surface: (i) release of phytoregulators upon molecular disaggregation; (ii) auxin structural analogues in the structural humic domains; (iii) mild stress caused by the accumulation of humic fractions at root surface and molecular disaggregation processes (from *Olaetxea et al., 2018*).

The treatment of Basil plant with organic materials extracted from different on-farm composts revealed common positive effects of applied substances on the plant development with a modulation in the intensity related to specific chemical features. Both HS and CT samples were in fact characterized by defined hydrophobic conformation with a suitable incorporation of polar O-alkyl compounds that may have hence allowed a suitable conformational flexibility and interaction with plant toots The specific chemical composition elucidated by NMR analyses suggested that the steady biostimulant effect found for HS-artichoke may be associated with the larger content of aromatic and lignin units, stressed by the respectively higher and lower values of ARM and LigR parameters, in the order (**Table 1**). The differential response of other organic extracts may be also associated with the lower abundance of bioavailable aromatic molecules and with preferential inclusion of bioactive peptidic clusters indicated by the progressive increase of LigR ratios for the CT samples and for HS-Coffee (**Table 1**).

3.3. Metabolomics approaches: metabolic profiling and PCA analyses

In order to investigate the biosynthesis of biochemical intermediates and products in Basil plants treated with HS and CTs at different concentrations, the primary and secondary metabolites were analyzed by the high-resolution mass spectrometry technique using Thermo Scientific Q Exactive HF quadrupole-Orbitrap equipped with an electrospray source. The **Figure 5** shows the chromatogram of the metabolites extracted from the *Basil* leaves of the representative Control sample. The combined application of two software such as Compound Discovered and Skyline has enabled to singling out of 149 metabolites in Basil leaves extracts, with accurate identification according to the similarity with the chemical standard used in this work (**Table 2**). Briefly, the identified compounds were mainly of carbohydrates (30%), organic acids (16%), lipids (17%), amino acids (9%), aromatics (11%), others nitrogenous compounds besides amino acids (12%) and other compounds (2%). Overall, the humic substances from artichoke and compost tea from coffee induced

increasing yield for the larger majority of compounds in respect to the control, particularly for aromatics metabolites. Hence, in order to understand how many and which metabolites were involved in the differentiation between the analyzed samples, the metabolic profiles of each treatment resulting from the use of analytical techniques was examined as unique data matrix by Principal Components Analysis (PCA). The PCA outputs consisted in score-plot and loading-plots, where the formers highlighted the differences existing among samples, while the seconds described the involvement of variables in such differentiations.

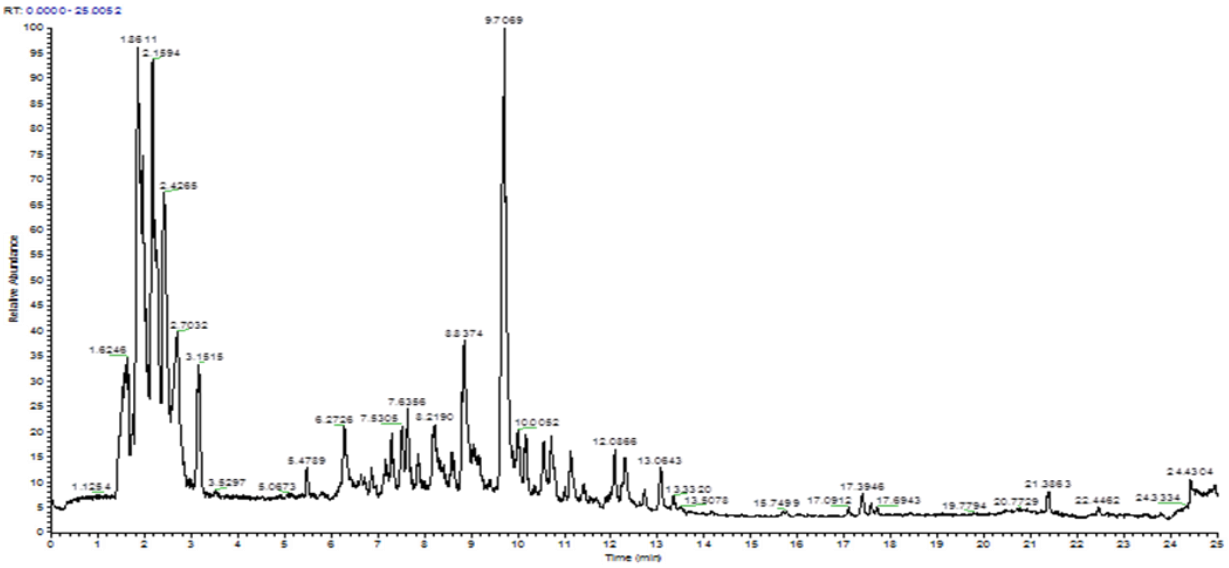


Figure 5: Total Ion Chromatogram (TIC) by Liquid Chromatography High Resolution Mass Spectrometry (LC/HRMS) of Basil leaves extract (CTRL sample).

Table 2: List of primary and secondary metabolites from Basil leaves identified by Liquid Chromatography High Resolution Mass Spectrometry (LC/HRMS).

	Metabolite	Molecular Formula	M.W.[a]	R.T.[a]
1	Arginine	C6 H14 N4 O2	174.11145	1.599
2	α-Trehalose	C12 H22 O11	342.11597	1.621
4	Serine	C3 H7 N O3	105.04243	1.626
5	Arginine	C6 H14 N4 O2	174.11148	1.633
6	5,5-Dimethylhydantoin	C5 H8 N2 O2	128.05845	1.666
7	Glutamine	C5 H10 N2 O3	146.06899	1.666
8	Threonine	C4 H9 N O3	119.05804	1.668
9	Aspartic acid	C4 H7 N O4	133.03737	1.669
11	Glutamic acid	C5 H9 N O4	147.053	1.691
12	Glucose	C6 H12 O6	226.06844	1.716
13	α-Lactose	C12 H22 O11	388.12125	1.729
14	Mannose	C6 H12 O6	180.06319	1.732
15	Gluconic acid	C6 H12 O7	150.05233	1.74
16	Threonic acid	C4 H8 O5	136.03706	1.796
17	7-Methylxanthine	C6 H6 N4 O2	166.0475	1.824
18	N-Acetylornithine	C7 H14 N2 O3	174.10019	1.827
19	Quinic acid	C7 H12 O6	192.06329	1.832
20	2,3-Dihydroxypropanoic acid	C3 H6 O4	106.0265	1.883
21	δ-Gluconic acid δ-lactone	C6 H10 O6	132.04214	1.942
22	2,4-Dinitrophenol	C6 H4 N2 O5	184.01361	1.998
23	Purine	C5 H4 N4	120.04204	2.005
24	Quinic acid	C7 H12 O6	192.06313	2.008
25	Isocitric acid	C6 H8 O7	146.02134	2.019
26	Malic acid	C4 H6 O5	134.02135	2.036
27	Fructose	C6 H12 O6	180.06319	2.085
28	Tartaric acid	C4 H6 O6	150.01622	2.092
29	Fumaric acid	C4 H4 O4	116.01082	2.177
30	Uridine monophosphate (UMP)	C9 H13 N2 O9 P	324.0353	2.179
31	Adenosine 5'-monophosphate	C10 H14 N5 O7 P	347.06244	2.211
32	2-Oxobutyric acid	C4 H6 O3	102.03161	2.253
33	Citric acid	C6 H8 O7	192.02682	2.413
34	Pseudouridine	C9 H12 N2 O6	244.06903	2.421
35	2-Oxoglutaric acid	C5 H6 O5	146.0213	2.585
36	2-Furoic acid	C5 H4 O3	112.01592	2.608
37	Mesaconic acid	C5 H6 O4	130.0264	2.609
38	Uridine	C9 H12 N2 O6	244.06928	2.624
39	4-Oxoproline	C5 H7 N O3	129.04251	2.64
40	Adenosine	C10 H13 N5 O4	267.09655	2.668
41	2-Aminoadipic acid	C6 H11 N O4	161.06869	2.675
42	Tyrosine	C9 H11 N O3	181.07375	2.728

43	N-Acetyl-DL-glutamic acid	C7 H11 N O5	189.06355	2.761
44	Endothal	C8 H10 O5	186.05261	2.773
45	3-Hydroxybutyric acid	C4 H8 O3	104.04733	2.801
46	Guanosine	C10 H13 N5 O5	283.09138	2.838
47	Citraconic acid	C5 H6 O4	130.02639	2.849
48	cis-Aconitic acid	C6 H6 O6	174.01617	2.859
49	Leucine	C6 H13 N O2	131.09447	2.86
50	δ-Ribono-1,4-lactone	C5 H8 O5	148.03704	2.982
51	Succinic acid	C4 H6 O4	118.02647	3.038
52	4-Acetamidobutanoic acid	C6 H11 N O3	145.07376	3.523
53	N-Ethylglycine	C4 H9 N O2	103.06328	3.53
54	4-Pyridoxic acid	C8 H9 N O4	183.05306	3.551
55	Propanoic acid	C16 H22 O9	358.12629	3.605
56	Catechol	C6 H6 O2	110.03665	3.755
57	Xanthosine	C10 H12 N4 O6	284.07547	3.769
58	Gallic acid	C7 H6 O5	170.02144	4.183
59	Itaconic acid	C5 H6 O4	130.02649	4.366
60	N-Acetyl-4-aminosalicylic acid	C9 H9 N O4	195.05298	4.45
61	2-Aminooctanedioic acid	C8 H15 N O4	189.10001	4.497
62	Glutaric acid	C5 H8 O4	132.04211	4.554
63	Galactose	C6 H12 O6	180.06323	4.625
64	Pantothenic acid	C9 H17 N O5	219.1105	4.639
65	Phenylalanine	C9 H11 N O2	165.07885	4.882
66	Pyrogallol	C6 H6 O3	126.03157	4.939
67	Porphobilinogen	C10 H14 N2 O4	226.09514	5.207
68	Sorbic acid	C6 H8 O2	112.05237	5.215
69	N-Acetylvaline	C7 H13 N O3	159.08938	5.219
70	3-Anisic acid	C8 H8 O3	152.04714	5.257
71	Gentisic acid	C7 H6 O4	154.0264	5.258
72	Methylsuccinic acid	C5 H8 O4	132.04215	5.399
73	Ethylmalonic acid	C5 H8 O4	132.04211	5.4
74	2-Methylbenzoic acid	C8 H8 O2	136.05233	5.424
75	4-Hydroxyphenylacetic acid	C8 H8 O3	152.04717	5.424
76	6-Methoxysalicylic acid	C8 H8 O4	168.04214	5.514
77	Methyl salicylate	C8 H8 O3	152.04713	5.525
78	4-Hydroxybenzylalcohol	C7 H8 O2	124.05237	5.536
79	Xanthurenic acid	C10 H7 N O4	205.03731	5.614
80	Benzoic acid	C7 H6 O2	122.03664	5.687
81	Adenine	C5 H5 N5	135.0544	5.825
82	5'-S-Methyl-5'-thioadenosine	C11 H15 N5 O3 S	297.08929	5.826
83	Adipic acid	C6 H10 O4	146.05777	6.024
84	Tryptophan	C11 H12 N2 O2	204.08977	6.081
85	2,4-Dihydroxybenzoic acid	C7 H6 O4	154.02647	6.095
86	Caffeic acid	C9 H8 O4	134.03656	6.182
87	Syringic acid	C9 H10 O5	198.0526	6.245
88	Esculin	C15 H16 O9	340.07913	6.274

89	Geniposide	C17 H24 O10	388.13684	6.321
90	4-Methylumbelliferone	C10 H8 O3	176.0471	6.357
91	4-Hydroxyphenyllactic acid	C9 H10 O4	182.0578	6.365
92	N-Acetyl-L-methionine	C7 H13 N O3 S	191.06143	6.367
93	Mesalamine	C7 H7 N O3	153.04228	6.603
94	Kynurenic acid	C10 H7 N O3	189.04248	6.651
95	Triethyl 4-phosphonocrotonate	C10 H19 O5 P	250.09683	6.716
96	2-Hydroxycinnamic acid	C9 H8 O3	164.04722	6.764
97	Salicylic acid	C7 H6 O3	138.03154	7.072
98	2,5-Dihydroxybenzaldehyde	C7 H6 O3	138.03137	7.207
99	Pimelic acid	C7 H12 O4	160.07339	7.285
100	Ferulic acid	C10 H10 O4	194.05771	7.37
101	Vanillic acid	C8 H8 O4	168.04208	7.454
102	Phenylglyoxylic acid	C8 H6 O3	150.03148	7.649
103	Astragalin	C21 H20 O11	448.10039	7.814
104	Rutin	C27 H30 O16	610.15302	8.027
105	Cyclopentylacetic acid	C7 H12 O2	128.0836	8.086
106	Lariciresinol 4-O-glucoside	C26 H34 O11	568.2154	8.249
107	Quercetin-3β-D-glucoside	C21 H20 O12	464.09515	8.392
108	Eriodictyol	C15 H12 O6	288.06325	8.406
109	N-Acetyl-L-phenylalanine	C11 H13 N O3	207.08929	8.496
110	Suberic acid	C8 H14 O4	174.08899	8.541
111	Dehydroacetic acid	C8 H8 O4	168.04205	8.908
112	Vanillin	C8 H8 O3	152.04714	8.989
113	Afzelin	C21 H20 O10	432.10545	9.085
114	Corey lactone	C8 H12 O4	172.07328	9.125
115	N-Acetyl-DL-tryptophan	C13 H14 N2 O3	246.10018	9.187
116	3-O-β-D-Glucopyranosylandrographolide	C26 H40 O10	558.26991	9.268
117	Dodecanedioic acid	C12 H22 O4	230.15149	9.359
118	6-Hydroxycaproic acid	C6 H12 O3	132.07847	9.512
119	Azelaic acid	C9 H16 O4	188.10465	9.738
120	3-Phenyllactic acid	C9 H10 O3	120.0573	9.975
121	trans-Cinnamic acid	C9 H8 O2	148.05227	9.975
122	4-Indolecarbaldehyde	C9 H7 N O	145.05259	10.029
123	Isoferulic acid	C10 H10 O4	194.05764	10.375
124	Eugenol	C10 H12 O2	164.08363	10.46
125	4-Acetamidobenzoic acid	C9 H9 N O3	179.05801	10.845
126	4-Nitrophenol	C6 H5 N O3	139.02676	10.931
127	Abscisic acid	C15 H20 O4	264.13598	10.974
128	2-Hydroxyphenylalanine	C9 H11 N O3	181.07379	11.088
129	Hydrocinnamic acid	C9 H10 O2	150.06786	11.657
130	Genipin	C11 H14 O5	208.07338	11.661
131	Genistein	C15 H10 O5	270.0526	12.017
132	Naringenin	C15 H12 O5	272.0682	12.024
133	Formononetin	C16 H12 O4	268.07336	12.054
134	Hispidulin	C16 H12 O6	300.06317	12.215

135	3',5,7-Trihydroxy-4'-methoxyflavanone	C16 H14 O6	302.07883	12.231
136	Trihydroxy-octadecenoic acid	C18 H34 O5	330.24048	12.329
137	Helvolic acid	C33 H44 O8	568.30693	12.35
138	Jasmonic acid	C12 H18 O3	210.12539	12.354
139	4-Hydroxycoumarin	C9 H6 O3	162.03157	12.37
140	3-Hydroxyanthranilic acid	C7 H7 N O3	153.04245	12.568
141	Arjungenin	C30 H48 O6	504.34491	12.883
142	Hydroxydodecanoic acid	C12 H24 O3	216.172	13.449
143	Hexadecanedioic acid	C16 H30 O4	286.21388	13.851
144	Butylparaben	C11 H14 O3	194.0941	13.962
145	2,5-di-tert-Butylhydroquinone	C14 H22 O2	222.16152	14.953
146	Asiatic acid	C30 H48 O5	488.34983	15.825
147	Hydroxyhexadecanoic acid	C16 H32 O3	272.23501	17.717
148	Abietic acid	C20 H30 O2	302.22436	18.161
149	Ostruthin	C19 H22 O3	298.16003	24.186

a MW= molecular weight; RT= Retention time (minutes)

3.4. Metabolomics approaches: metabolic analysis of Basil plants treated with the application of HS and CTs from artichoke and coffee composted biomasses

The PCA score-plots of metabolomic analyses performed on Basil plants following the application of HS and CT extracts are reported in **Figure 6 and 7**, respectively. For both humic and water extracts the statistical analysis allowed a noticeable separation between treatments as related to either sources and application rates (**Figures 6, 7**.).The PCA models revealed a good grouping of all treated plants with a suitable reproducibility between replicates with high statistical significance ($p < 0.05$) for both principal components .The differentiation of plant samples along the two principal components (PC1 and PC2) were based on similar distribution of metabolites with minor differences for HS and CT treatments (**Figures 6, 7**) thus indicating the involvement of the same metabolic pathways in the biostimulant activities of applied organic extracts. The first main components (PC1) representing the 33.55% and the 29.3% of the total variance for HS and CT fractions, respectively, included as main variables the glucose, glucose-1-phosphate, mannose, raffinose, galactose-6-

phosphate, rosmarinic acid, caffeic acid proline, glutamine, shikimic acid, tryptophan, tyrosine, phenylalanine, eugenol and coumaric acid, that showed increasing concentrations in plant leaves placed along the positive values of PC1. The presence of metabolites such as raffinose and proline may indicate the activation of metabolic adaptation to a stress signal (*Vinci et al., 2018*). Particularly, raffinose is synthesized in response to some biotic or abiotic stresses (*Sengupta et al., 2015*), although its accumulation in plant cells also suggests a carbon storage mechanism (*ElSayed et al., 2014*). Proline is also commonly identified as produced in high concentration in response to a variety of abiotic stress, while its catabolism can provide an energy supply to drive plant growth when stress mitigation is applied (*Kavi Kishor et al., 2014*).

The metabolic profiles of Basil leaves were further discriminated along the positive values of PC2 that explained the 18.9% and 16.8% of total variance for HS and CT. The various samples were statistically partitioned according to the concentrations of glucose-6-phosphate, fructose-6-fosfate, aconitic acid, mannose, proline, glutamine, salicylic acid, abscissic acid, jasmonic acid, cinnamic acid, naringenin, resveratrol with minor differences in metabolite types among humic and water extracts (**Figures 6 ,7**).

With respect to the structural-activity relationship in the stimulation of metabolites synthesis, the data of PC1 component delineate a significant discrimination of HS and CT treatments in respect to control samples, with lower effect associated to the applied concentrations (**Figures 6, 7**). This behavior may be related to the common structural features of organic extracts such as the hydrophobic/hydrophilic ratios (**Table 1**) with the prevalence of apolar character combined with a significant inclusion of O-alkyl-C components. These features may determine a pliable conformational behavior with a more feasible interaction with plant roots. Moreover, the micro-hydrophobic environment promote a useful preservation and release in the rhizosphere environments of bioactive components that may entail a biostimulation effect of metabolic processes. In fact, even though the main HS functions as bio-effectors are mediated by the interaction with root systems, the bio-stimulant activities of humic molecules may also trigger the physiological processes linked with

the development of shoot and leaves tissues in various crop species (*Canellas and Olivares, 2014; Vaccaro et al., 2015; Nardi et al., 2017*).

The separation between different organic materials for both HS and CT treatments was highlighted along the PC2. The larger positive values were found for HS-artichoke and CT coffee at the application dose of 50 g L^{-1}. For the humic extracts, the best performance of HS artichoke may be ascribed to the significant larger content of bioavailable aromatic and lignin derivatives, conversely, no specific structural properties were shown by NMR analyses to support the results of PCA analyses of CT samples (**Table 1**). However, although both water extract from artichoke and coffee composts had similar amount of lignin and aromatic compounds, the outputs of thermochemolysis reported in the previous Chapter 3 stressed the lower degradation level of lignin fraction of CT-artichoke. It is hence conceivable that the larger decomposition of lignin biopolymers in the original source material of coffee residues may have promoted a preferential accumulation of small and active aromatic fragments, which allowed a prompt plant physiological response due to their hormone-like activity (*de Aquino et al., 2019; Spaccini et al., 2019*)

With respect to plant response to applied doses, steady larger effects were shown by the soil addition of the 50 g L^{-1} rates for both HS and CT samples. It has been previously noted that the bioactivity of dissolved organic fractions depend on the structural conformation which may act as effective sinks able to incorporate, preserve, transport and release the active molecules The dynamic equilibrium of contiguous hydrophilic and hydrophobic domains of dissolved HS and CT components, iterative regulated by the environmental condition (pH, soil moisture, ionic strength, root exudates, active mineral surfaces, etc), is the driving parameter for the mobility, molecular conformation and functional group distribution which determine the HS/CT-mediated bio-stimulant activity (*de Aquino et al., 2019; Spaccini et al., 2019; Bento et al., 2020*) The structural conformation of dissolved HS and CT in soil circulating solution seemed to influence the response observed for increasing concentrations for either plant development and metabolic effects. It is conceivable the application of higher concentrations (100 g L^{-1}) possibly promoted a closer aggregation of aromatic

and alkyl hydrophobic cluster or integer ligno cellulose structures with a small chance of structural modification and irregular release of bioactive molecules (*de Aquino et al., 2019*; *Bento et al., 2020*). In this respect, a positive alternative mechanism on plant roots have been related to the activation of an array of physiological responses, such as reduced root hydraulic conductivity, and induced mild stress signals (*Garcia et al., 2016; Olaetxea et al.,2018*). Conversely, the average dissolved amount (50 g L^{-1}) of compost extracts may have underwent the above-sketched structural rearrangement with a suitable interaction with plant rhizosphere and unfolding of bio-stimulants depending on the specific molecular composition. The uneven stimulating effects observed for the lower concentration (10 g L^{-1}) suggested the occurrence of a limiting interference of the two mechanisms.

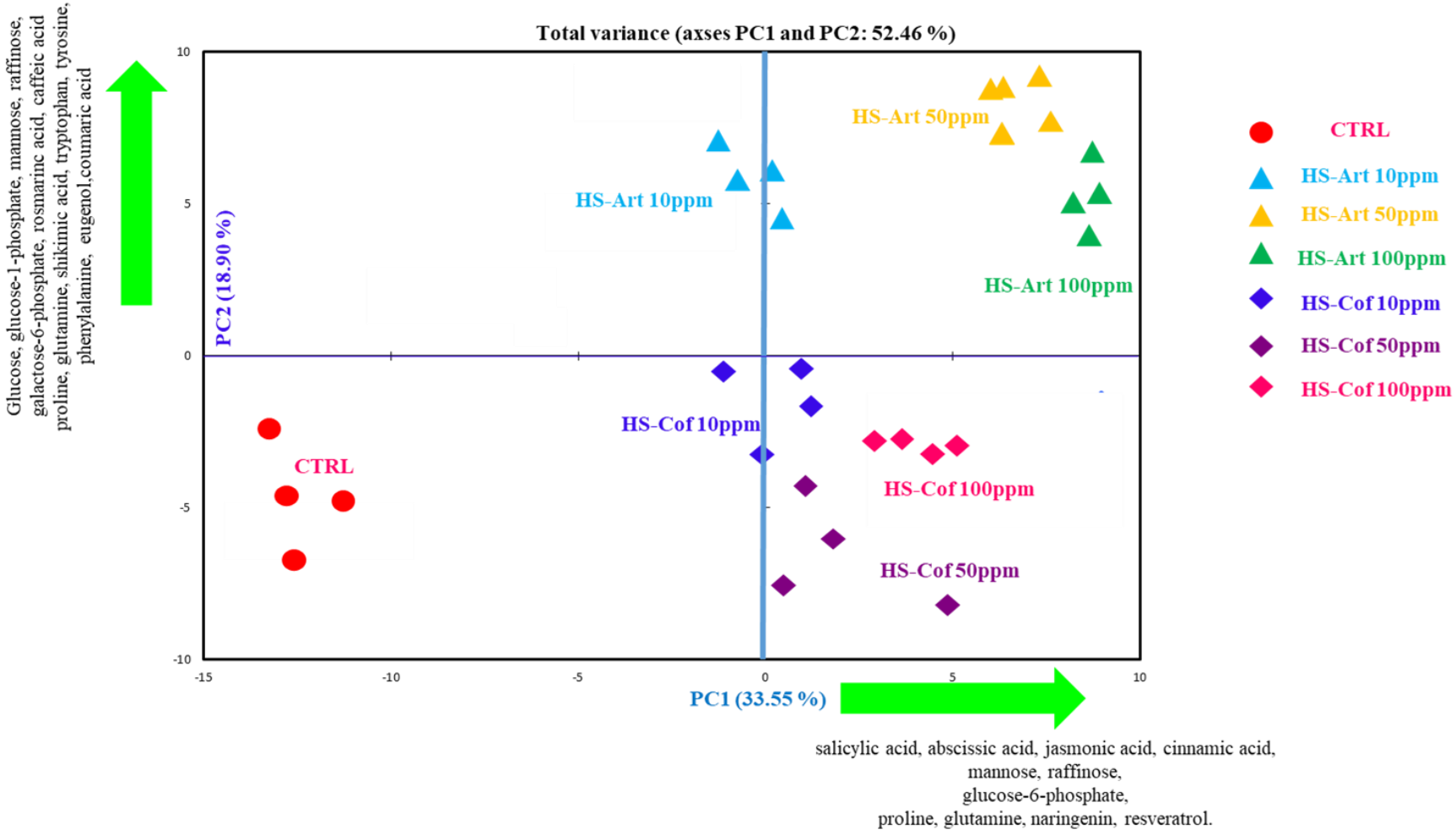


Figure 6: PCA of plant metabolites treated with humic substances (HS) from artichoke and coffee composted vegetable wastes.

PCA score-plot based on data obtained using Liquid Chromatography High Resolution Mass Spectrometry (LC/HRMS) of Basil leaf extracts.

Names and direction of most significant PCA loading vectors involved in the differentiation of treatments are reported along the score-plot borders.

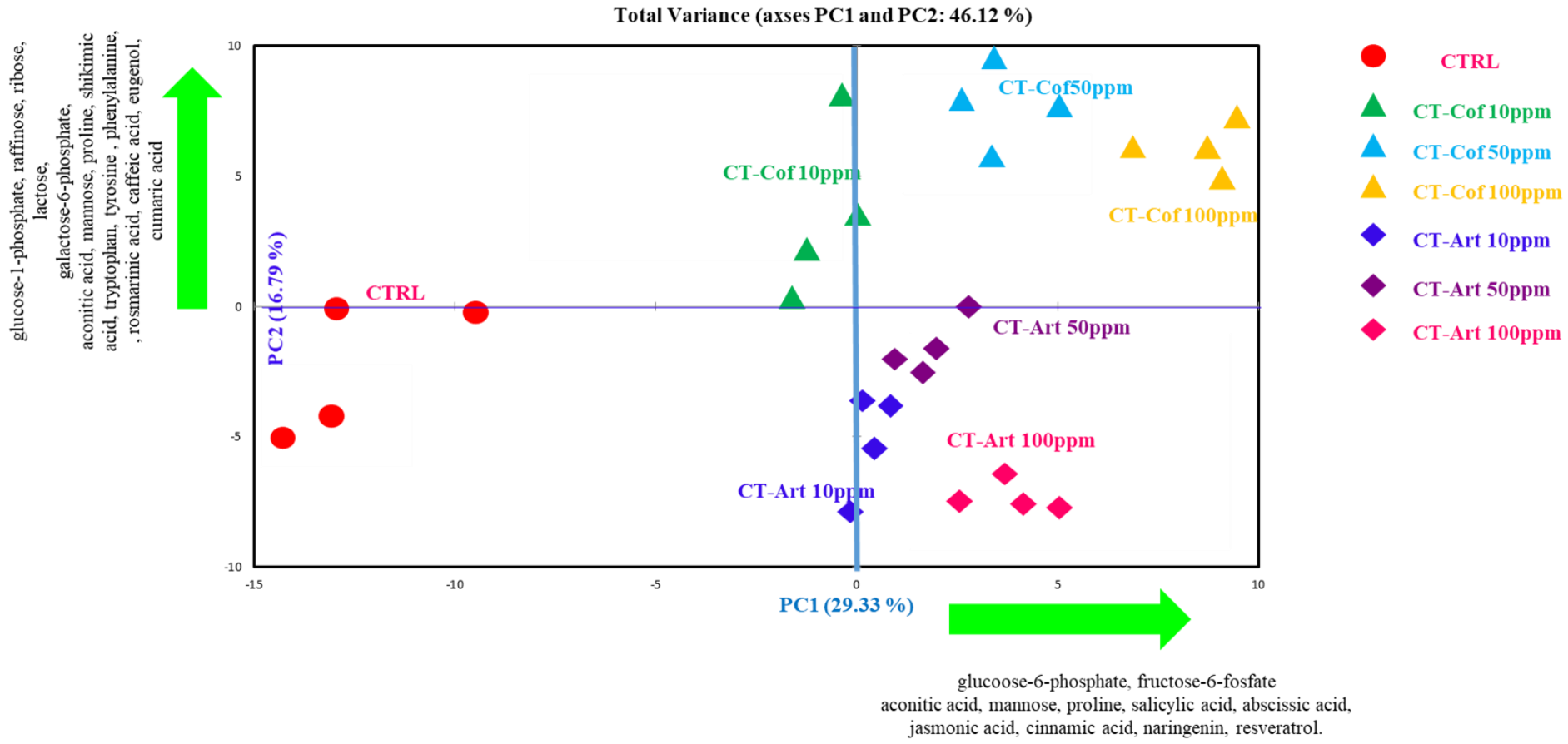


Figure 7: PCA of plant metabolites treated with Compost Teas (CTs) from artichoke and coffee composted vegetable wastes.

PCA score-plot based on data obtained using Liquid Chromatography High Resolution Mass Spectrometry (LC/HRMS) of Basil leaf extracts.

Names and direction of most significant PCA loading vectors involved in the differentiation of treatments are reported along the score-plot borders

3.5. Metabolomics approaches: implication of metabolic pathways

Besides the proposed hypothesis on structural-activity relationship between Basil plant development and compost extracts, the metabolomic analyses have confirmed the occurrence of multiple interactions with different metabolic pathways (*Canellas et al., 2019*). The biostimulant properties of complex organic fractions may in fact activate an array of different enzymatic reactions and biochemical processes on both roots and shoot tissues with not univocal or simple correlations among chemical composition and physiological activities (**Figure 8**).

The comprehensive understanding of the mechanisms that underpin the stimulation of alternative metabolic biosynthetic reactions may provide useful information to attempt a tailored application of specific organic materials on selected crops. The application of organic materials such as HS-artichoke and CT-coffee among other metabolites improved the synthesis of narigenin and resveratrol, two natural molecules widely used in the pharmaceutical, nutraceutical and cosmetic fields. In detail, narigenin is a flavonoid belonging to the class of flavanones that has a bioactive effect on human health as an antioxidant, free radical scavenger, anti-inflammatory, and enhancer of carbohydrate metabolism and modulator of immune system (*Felgines et al., 2000*). The resveratrol belongs to polyphenols' stilbenoids group, is made up by two phenol rings linked to each other by an ethylene bridge; it exhibits antitumor activity, and is considered a potential candidate for prevention and treatment of several types of cancer. Other bioactive effects of this metabolite as anti-inflammatory, anticarcinogenic, cardioprotective, vasorelaxant, phytoestrogenic and neuroprotective have also been reported (*Salehi et al., 2018*). Furthermore, the application of HS-artichoke showed an improvement in the yield of following organic acids: citric, alpha-ketoglutaric, maleic, ribonic, oxalic, succinic and lactic compared to the control. In addition to the most abundant metabolites used in the PCA, the tested organic materials promoted the biosynthesis of intermediates represented by erythrosis-4-P and phosphoenolpyruvate **(Figure 9)**. These compounds are acknowledged as intermediates of the primary carbon metabolism as well as signaling the activation of shikimic or

mevalomic acid biosynthetic pathways related to the synthesis of phenolic compounds or terpenes. The results obtained in this work showed a clear effect of compost derivatives on plant metabolism according to previous studies which reported that shikimic pathway compounds (flavonoids, some alkaloids such as isoquinoline alkaloids, tocopherols and phenols) are affected by humic acid (*Nardi et al., 2002, Canellas et al., 2014, Vaccaro et al., 2015, Aguiar et al., 2018*). Additionally, *Schiavon et al. (2010)* showed that humic acid increased the expression of phenylalanine ammoniacalase, which catalyzes the first phase in phenylpropanoid biosynthesis, transforming tyrosine with *p*-cumaric acid and phenylalanine with trans-cinnamic acid. The expression of these enzymes was related to an up-take of phenol compounds in plant leaves. Furthermore, the increase in the relative content of sugars such as glucose and its two phosphorylated forms as well as fructose and galactose are reflected by an increase of photosynthetic efficiency.

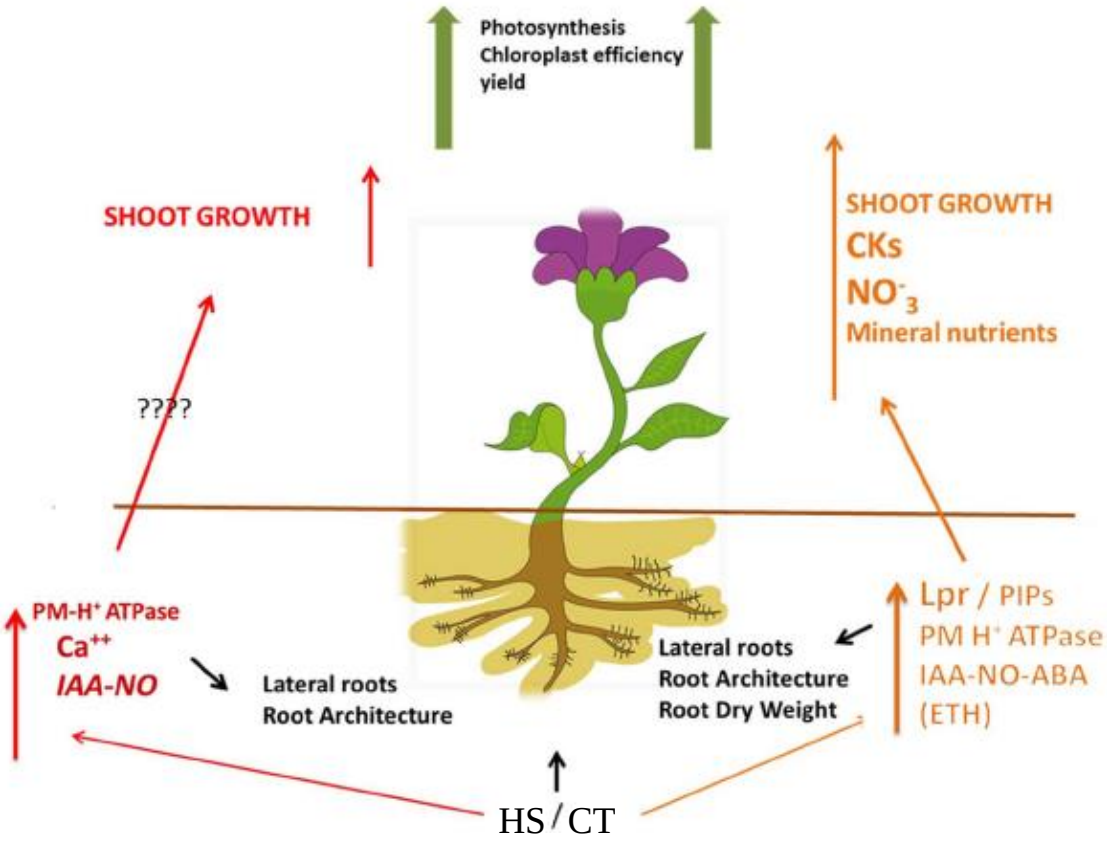
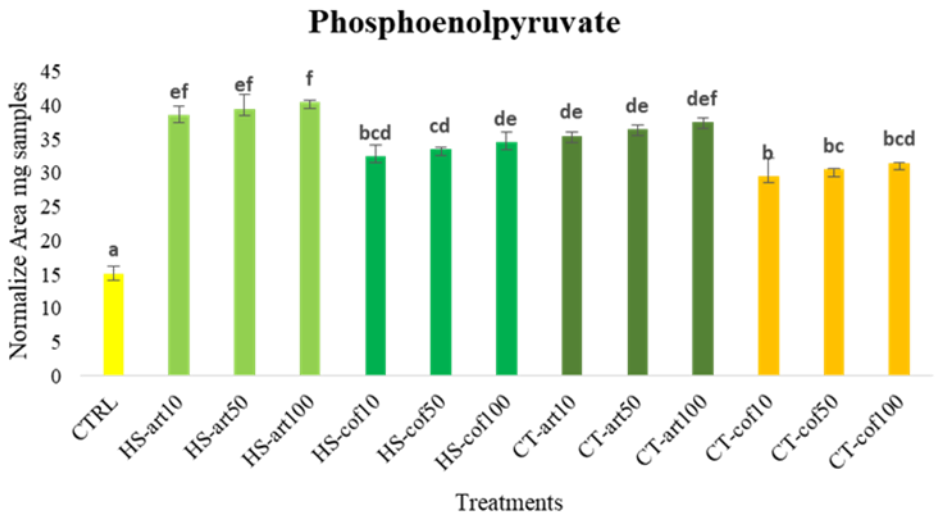


Figure 8: Root-shoot signalling pathways involved in the crop growth promoting action of organic substances with diverse origin: HS/CT humic substances/compost teas obtained from compost (modified from *Olaetxea et al., 2018*).



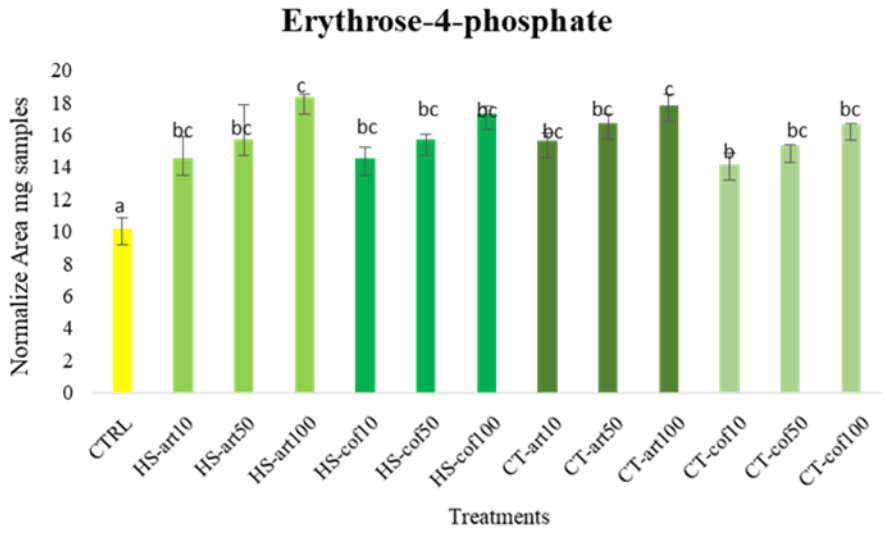


Figure 9: Quantification of Erytrose-4-phospate and Phosphoenolpyruvate in Basil plants treated with humic substances (HS) and compost teas (CTs) extracted from artichoke and coffee composted vegetable wastes. Vertical bars represent the standard deviation of the mean. Columns (mean ± S.D.) followed by different letters indicate significant difference according to LSD test ($p \leq 0.05$).

Glucose-1-P is a key intermediate in several major carbon anabolic fluxes, such as sucrose, starch and cellulose biosynthesis while Glucose-6-P showing effect on oxidative pentose phosphate cycle (*Aguiar et al., 2018*). Our study suggests an increased photosynthesis activity and protein synthesis of plants, thus explaining the high amino acid content due to the use of humic bio-effectors. The large amount of abscisic acid present in the samples may be an optimal indicator of the energetic state of the cells. In plants, medium stress conditions induce the activation of physical signal transduction process where the increase of Ca2+ cytosolic triggers Ca2+/calmodulin activity related to abscisic acid synthesis. The accumulation of abscisic acid is probably mainly facilitated by glutamate decarboxylase (*Shelp, 1999*). This metabolite works as a signal to stimulate the plant tissue to accumulate more energy, or to reduce excess energy (*Michaeli and Fromm, 2015*). In addition, the high content of amminoacids such as tryptophan, tyrosine and phenylalanine found in plants treated with HS-artichoke and CT-coffee, suggests a higher production of secondary metabolites. Notably, the significant presence of phenylalanine and tryptophan provides a substrate for the phenylpropanoid pathway (*Vogt, 2010*), that is a critical step for the biosynthesis of many secondary plant products, such as anthocyanins, lignin and phenols (*Tzina and Galili, 2010*). In addition, the presence of chlorogenic and shikimic acids also confirms a better synthesis of secondary metabolites.

Phenylalanine in the phenylpropanoid pathway is a precursor for a variety of secondary plant products, such as anthocyanins, lignin and phenols. On the other hand, tryptophan is the precursor of indolacetic acid, which is involved in cell expansion and many other regulatory processes (*Tzina and Galili, 2010*). Synthesis occurs through the shikimate pathway, followed by the metabolic pathway of branched aromatic amino acids (*Vogt, 2010*). The high content of chlorogenic acid, shikimic acid, quinic acid in plant leaves indicates an increased synthesis of secondary metabolites, even partially involved in lignin biosynthesis (*Schoch et al., 2001*), which are probably due to various biotic and abiotic stress conditions, such as wounds, resistance to infection by pathogens, metabolic stress or disruption of the cell wall structure (*Caño-Delgado et al., 2003; Tronchet et al., 2010*). The improvement of secondary metabolism is also shown by a higher production of phenolic compounds

such as caffeic acid, rosmarinic acid, eugenol, coumaric acid and salicylic-acid. Pharmacological activities of phenolic metabolites were previously reported (*Sing et al., 2018*). In detail, caffeic acid shown antioxidant (*Anwar et al. 2013*), anti-cancerogenesis (*Cho et al., 2010*) and anti-obesity effects (*Piazzon et al., 2012*). Conversely, rosmarinic acid displays important biological activities as anti-carcinogenic, antiviral, antibacterial, antimicrobial, and antidepressant benefits. This metabolite is also employed for memory enhancement, circulation improvement, strengthening of vulnerable blood vessels, inflammation, and central nervous system disorders (*Wu et al., 2012, Bhat et al., 2013*). Eugenol displays a considerable antioxidant, anti-inflammatory and cardiovascular activity, as well as analgesic and local anaesthetic properties (*Pramod et al., 2010*) whereas *p*-coumaric acid has shown immunomodulatory and anti-inflammatory effect (*Pragasam et al., 2013*). Conversely, salicylic acid and its derivatives (collectively called salicylates) as pharmacological agents has not long been investigated although this metabolite exhibits a phyto-hormonal activity associated to the regulation of advanced mechanisms such as seed germination, root initiation, stomatal closure, and abiotic and biotic stresses. (*Klessig et al., 2016*).

3.5. Antioxidant activity and total phenolic content of Basil leaves

Antioxidant activity and total phenolic content of *Basil* leaves treated with humic substances and Compost teas obtained from composted vegetable wastes are reported in **Figure 10**. Generally, antioxidant capacity is directly correlated with content of phenolic compounds (*Koh et al., 2009*). In this study, the treatment of Basil plants with compost derivates induced an increase of antioxidant activity and total phenolic content with clear dose-response effect. The leaves extracts from the plants treated with HS-artichoke showed the best TEAC value (55) at the maximum concentration applied (100 g/L) compared to the control followed by treatment with CT-coffee which, as the same concentration, exhibited a comparable effective TEAC response (49). Conversely, the treatments with HS- coffee and CT-artichoke did not significantly influence the antioxidant activity (**Figure 10**).

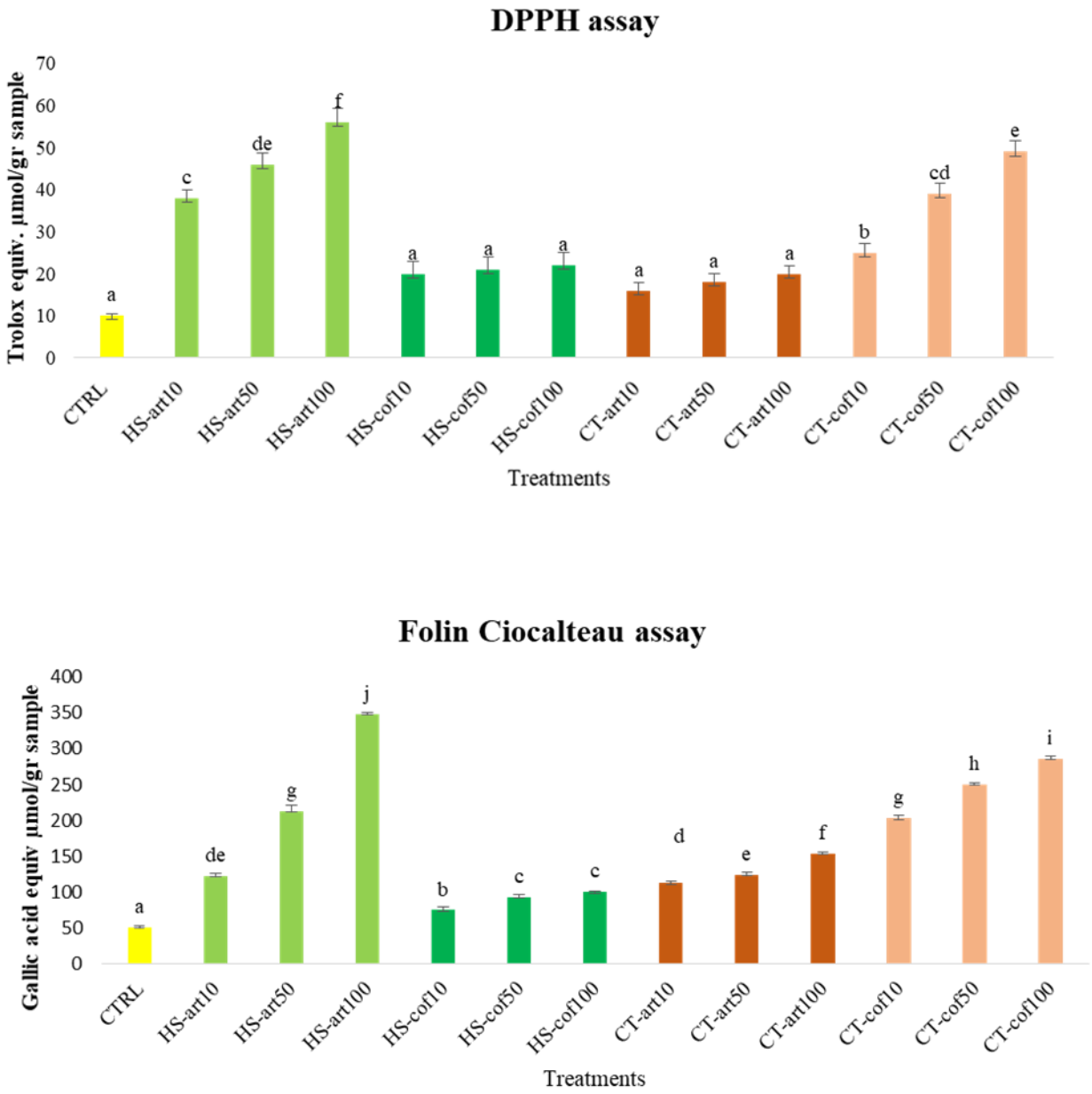


Figure 10: Antioxidant activity and Total Phenolic Content of Basil leaves from plants treated with humic substances (HS) and compost teas (CTs) extracted from artichoke and coffee composted vegetable wastes at different concentration (10-50-100 g L^{-1}). Vertical bars represent the standard deviation of the mean. Columns (mean ± S.D.) followed by different letters indicate significant difference according to LSD test ($p \leq 0.05$).

Furthermore, the same trend was observed for the Total phenol content (TPC) measured using Folin-Ciocalteau assay, where larger values of TPC were exhibited in the leaves of Basil plant treated with HS-artichoke and CT-coffee (**Figure 10**). Antioxidants play an important role in the prevention of many diseases due to their effect on formation of reactive oxygen species (ROS) and lipid peroxidation (*Sgherri et al., 2010*). Previous studies have shown that phenolic compounds contained in *Ocimum basilicum* leaves can be used as powerful antioxidants and free radical scavengers (*Rice-Evans et al., 1996, Tarchoune et al., 2009*). Antioxidant components of *Basil* leaves have a benefits effect for human health reducing heart disease and potential risk of development cancer and diabetes (*Lee and Scagel, 2009; Flanigan and Niemeyer, 2014 ; Mastaneh et al., 2014*,). As indicated for the previous analysed parameters, the antioxidant activity was influenced by the chemical composition of applied compost derivatives, suggesting a structure activity relationship (*Muscolo et al., 2013, Garciá et al., 2016*).

Effectively, HS-artichoke and CT-coffee, which have shown a positive effect on Basil scavenger activity, are characterized by a larger content of bioavailable aromatic and phenolic compounds compared to HS-coffee and CT-artichoke (**Figure 1, Table 1**). Furthermore, the results related to antioxidant activity may also be related to improvement production of secondary metabolites as a response to treatment with humic materials. Bioactive compounds involved in antioxidant activity of Basil are chlorogenic, p-hydroxybenzoic, caffeic, vanillic and rosmarinic acids, as well as apigenin, quercetin and rutin (*Kwee et al., 2011*). As reported in this study, HS-artichoke and CT-coffee have shown a bioactive effect stimulating the production of secondary metabolites such as caffeic acid and rosmarinic acid related to the antioxidant activity of plant extracts.

Conclusions

Basil is a medicinal plant commonly used in different industrial fields such as nutraceuticals, cosmetics and pharmaceuticals. Generally, nutraceutical and antioxidant properties of basil extract

are related to the presence of bioactive phenolic compounds also recognized as secondary metabolites. In this study, the effect of HS and CT extracted from composted wastes biomasses was evaluated through the analyses of phenotypic parameters (weight of fresh/dry biomass and chlorophyll content) and metabolomic approach to identify possible changes associated with biosynthetic pathways. The use of advanced liquid chromatography techniques combined with mass spectrometry (Orbitrap Q elite) have enabled the identification of 147 metabolites contained in Basil leaves. Moreover, a positive effect related to the increased production of compounds with possible nutraceutical applications such as caffeic acid, rosmarinic acid, narigenin, resveratrol were related to the application of HS- artichoke and CT-coffee characterized by a larger content of available aromatic component. This work confirmed a strong correlation between chemical structure and bio-activity of organic extracts from composted biomasses with a direct effect on secondary plant metabolism suggesting a potential application of these eco-sustainable materials in nutraceutical and cosmetical fields for the increase of natural bioactive products in medicinal plants

Acknowledgments

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CHAPTER 5

Research in progress

Lipidomics approach to evaluate the effect of humic derivates on lipids with nutraceutical activity in Basil plants.

1. Introduction

Lipids play crucial roles in plants as structural components of membranes, reserves in seeds, pigments for energy capture in leaves and signaling molecules (*Shulaev et al., 2017*). Lipidomics is defined as a new field of metabolomics that allows to identify different lipids in human or vegetable cells. The increased performance of lipidomic analysis has led to recent developments in electrospray ionization mass spectrometry (ESI/MS) and rapid scan tandem spectrometers that are able to detect and quantify high-sensitivity lipids in high-performance online chromatography (*Fouillen et al., 2013*). Plant lipidomics is often applied to the analysis of different polar glycerolipids and sphingolipids using highly selective mass spectrometry applications that include scanning precursor ions or monitoring multiple reactions based on clearly characterized mass fragmentation patterns (*Okazaki et al., 2013*). Plants are the basic and natural source of all types of phytochemicals (*Jaiwal, 2006*) including Coenzyme Q10 (CoQ10). CoQ10 is benzoquinone used as electron carrier in aerobic respiration and found extensive application as ingredient in food supplements, nutraceuticals and functional foods, as well as in anti-aging creams. To satisfy its growing demand of pharmaceutical,

cosmetic and food industries, there is great interest in the commercial production of CoQ10. However, the production of CoQ10 is very limited in all food crops and decreases during food processing (*Pravst et al., 2009, 2010*). In plants, CoQ 10 has been shown to be essential for the development of Arabidopsis (*Okada et al., 2004*), improved tolerance to oxidative and saline stress (*Ohara et al., 2004*), biotic stress (*Bajda et al., 2009*) and the development of functional foods rich in CoQ10 (*Takahashi et al., 2009*). Therefore, CoQ10 seems to be an important target not only to enhance the performance of crops in adverse habitats, but also to improve their nutritional value (*Pravst et al., 2010*). Although microbial production is the main industrial source of CoQ10, due to the low yield and high cost of production, other alternative and cost-effective sources need to be explored (*Parmar et al., 2015*). In this context, natural organic components as humic substances and compost teas from recycled agricultural biomasses could be potential candidates considering their effect on plants metabolism already discussed in **Chapter 4** to increase the production of CoQ10 in plants. In this work, we evaluate the effects of humic substances (HS) and compost teas (CTs) obtained from artichoke and coffee composted biomasses in the biosynthesis of CoQ10 in Basil plants using a Lipidomic approach.

2. Materials and methods

2.1. Experimental design, plant growth, sampling, analyses and ^{13}C-CPMAS- NMR of humic substances and compost teas

Composting process, extraction and chemical characterization of organic materials by ^{13}C-CPMAS- NMR used in this experiment were previously described in **Chapter 3 and 4.** Moreover, the experimental design and plant growth have been reported in **Chapter 4**. Briefly, Basil plants were

treated with different concentration of humic substances and compost teas extracted from artichoke and coffee biomasses composted.

2.2. Lipidomic approach: extraction method, standards and solvents

Basil leaves stored at -80 °C were homogenized by using a mortar and pestle under liquid nitrogen. Then, 60±0.5 mg of homogenized plant material was weighed into 2 ml Eppendorf tube. The extraction was conducted by adding 1 mL of water/methanol/chloroform mixture (1:3:1 ratio) pre-cooled at -20°C. The samples were mixed for 60 s and incubated for 30 min at 70 °C in order to inhibit the activity of possible co-extracted enzymes. The mixtures were then centrifuged for 10 min at 10000 rpm and 4 °C, and the supernatants were recovered and transferred into 2 mL Eppendorf tubes. Milli Q water (400 µL) was added to allow the separation of polar and apolar phases corresponding to the methanol/water (upper) and chloroform (lower) phases, respectively. All extracts were finally stirred for 30 s and centrifuged for 10 min at 4°C at 10000 rpm. A volume of 200 µL was collected from the lower phase, transferred into 1.5 ml glass tubes for LC–MS analyses, dried using Nitrogen Flow and stored at -80 °C. Extracted lipids were dissolved in 180 µL of LC-MS starting buffer (30% B). Additionally, a quality control (QC) mix a quantity of 20 µl of each samples was prepared. Lipid Standards (ceramide (Cer) d36:1, diglyceride (DG) 34:1, lysophosphatidylcholine (LPC) 16:0, LPC 18:0, phosphatidylcholine (PC) 34:0, PC 34:1, PC 34:2, phosphatidylethanolamine (PE) 34:1, PE 36:2, phosphatidic acid (PA) 34:1, phosphatidylserine (PS) 34:1, PS 36:1, triglyceride (TG) 52:2) and CoQ10 were obtained from Avanti Polar Lipids, Inc. (Alabaster, Alabama, USA). Ammonium bicarbonate, ammonium formate, ammonium hydroxide and formic acid were used as eluent components for LC-MS analyses.

2.3. Lipidomic approach: Liquid Chromatography High Resolution Mass Spectrometry (LC/HRMS)

For the separation of lipids an Aquity UPLC HSS T3 column (150 × 2.01 mm, particle size 1.8 µm; Waters) column was used. A 27 min gradient was applied at a flow rate of 250 µl/min. Eluent A was acetonitrile-water (6/4 [v/v]) and eluent B was isopropanol-acetonitrile (9/1 [v/v]. The following gradient program was applied: 0-2 min isocratic at 70% A; 2-15 min linear gradient 70% A to 25% A; 15-17 min linear gradient 25% A to 0% A; 17-22 min isocratic at 0% A; 22-27 min re-equilibration at 70% A. The injection volume was 5 µL. All mass spectrometric measurements were performed at the Department of Analytical Chemistry at the University of Vienna. The analyses were carried out on a Q Exactive HF mass spectrometer (Thermo Fisher Scientific) using electrospray ionization (ESI). For ddMSMS experiments a higher energy collisional dissociation (HCD) cell was used for fragmentation. Lipids were detected in positive ionization mode in the range between 200 and 2000 m/z. For the identification of lipids full MS experiments were performed with subsequent ddMSMS. Collecting time during full MS was 200 ms, during ddMSMS it was 60 ms. For full MS runs, positive and negative mode data were acquired in the polarity switching mode with a resolution of 120 000.

For lipid analysis, a standard panel covering all lipid classes, i.e. monoglycerides (MG), diglycerides (DG), triglycerides (TG), ceramides (Cer), hexosyl ceramides (HexCer), dihexosyl ceramides (Hex2Cer), cholesteryl esters (CE), phosphatidic acids (PA), phosphatidylcholines (PC), phosphatidylethanolamines (PE), phosphatidylglycerols (PG), phosphatidylserines (PS), lysophosphatidylcholines (LPC), lysophosphatidylethanolamines (LPE), fatty acids (FA), sphingomyelins (SM), and acyl carnitines (AcCa) (data no shown) was used to define the condition for the best chromatographic separation and assess the limits of detection. Data evaluation was performed using Lipid Search 4.1 (Thermo Fisher Scientific) for the ddMS2 identification runs (n = 3 samples and one sample was measured twice as analytical replicate, in positive and negative mode).

Lipid Search results were filtered for 5 g L^{-1} in MS^1, 7 g L^{-1} in MS^2 and the lipids were only considered if the areas were 3 × higher than in the blank samples or not present in the blanks at all. The main adduct ions in positive mode was set to H+ for PC, PS, PE, PA, HexCer, SM, AcCa, for MG, DG, TG, PG, PI, CE the main adduct ions were set to M + NH_4/Na, for Cer, and HexCer additionally adduct ion with loss of H_2O were considered. The main adduct ions in negative mode was set to H- for PS, PE, PA, Cer, HexCer, SM, AcCa, for PC and SM the main adduct ion was set to HCOO–, for Cer, and HexCer additionally adduct ion with loss of H_2O were considered. The main grade was set to A (lipid class and fatty acids are completely identified) and B (lipid class and some fatty acids are identified) for all lipid classes except PC, Cer, HexCer and SM, there A, B and C (lipid class or fatty acids are identified) grade were allowed. Skyline (Version 3.7) was used for Full-MS quantification of lipid standards based on peak areas obtained from extracted ion chromatograms (±5 g L^{-1}) with external calibration. The calibration was performed over four orders of magnitude (0.01–10 μM) for all lipids analysed. For lipid chromatograms was used. All calibration curves were linear and weighted 1/x *(Peng et al., 2016; Rampler et al., 2018*).

2.4. Statistical analysis

All measurements were normally distributed, according the Shapiro-Wilk test ($p < 0.05$), and the One-Way ANOVA and the post hoc Tukey's range tests were used to compare means among treatments ($p < 0.05$). Data obtained from this study were processed using XLSTAT software. All tests were performed at a level of significance of $\alpha = 0.05$.

3. Preliminary Results

In order to investigate the different production of lipids in Basil plants treated with HS and CTs at different concentrations, lipid fraction was analyzed applied a High-resolution mass

spectrometry technique using Thermo Scientific Q Exactive HF quadrupole-Orbitrap equipped with an electrospray source. **Figure 1** shows TIC chromatogram of the lipid components extracted from the Basil leaves of a representative sample (CTRL) whereas the TIC chromatograms relative to lipids extract obtained from plants treated with HS-artichoke at different concentration are reported in **Figure 2**. Lipidomic analysis carried out on Basil leaves with the application of two different software *Lipid search* and *Skyline* has allowed the identification of 157 lipids from different structural classes as reported in **Table 1**. In this case, our attention was focused on the quantification of a lipid with a possible nutraceutical application such as Coenzyme Q10. The quantification using external calibration of Coenzime Q10 was reported in **Figure 3**. Calibration curve was linear and weighted 1/x. Analysis of target data shown an increase of Coenzyme Q10 concentration in *Basil* leaves treated with humic material as summarized in **Figure 4**. Particularly, the application of HS-artichoke at concentration of 50 and 100 g/L induced an improvement of production among 7 and 18 % compared to the control whereas the treatment with CT-coffee shown a low effect bio-active with an increment of 3 % and 7 % at the same concentration, respectively. These results suggest that the content of target lipid molecules may be correlated with the aromatic components of HS and CT samples that may influence the biosynthetic pathways.

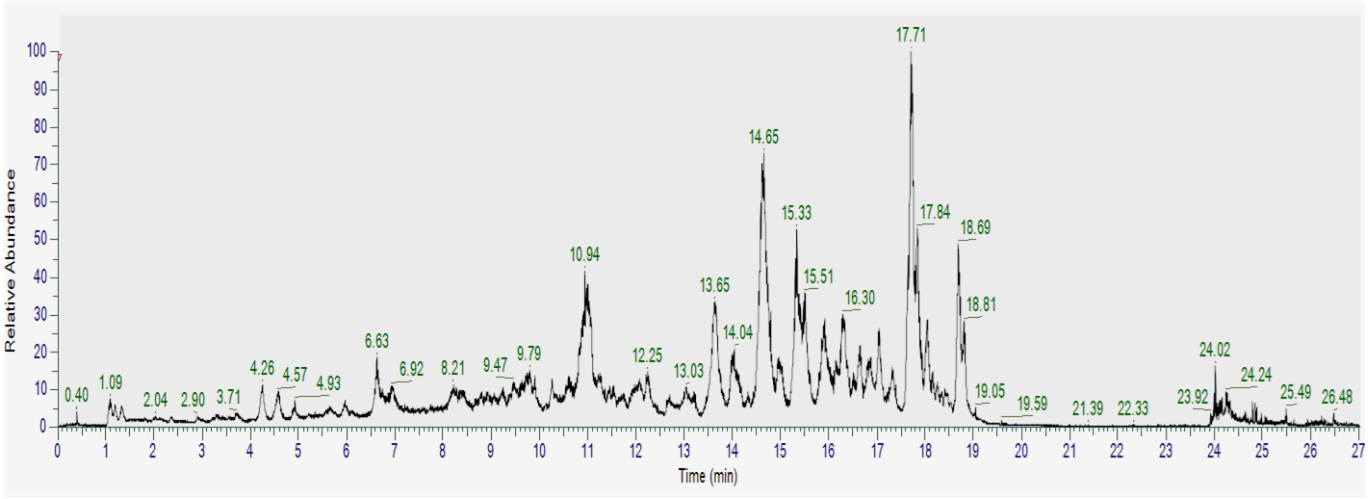

Figure 1: Total Ion Chromatogram (TIC) by Liquid Chromatography High Resolution Mass Spectrometry (LC/HRMS) of *Basil* leaves extract (CTRL sample).

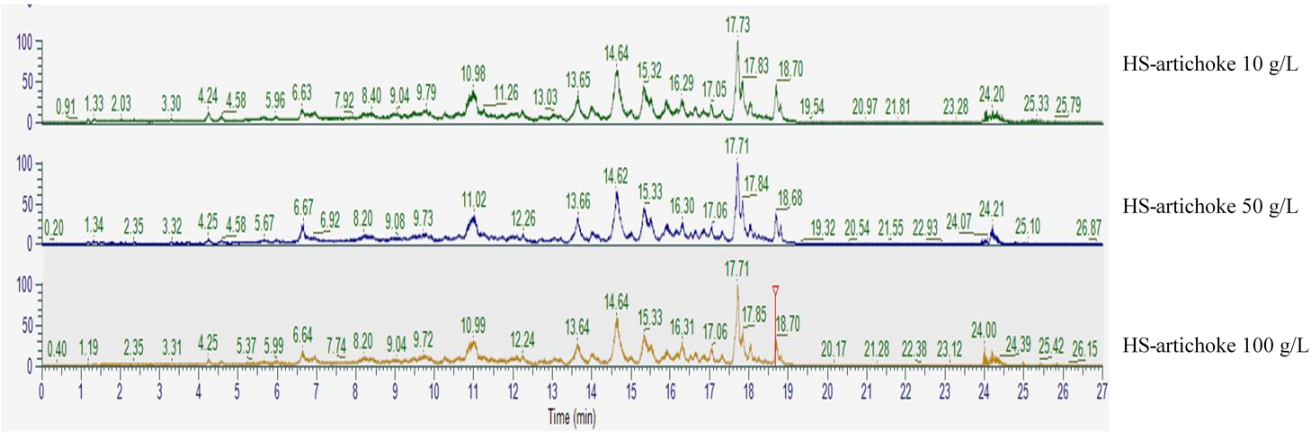

Figure 2: Total Ion Chromatogram (TIC) by Liquid Chromatography High Resolution Mass Spectrometry (LC/HRMS) of Basil leaf extracts treated with different concentration of HS-artichoke (10-50-100 g L^{-1}).

Table 1: List of lipids from Basil leaves identified by Liquid Chromatography High Resolution Mass Spectrometry (LC/HRMS).

	Ret time (min)	Lipid Molecules	Class[a]	Calc Mass	Formula
1	1.383	AcCa(6:0)	AcCa	259.1784	C13 H25 O4 N1
2	18.688	Cer(d18:1_24:1)	Cer	647.6216	C42 H81 O3 N1
3	18.438	Cer(t18:1_23:0+O)	Cer	667.6115	C41 H81 O5 N1
4	18.706	Cer(t38:0)	Cer	611.5853	C38 H77 O4 N1
5	16.734	DG(14:0_18:3)	DG	562.4597	C35 H62 O5
6	18.316	DG(16:0_16:0)	DG	568.5067	C35 H68 O5
7	17.956	DG(16:0_16:1)	DG	566.491	C35 H66 O5
8	18.03	DG(16:0_18:2)	DG	592.5067	C37 H68 O5
9	13.766	DG(16:0_18:3)	DG	590.491	C37 H66 O5
10	14.829	DG(16:0_18:3)	DG	590.491	C37 H66 O5
11	17.656	DG(16:0_18:3)	DG	590.491	C37 H66 O5
12	18.037	DG(16:0_20:5)	DG	614.491	C39 H66 O5
13	17.303	DG(16:1_18:3)	DG	588.4754	C37 H64 O5
14	17.517	DG(17:1_18:3)	DG	602.491	C38 H66 O5
15	18.849	DG(18:0_18:0)	DG	624.5693	C39 H76 O5
16	18.167	DG(18:0_18:3)	DG	618.5223	C39 H70 O5
17	18.335	DG(18:0_20:4)	DG	644.538	C41 H72 O5
18	18.392	DG(18:1_18:1)	DG	620.538	C39 H72 O5
19	16.363	DG(18:1_18:3)	DG	616.5067	C39 H68 O5
20	17.645	DG(18:2_18:2)	DG	616.5067	C39 H68 O5
21	14.394	DG(18:3_18:2)	DG	614.491	C39 H66 O5
22	15.523	DG(18:3_18:2)	DG	614.491	C39 H66 O5
23	17.034	DG(18:3_18:2)	DG	614.491	C39 H66 O5
24	14.646	DG(18:3_18:3)	DG	612.4754	C39 H64 O5
25	16.255	DG(18:3_18:3)	DG	612.4754	C39 H64 O5
26	17.245	DG(18:3_20:3)	DG	640.5067	C41 H68 O5
27	13.489	DG(18:4_18:2)	DG	612.4754	C39 H64 O5

28	13.754	DG(18:4_18:2)	DG	612.4754	C39 H64 O5
29	10.625	DG(18:4_18:3)	DG	610.4597	C39 H62 O5
30	10.958	DG(18:4_18:3)	DG	610.4597	C39 H62 O5
31	12.774	DG(18:4_18:3)	DG	610.4597	C39 H62 O5
32	18.16	DG(20:1_18:3)	DG	644.538	C41 H72 O5
33	17.653	DG(20:5_18:2)	DG	638.491	C41 H66 O5
34	17.395	DG(30:0e)	DG	526.4961	C33 H66 O4
35	18.031	DG(32:1e)	DG	552.5118	C35 H68 O4
36	12.041	DG(34:4)	DG	588.4754	C37 H64 O5
37	18.024	DG(34:4e)	DG	574.4961	C37 H66 O4
38	17.347	DG(35:4)	DG	602.491	C38 H66 O5
39	11.996	DG(36:6)	DG	612.4754	C39 H64 O5
40	15.816	Hex1Cer(d18:1_16:0+O)	Hex1Cer	715.5598	C40 H77 O9 N1
41	15.454	Hex1Cer(d34:2+O)	Hex1Cer	713.5442	C40 H75 O9 N1
42	18.422	Hex1Cer(t18:1_25:0+O)	Hex1Cer	857.6956	C49 H95 O10 N1
43	15.831	Hex1Cer(t34:1)	Hex1Cer	715.5598	C40 H77 O9 N1
44	14.906	Hex1Cer(t34:1+O)	Hex1Cer	731.5547	C40 H77 O10 N1
45	17.233	Hex1Cer(t38:1+O)	Hex1Cer	787.6173	C44 H85 O10 N1
46	17.891	Hex1Cer(t40:1+O)	Hex1Cer	815.6486	C46 H89 O10 N1
47	18.095	Hex1Cer(t41:1+O)	Hex1Cer	829.6643	C47 H91 O10 N1
48	6.146	LPC(16:0)	LPC	495.3325	C24 H50 O7 N1 P1
49	8.73	LPC(18:0)	LPC	523.3638	C26 H54 O7 N1 P1
50	4.912	LPC(18:2)	LPC	519.3325	C26 H50 O7 N1 P1

51	3.732	LPC(18:3)	LPC	517.3168	C26 H48 O7 N1 P1
52	11.22	LPC(20:0)	LPC	551.3951	C28 H58 O7 N1 P1
53	8.945	LPE(20:3)	LPE	503.3012	C25 H46 O7 N1 P1
54	4.312	LPG(16:1)	LPG	482.2645	C22 H43 O9 N0 P1
55	4.706	LPG(16:1)	LPG	482.2645	C22 H43 O9 N0 P1
56	3.228	LPG(18:3)	LPG	506.2645	C24 H43 O9 N0 P1
57	2.757	LPS(18:3)	LPS	519.2597	C24 H42 O9 N1 P1
58	9.786	MG(18:1)	MG	356.2927	C21 H40 O4
59	7.711	MG(18:2)	MG	354.277	C21 H38 O4
60	2.676	MG(18:4)	MG	350.2457	C21 H34 O4
61	17.602	MGDG(16:0_16:0)	MGDG	730.5595	C41 H78 O10
62	17.668	MGDG(16:0_18:1)	MGDG	756.5752	C43 H80 O10
63	17.044	MGDG(16:0_18:2)	MGDG	754.5595	C43 H78 O10
64	16.303	MGDG(16:0_18:3)	MGDG	752.5438	C43 H76 O10
65	15.26	MGDG(16:1_18:3)	MGDG	750.5282	C43 H74 O10
66	16.84	MGDG(17:0_18:3)	MGDG	766.5595	C44 H78 O10
67	17.787	MGDG(18:0_18:2)	MGDG	782.5908	C45 H82 O10
68	17.334	MGDG(18:0_18:3)	MGDG	780.5752	C45 H80 O10
69	17.134	MGDG(18:1_18:2)	MGDG	780.5752	C45 H80 O10
70	16.407	MGDG(18:1_18:3)	MGDG	778.5595	C45 H78 O10
71	15.513	MGDG(18:2_18:3)	MGDG	776.5438	C45 H76 O10
72	15.681	MGDG(18:2_18:3)	MGDG	776.5438	C45 H76 O10
73	13.478	MGDG(18:3_16:3)	MGDG	746.4969	C43 H70 O10
74	1.998	MGDG(18:3_18:3)	MGDG	774.5282	C45 H74 O10
75	14.526	MGDG(18:3_18:3)	MGDG	774.5282	C45 H74 O10

76	14.774	MGDG(18:3_18:3)	MGDG	774.5282	C45 H74 O10
77	16.313	MGDG(18:3_18:3)	MGDG	774.5282	C45 H74 O10
78	24.179	MGDG(18:3_18:3)	MGDG	774.5282	C45 H74 O10
79	14.268	MGDG(18:3_18:4)	MGDG	772.5126	C45 H72 O10
80	15.749	MGDG(18:3_20:3)	MGDG	802.5595	C47 H78 O10
81	14.52	MGDG(18:3_20:6)	MGDG	796.5126	C47 H72 O10
82	14.753	MGDG(18:3_20:6)	MGDG	796.5126	C47 H72 O10
83	17.164	PA(18:1_18:1)	PA	700.5043	C39 H73 O8 N0 P1
84	11.904	PA(18:4_16:0)	PA	668.4417	C37 H65 O8 N0 P1
85	16.542	PA(34:2)	PA	672.473	C37 H69 O8 N0 P1
86	15.326	PA(34:3)	PA	670.4574	C37 H67 O8 N0 P1
87	16.253	PA(36:3)	PA	698.4887	C39 H71 O8 N0 P1
88	15.307	PA(36:4)	PA	696.473	C39 H69 O8 N0 P1
89	14.425	PA(36:5)	PA	694.4574	C39 H67 O8 N0 P1
90	13.717	PA(36:6)	PA	692.4417	C39 H65 O8 N0 P1
91	15.95	PC(16:0_18:3)	PC	755.5465	C42 H78 O8 N1 P1
92	16.626	PC(16:0_20:4)	PC	781.5622	C44 H80 O8 N1 P1
93	15.166	PC(17:1_22:3)	PC	823.6091	C47 H86 O8 N1 P1
94	16.015	PC(18:2_18:2)	PC	781.5622	C44 H80 O8 N1 P1
95	19.634	PC(36:1)	PC	787.6091	C44 H86 O8 N1 P1

96	16.934	PC(36:3)	PC	783.5778	C44 H82 O8 N1 P1
97	14.966	PC(36:5)	PC	779.5465	C44 H78 O8 N1 P1
98	24.208	PC(36:5)	PC	779.5465	C44 H78 O8 N1 P1
99	15.943	PC(36:6)	PC	777.5309	C44 H76 O8 N1 P1
100	16.194	PC(40:9)	PC	827.5465	C48 H78 O8 N1 P1
101	15.923	PE(16:0_18:3)	PE	713.4996	C39 H72 O8 N1 P1
102	13.684	PE(18:2_18:2)	PE	739.5152	C41 H74 O8 N1 P1
103	13.821	PE(18:2_18:2)	PE	739.5152	C41 H74 O8 N1 P1
104	15.881	PE(18:2_18:2)	PE	739.5152	C41 H74 O8 N1 P1
105	5.586	PE(18:3_18:3)	PE	735.4839	C41 H70 O8 N1 P1
106	16.701	PE(34:2)	PE	715.5152	C39 H74 O8 N1 P1
107	17.59	PE(36:2)	PE	743.5465	C41 H78 O8 N1 P1
108	15.816	PG(16:0_16:1)	PG	720.4941	C38 H73 O10 N0 P1
109	15.036	PG(16:0_18:3)	PG	744.4941	C40 H73 O10 N0 P1
110	14.172	PG(16:1_18:3)	PG	742.4785	C40 H71 O10 N0 P1
111	16.354	PG(33:1)	PG	734.5098	C39 H75 O10 N0 P1
112	16.33	PG(34:1)	PG	748.5254	C40 H77 O10 N0 P1

113	14.693	PG(34:3)	PG	744.4941	C40 H73 O10 N0 P1
114	11.524	PG(34:4)	PG	742.4785	C40 H71 O10 N0 P1
115	11.597	PI(16:0_18:3)	PI	832.5102	C43 H77 O13 N0 P1
116	11.825	PI(16:0_18:3)	PI	832.5102	C43 H77 O13 N0 P1
117	14.32	PI(16:0_18:3)	PI	832.5102	C43 H77 O13 N0 P1
118	16.642	PIP(16:0_16:0)	PIP	890.4922	C41 H80 O16 N0 P2
119	18.212	PMe(18:0_16:0)	PMe	690.52	C38 H75 O8 N0 P1
120	16.425	PS(16:0_18:1)	PS	761.5207	C40 H76 O10 N1 P1
121	16.507	PS(40:8)	PS	831.505	C46 H74 O10 N1 P1
122	16.878	PS(43:1)	PS	887.6615	C49 H94 O10 N1 P1
123	3.564	SPH(t18:1)	SPH	315.2773	C18 H37 O3 N1
124	15.539	TG(12:1e_6:0_18:4)	TG	614.491	C39 H66 O5
125	19.227	TG(14:0_14:0_14:0)	TG	722.6424	C45 H86 O6
126	19.299	TG(15:0_14:0_16:1)	TG	762.6737	C48 H90 O6
127	19.586	TG(15:0_16:0_16:0)	TG	792.7207	C50 H96 O6
128	19.601	TG(15:0_16:0_18:1)	TG	818.7363	C52 H98 O6
129	17.987	TG(16:0_10:1_12:2)	TG	660.5329	C41 H72 O6
130	19.374	TG(16:0_14:0_14:0)	TG	750.6737	C47 H90 O6
131	19.52	TG(16:0_14:0_16:0)	TG	778.705	C49 H94 O6
132	19.663	TG(16:0_16:0_16:0)	TG	806.7363	C51 H98 O6
133	19.66	TG(16:0_16:0_18:1)	TG	832.752	C53 H100 O6
134	19.662	TG(16:0_18:1_18:1)	TG	858.7676	C55 H102 O6

135	19.162	TG(16:0_18:3_18:3)	TG	850.705	C55 H94 O6
136	19.269	TG(16:1_14:0_16:1)	TG	774.6737	C49 H90 O6
137	19.801	TG(18:0_16:0_16:0)	TG	834.7676	C53 H102 O6
138	20.007	TG(18:0_16:0_18:0)	TG	862.7989	C55 H106 O6
139	19.849	TG(18:0_16:0_18:1)	TG	860.7833	C55 H104 O6
140	19.673	TG(18:1_18:1_18:1)	TG	884.7833	C57 H104 O6
141	19.538	TG(18:1_18:1_18:2)	TG	882.7676	C57 H102 O6
142	19.666	TG(18:1_18:1_20:4)	TG	906.7676	C59 H102 O6
143	13.783	TG(18:2_10:1_10:4)	TG	652.4703	C41 H64 O6
144	19.267	TG(18:2_18:2_18:2)	TG	878.7363	C57 H98 O6
145	12.8	TG(18:3_10:1_10:4)	TG	650.4546	C41 H62 O6
146	19.037	TG(18:3_18:2_18:3)	TG	874.705	C57 H94 O6
147	18.91	TG(18:3_18:3_18:3)	TG	872.6894	C57 H92 O6
148	18.921	TG(18:4_18:3_20:5)	TG	894.6737	C59 H90 O6
149	19.27	TG(20:5_18:2_18:2)	TG	900.7207	C59 H96 O6
150	12.731	TG(36:5)	TG	628.4703	C39 H64 O6
151	17.634	TG(4:0_18:2_18:3)	TG	684.5329	C43 H72 O6
152	17.098	TG(4:0_18:3_18:3)	TG	682.5172	C43 H70 O6
153	14.405	TG(6:0_14:4_18:2)	TG	654.4859	C41 H66 O6
154	16.523	TG(8:0_10:0_10:0)	TG	526.4233	C31 H58 O6
155	11.039	TG(8:0_10:3_18:3)	TG	626.4546	C39 H62 O6
156	13.577	TG(8:0_8:0_8:0)	TG	470.3607	C27 H50 O6
157	19.371	Coenzyme Q10	/	863.3769	C59H90O4

[a] Different class of lipids: monoglycerides (MG), diglycerides (DG), triglycerides (TG), ceramides (Cer), hexosyl ceramides (HexCer), dihexosyl ceramides (Hex2Cer), cholesteryl esters (CE), phosphatidic acids (PA), phosphatidylcholines (PC), phosphatidylethanolamines (PE), phosphatidylglycerols (PG), phosphatidylserines (PS), lysophosphatidylcholines (LPC), lysophosphatidylethanolamines (LPE), fatty acids (FA), sphingomyelins (SM), and acyl carnitines (AcCa).

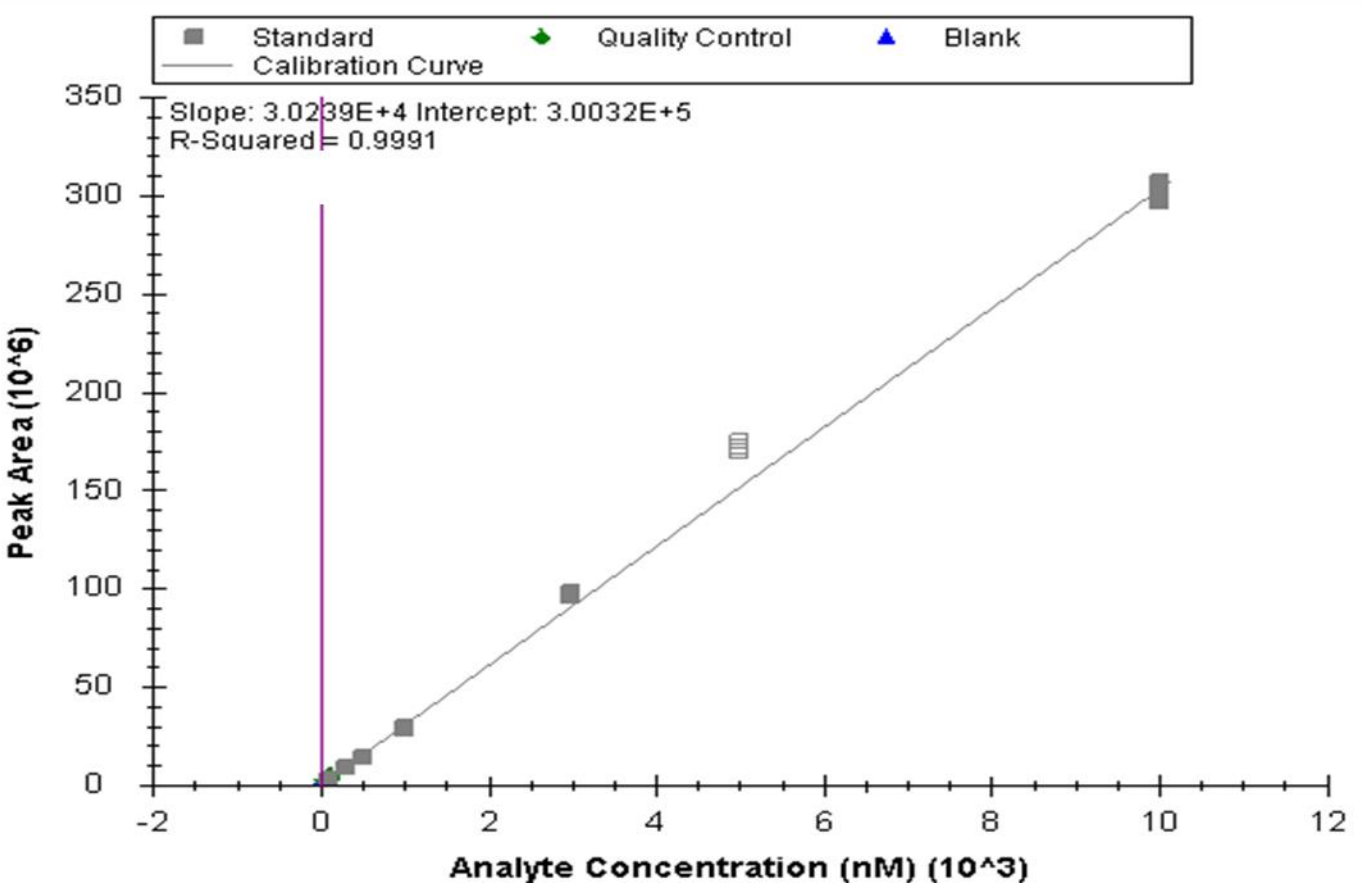

Figure 3: Calibration curves of Coenzyme Q 10 lipid using external calibration with standards applied Skyline software.

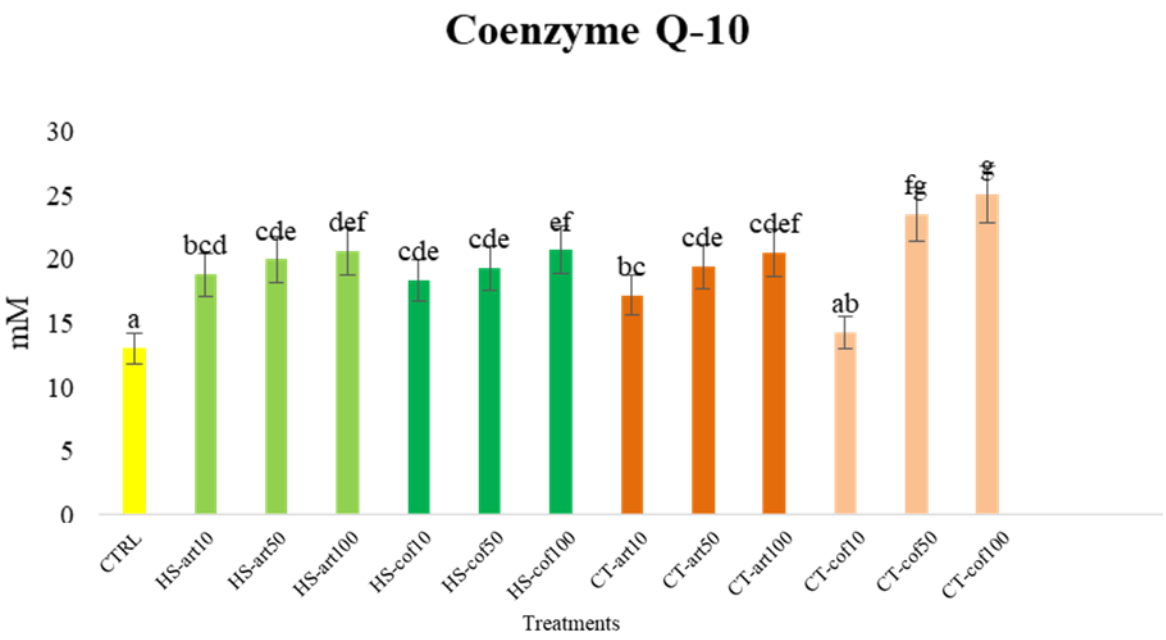

Figure 4: Absolute mM concentration of Coenzyme Q 10 in lipid extract from plants treated with different humic materials at 10, 50 and 100 g L-1 concentration. Vertical bars represent the standard deviation of the mean. Statistical analysis are performed using Tukey's test ($p \leq 0.05$).

4. Further analyses to be conducted

The analysis of full lipidomic profile of Basil plants treated with humic materials will be carried out to identify possible changes of lipids production such as monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) involved in stress mechanisms investigating the biochemical pathways that induce this response in plants.

5. Preliminary conclusions and future application

These preliminary results promote the application of humic materials extracted from compost as bio-active molecules to increase the production yield of CoQ10 in plants in order to obtain a potential sustainable production levels without using genetic engineering strategies.

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CHAPTER 6

Effect of humic substances on the chemical composition and antimicrobial activity of Basil Essential oils (EOs).

Abstract

The ever-growing demand for pharmaceutical and cosmetic applications of Essential oils (EOs), induces the necessity to find new sustainable methods to increase the production of bioactive compounds in aromatic plants. Sweet Basil (*Ocimum basilicum L.*) is the one of the major essential oils producing species of Labiatae family. Environmental factors induce changes in growth of medicinal plants as well as the quantity and quality of their essential oil composition. In this context, a possible sustainable agricultural practice is the use of compost as a source of organic matter. In particular, humic substances (HS) and compost tea (CT) extracted from composted vegetable waste are increasingly regarded as efficient biostimulants to improve crop productivity and induce positive responses to biotic and abiotic stress in plants. Moreover, the combination effect between HS and arbuscular mycorrhizal fungus (AMF) to increase the yields and antimicrobial activity of EOs in Basil plant was already investigated (*Morelli et al., 2017*). The aim of this work was to characterize the molecular composition of HS extracted from artichoke composted biomasses and evaluate their bioactive effect on *Ocinum basilicum* plant focusing on yield, chemical composition, antioxidant proprieties and antimicrobial activity of EOs. Green composts were processed in the composting plant at the Castel Volturno experimental farm of the University of Napoli Federico II. The EOs extraction were performed using steam distillation. The EOs chemical composition was carried out by GC-MS analysis. The antioxidant proprieties of EOs was evaluated used DPPH assay and antimicrobial

activities was performed against human common bacterial strains using MIC assay. The results showed that humic materials applied to soil increase the yield of Essential oils (EOs). A total of 29 chemical compounds were found in the basil EOs. Eugenol, methyl-Chavicol and α-trans-Bergamotene were the major compounds identified. HS addition increased the contents of eugenol and α-trans-Bergamotene. The antimicrobial activity (MIC) of EOs Basil was generally significative. In detail, Gram-positive bacteria such as *Staphilococcus Aureus* was inhibited at 10 μg mL^{-1} of EOs obtained by plants treated with HS, whereas in the case of Gram-negative bacteria such as *Pseudomonas aeuriginosa* and *Klebsiella pneumonia*, the antimicrobial effect of EOs was shown at 4 and 2 μg mL^{-1}, respectively. The antioxidant activity of EOs was higher in the case of Basil plant treatment using HS. These results support the use of compost extracts as promising potential applications in the biomedical field to increase the quality and the yield of aromatic plant EOs.

Keywords: *Humic substances, Basil, Essential oil, steam distillation, antioxidant, antimicrobial.*

1. Introduction

Aromatic plants have been used since antiquity around the world due to their medical proprieties (*Petrovska, 2012*). The definition of aromatic character attains from the usual specific synthesis of organic components derived from secondary metabolism which determine the specific aroma and the flavour of these plants (*Toncer et al., 2017; Özcan et al., 2018*). Many species of aromatic plants classified in the *Lamiaceae sp*. which includes about 200 species are typical of Mediterranean distribution area (*Marotti et al., 1996; Martins et al., 1999*). Sweet Basil (*Ocimum basilicum L.)* also belongs to the Lamiaceae family, and occur in various botanic varieties and has been used as a medicinal plant in the treatment of headaches, coughs, diarrhea, constipation, warts, worms, kidney malfunctions, in control the blood glucose or in the case of diabetic disease (*Mousavi*

et al., 2018). Furthermore, this medical plant represents a source of Essential Oils (EOs) extracted from the leaves and the flowering tops, which are usually applied in the production of fragrances. The characteristic of aromatic compounds of basil plant is related to the specific genotype which determine the composition of EOs (*Lupton et al., 2012*). The EOs are mainly composed by a mixture of different secondary metabolites pertaining to terpenes and phenylpropanoids classes (*Tateo et al., 1989; Marotti et al., 1996*). In order to improve the extraction yield, several methods are applied for the isolation of EOs such as steam distillation (*Gabri and Chialva, 1981*), organic solvent extraction and soxhlet apparatus (*Rassem et al., 2016*).

Besides the quantitative output, the type of extraction method affects the quality of essential oil depending on the applied pressure and temperatures. The conventional techniques to extract essential oils from medicinal and aromatic plants relies on the large removal properties of organic solvents. However, the process requirement to combine lower environmental impact and the improvement of final products recovering have promoted the research of new eco-friendly approach to increase the yield and quality of EOs (*Tongnuanchan and Benjakul, 2014*). In this respect, water is increasingly regarded as useful green solvent for the EOs extraction methods (*Chemat et al., 2019*). Effectively, steam distillation combined with microwave extraction may reduce the occurrence of secondary chemical reactions and the formation of by-products thus allowing to obtain a suitable yield at low cost associated with an eco-compatible approach (*Filly et al., 2016*). The curative and therapeutical properties of EOs have been related to anti-bacterial (*Suppakul et al., 2003;Gaio et al., 2015*), anti-fungal (*Beatovi et al., 2015*) and antioxidant activities (*Politeo et al., 2007*). This features are the key factors that for their use in various industries such as pharmaceutical (*Shiwakoti et al., 2017*), cosmetic and aromatherapy, as well as in food industry (*Padalia et al., 2013*) and for fresh commodity postharvest protection (*Chowdharya et al., 2017*).

The ever-growing demand for industrial applications of EOs, induces the necessity to find new sustainable methods to increase the production of these bioactive compounds in aromatic plants (*Perricone. et al., 2015*). The stability of both composition and quantity of EOs active components

are a market requirement for medical aromatic plants such as basil. Generally, the synthesis of these bioactive compounds in medicinal plants are affected by different factors such as environmental conditions and soil management practices and inputs, (e.g. fertilizers, water, pesticides) that determine the biosynthesis and accumulation of secondary metabolites (*Morelli et al., 2017*). Moreover, the use of synthetic agrochemical products in agriculture is currently under pressure, since consumer, economic and environmental considerations have resulted in an increasing number of regulations limiting their applications. On the other hand, one of the current challenges of agriculture is to guarantee high crop yields and productivity, while concomitantly matching the stringent environmental regulations devised for agro- and forestry-ecosystems. Therefore, less intensive and sustainable cultivation methods are highly recommended for the cultivation of aromatic plants to improve biomass yields with large and steady amount of medicinal substances with the decrease of mineral fertilizers /or agrochemicals (*Urcoviche et al., 2015; Lermen et al., 2017*).

In this context, a reliable agricultural practice is represented by the use of recycled biomasses such as compost for the sustainable and comprehensive management of cultivated lands (*Scotti et al., 2016*). The recent indications of the EC on the relation between circular economy and rural development (*COM (2017) 33 final*) strongly support the reutilization of different organic biomasses in order "*to recycling of bio-waste in organic-based fertilizers*", thereby "turning waste management problems into economic opportunities" and improving the environmental resilience and circular economy of the agricultural sector. In particular, the humic substances (HS) extracted from composted vegetable waste are increasingly regarded as efficient biostimulants to improve crop productivity while inducing positive responses to biotic and abiotic stress in plants with a concomitant decrease of energetic inputs such as agrochemical and water (*Garcia et al., 2014; Jindo et al., 2016; da Piedade Melo et al., 2017*).

These positive effects can be explained by the direct action of humic substances as hormone like molecules on physiological and metabolic processes (*Canellas et al., 2015*). The aim of this work was to evaluate the bioactive effect of humic substances extracted from artichoke composted

biomasses on the synthesis of EOs in *Ocinum basilicum* plant. The isolation of EOs extractives was performed with water distillation methodology thereby analysing on yield, molecular composition, antioxidant and antimicrobial activities of EOs.

2. Materials and Methods

2.1. Composting process and extraction of humic substances (HS)

The green composts used in this study were produced in the on-farm composting facility of the Experimental Farm of University of Napoli Federico II at Castel Volturno (CE), as described in **Chapter 3**. The humic substances were isolated with alkaline water solution through the conventional extraction procedure for organic biomasses (*Canellas et al., 2015; Monda et al., 2018*): an aliquot of 100 g of each air-dried compost (2 mm sieved) was suspended in 500 ml 0.1 mol L^{-1} KOH and mechanically shaken for 24 h. The humic extracts were separated by by centrifugation (7000 rpm for 20 min) and subsequent filtration with glass wool filters (Whatman GF/C). In order to improve the yield of HS, the compost pellets were resuspended in the extracting solution for additional extraction steps (2 times x 1h). The supernatants were hence combined acidified to pH 7 with 6 mol L-1 HCL, and dialyzed against deionized water (Spectrapore 1-kD cutoff membrane). The HS solution were finally freeze-dried for subsequent application and analytical characterization.

2.2. Molecular characterization of HS : ^{13}C-CPMAS-NMR and TMAH-GC–MS

The molecular characterization of HS from artichoke-based compost was performed using ^{13}C-CPMAS-NMR Spectroscopy and Offline pyrolysis TMAH-GC–MS.

The solid-state NMR analysis was carried out with a 300 MHz Bruker Avance spectrometer, equipped with a 4 mm wide-bore MAS probe. Each fine-powdered sample was packed into a 4 mm zirconium rotor, provided with a Kel-F cap, and spun at a rate of 13000±1 Hz. Then, 13C NMR spectra was

acquired through Cross-Polarization Magic-Angle-Spinning (CPMAS) technique by using 2 s of recycle delay, 1 ms of contact time, 30 ms of acquisition time and 4000 scans. According to literature (*de Aquino et al., 2019; Spaccini et al., 2019*), the overall C distribution was split in six chemical shift regions assigned to the main organic functional groups: 0–45 ppm (aliphatic C), 45–60 ppm (O-substituted alkyl C), 60-110 ppm (O-alkyl-carbon), 110–140 ppm (aromatic C), 140-160 ppm (O-aryl-C), 160–190 ppm (carbonyl/carboxyl C). The area of each of the regions was determined by integration (MestreNova 6.2.0 software, Mestrelab Research, 2010), and expressed as percentage of the total area. In order to summarize the structural composition of humic derivatives, the following four dimensionless indices were calculated by the relative areas of various chemical shift ranges as described in **Chapter 3**: HB/HI hydrophobicity index, A/OA alkyl ratio, ARM aromaticity index, LigR lignin ratio. The, Offline pyrolysis TMAH-GC–MS was performed using 500 mg of dried HS placed in a quartz boat and treated with 1 ml of TMAH (25% in methanol) solution. After drying the mixture under a gentle stream of nitrogen, the quartz boat was introduced into a Pyrex tubular reactor (50 cm × 3.5 cm i.d.) and heated at 400 °C for 30 min in a round furnace (Barnstead Thermolyne 21,100). The products released by thermochemolysis were continuously transferred by a helium flow (20ml min^{-1}) into a series of two (50 ml) round flasks filled with chloroform and kept in ice/salt baths. The chloroform solutions were combined and concentrated by roto-evaporation. The residue was dissolved in 1 ml of chloroform and transferred in a glass vial for GC–MS analysis. The GC–MS analyses were performed with a Perkin- Elmer Autosystem XL by using a RTX-5MS WCOT capillary column (Restek, 30 m × 0.25 mm; film thickness, 0.25 μm), that was coupled, through a heated transfer line (250 °C), to a PE Turbomass-Gold quadrupole mass spectrometer. The chromatographic separation was achieved with the following temperature program: 60 °C (1 min isothermal), rate 7 ° min^{-1} to 320 °C (10 min isothermal). Helium was used as carrier gas at 1.90 ml min^{-1}, the injector temperature was at 250 °C, the split-injection mode had a 30ml min^{-1} of split flow.Mass spectra were obtained in EI mode (70 eV), scanning in the range 45–650 m/z, with a cycle

time of 1 s. Compound identification was based on comparison of mass spectra with the NIST library database, published spectra, and real standards.

2.3. Pots preparation and experimental design

To set up the pot experiment, we used the surface layer (0–15 cm) of soil collected at the Long-Term Experimental field site of the University Farm of Agricultural Dept. (University of Naples "Federico II"), located in Castel-Volturno (CE). Soil and compost pH and electrical conductivity (EC) were measured in a 1:5 soil/water suspension (w/v) after 1 h end-over-end shaking at 25 °C. Soil organic carbon (C) was determined by the Walkley–Black procedure (*Nelson and Sommers, 1982*) and total nitrogen (N) by the Kjeldahl digestion method (*Bremner and Mulvaney, 1982*). Available soil phosphorus (P) was extracted with sodium bicarbonate (Olsen method) and then determined by the molybdenum-blue method (*Murphy and Riley, 1962*). The soil was a clay loam (44.6%, 28% and 27.4% sand, silt and clay), alkaline (pH 8.6) and classified as a Vertic Xerofluvent, containing 1.11 g kg^{-1} total N, 10.5 g kg^{-1} organic carbon, 11 mg kg^{-1} of $NaHCO_3$-extractable P. The pot experiment was conducted from June to July 2018, under greenhouse conditions (25–33 °C, daily temperature range). For pot experiment the *Ocinum basilicum* plants were grown using a mixture of soil/sand substrate (1 kg pot $^{-1}$); the soil was sieved to 5 mm, mixed with quartz sand at 2:1 (w/w) ratio of and thoroughly homogenized. A basal nutrients supply was performed adding (N) nitrogen as ammonium nitrate (NH_4NO_3) at the rate of 150 mg N kg^{-1} dry substrate, (K) potassium as triple superphosphate (TSP) at the rate of 75 mg K kg^{-1} dry substrate and (P) phosphorus as potassium sulphate (K_2SO_4) at the rate of 160 mg P kg^{-1} dry substrate. The fertilizers were added in powder form to the substrate (soil/sand mixture) of each individual pot and mixed thoroughly. Furthermore, HS from composted artichoke were tested at three different concentration (10-50-100 g L^{-1}) and applied to the soil one time a week for 4 weeks to obtain a final concentration of 0.10, 0.20 and 0.40 g kg^{-1}.

The pot trial was designed as follows:

CTRL: Control (H_2O) + mineral fertilizers

A: HS-artichoke 10 g L^{-1}

B: HS-artichoke 50 g L^{-1}

C: HS-artichoke 100 g L^{-1}

Each treatment was replicated five times for a total of 20 pots. Harvested leaves were weighed, immediately frozen in liquid nitrogen, in order to quench the metabolism, and stored at -80 °C.

2.4. Determination of chlorophyll content

The chlorophyll content was determined as follows: 9 mm of fresh foliar tissue from each plant were ground in liquid nitrogen, homogenized in 5 ml of pure acetone, and then transferred into 15 mL glass centrifuge tubes to be centrifuged at 3000 x g for 5 min at 8°C. The supernatant absorbance at 645 and 663 nm were measured using a spectrophotometer and the concentrations of Chl a, Chl b, total Chl (Chl a + b) were calculated according the method of *Lichtenthaler* (1987) and expressed in mg of pigment per g of leaf fresh weight.

2.5. Extraction method of Basil Essential oils (EOs)

In this work, steam distillation method has been used to obtain EOs from Basil leaves. The system to perform the steam distillation was made up of a 1000 ml steam generator flask, a distilling flask, a condenser and a receiving vessel. In detail, 100 g of frozen Basil leaves was subjected to steam distillation and positioned in the distillation flask connected to the steam generator flask filled with 900 ml of distilled water and heated with a heating mantle. As water was vaporized, the steam

passed through the distillation flask containing the plant. The vapor passed through the cooled tube, where it condensed. The volatile components were collected into the receiving flask (250 ml) during 2.5 h of steam distillation. The final distilled steam is formed by a mixture of water/oil bi-layer system. In order to recover the organic phase, a liquid-liquid separation was performed adding 150 ml of exane and 2 g of sodium sulfate anhydrous to completely remove the trace of water from distillate that was stored at 0–4 °C until GC-MS analysis.

2.6. Chemical composition of Basil EOs

Qualitative chemical characterization of EOs obtained using the method described previously, was carried out by gas chromatography coupled to mass spectrometry (GC-MS) using a Perkin Elmer Autosystem XL coupled with a Turbomass Gold mass spectrometer and RTX-5MS WCOT capillary column (Restek,30 m × 0.25 mm; film thickness, 0.25 mm). The GC–MS operating conditions were as follows: initial temperature 60 °C held for 5 min, increased at a rate of 4 °C min^{-1} to 160 °C, increased at a rate of 15 °C min^{-1} to 240 °C. The temperature was then increased at a rate of 10 °C min^{-1} to a final isothermal step at 300 °C and held for 10 min. Injector and detector temperatures were 250 °C and 280 °C respectively. Helium (1.0 mL min^{-1}) was used as a carrier gas. The injection volume was 1µL. Mass spectra were obtained in electron ionization mode at 70 eV, scanning in the range included within 50 and 650 *m*/*z*, with a scan cycle rate of 0.2 s^{-1}. The compound peaks were integrated in manual mode and the identification was based on comparison of mass spectra with the NIST-library database and published spectra.

2.7. Antibacterial activity of Basil EOs

The bacterial strains used in this study included *Escherichia coli ATCC35218, Staphylococcus aureus ATCC6538P, Pseudomonas aeruginosa ATCC27355, Enterococcus Faecalis ATCC29212*

and *Klebisella pneumonie ATCC700503* provided by Department of Biology (University of Naples Federico II). The antimicrobial activity of EOs was performed in accordance to *Morelli et al., (2017)* with some modification. The tube-dilution method was used to determine the minimum inhibitory concentration (MIC) of the essential oil of *O. basilicum* against the studied microorganisms; the outputs indicate the lowest concentration of EOs causing complete growth inhibition (*CLSI, 2009*). A 40 μg ml $^{-1}$ EOs solution diluted with 2% Tween 80 in Mueller-Hinton broth with 2% glucose was prepared to treat the isolates. The culture medium (100 μL) was distributed in wells of a 96 micro-dilution plate, and then 200 μL of EOs was added to the second well. After, the solution was transferred to the third well, and so on until the tenth well. Thus, the final concentrations obtained after the serial dilution were: 40, 20, 10, 5, 2.5, 1.25, 0.625, 0.312, 0.156 and 0.078 μg ml $^{-1}$. A microbial suspension was prepared with turbidity equivalent to the tube 0.5 of the McFarland scale (1 x 10^8 UFC unit-forming colony ml $^{-1}$). Next, the 1:10 bacterial suspension was diluted with Mueller-Hinton broth to obtain 1 x 10^4 UFC ml $^{-1}$ as inoculums. Similarly, the 1:40 bacterial suspension was diluted 1:20 in Mueller-Hinton broth. The suspensions (100 μL) were inoculated in triplicate to each well containing the EOs concentrations. A mix solution of tetracycline and ampicilline was used as positive control. The combination of these two antibiotic has a wide range of antimicrobial activity against Gram-positive and Gram-negative bacteria. Then, 1 mL of each concentration of EOs was mixed with 1.0 ml of bacteria cell at 10^4 CFU ml $^{-1}$ concentrations. Tubes were incubated for 24 h at 37 °C. Finally, the MIC values were estimated measuring the absorbance of microtiter plates at 570 nm. Moreover, the lowest concentration at which no turbidity was observed was considered the MIC value. Three independent experiments were performed for each MIC value.

2.8. Antioxidant activity of Basil EOs

The free radical scavenging activity of the essential oils was carried out using DPPH (1,1-diphenyl-2- picrylhydrazyl) assay as described in *Beatovic et al., (2015)*. The antiradical capacity of

each essential oil was evaluated using a serial dilution of the sample, in order to obtain a large spectrum of sample concentrations. Essential oils were dissolved in methanol (500 μL) at final concentration of 1 mg/mL and mixed with 500 μL of a 150 μM DPPH solution. The resulting mixture (1 ml) was shaken vigorously and held for 20 minutes at room temperature in the dark. The absorbance of the samples was measured spectrophotometrically at 517 nm. All tests were performed in triplicate, with Trolox as positive controls. The percentage of inhibition was calculated using the following equation:

% Inhibition = $[(A_0-A_1)/A_0] \times 100$

where A_0 is the absorbance of the control solution (blank without samples) and A_1 is the absorbance of the samples.

2.9. Statistical analysis

All results are expressed as means ± SE (standard error). Data obtained from this study were processed using XLSTAT software. The effects of the treatments were tested by one-way analysis of variance (ANOVA) and the means (n=3) were tested appling the least-test difference (LSD) test at the 0.05 significance level.using XLSTAT software.

3. Results and Discussion

3.1 Molecular characterization of humic substances-artichoke

The ^{13}C-CPMAS-NMR spectrum of HS-artichoke is shown in **Figure 1**, while the **Table 1** reports the relative carbon distribution of main functional groups over chemical shift regions (*Spaccini et al., 2016, 2019*). The NMR data of HS indicated a predominance of aromatic carbons (110-145 ppm) followed by O-alkyl carbons (60–110 ppm) which relative amounts accounted for the

35 and 25 % of total signal area, respectively (**Table 1**).The former region of NMR spectra includes the alkyl-C resonances (0-45 ppm), associated to the presence of aliphatic chains (-CH_2- groups) pertaining to various lipid compounds, such as fatty acids, plant waxes and bio-polyesters. The most prominent peak at 33 ppm, is mainly due to the bulk methylene segments of different molecules with specific structural conformation and rigidity such as wax and cutin components, while the shoulder around 24 ppm may indicate the CH_2 groups in γ position or in the proximity of alcoholic portion in aliphatic esters. The less intense but evident broader signals extended around 45-50 ppm suggested the inclusion of various methyl and methylene carbons in of branched short chain alkyl components, such as amino acids and oligopeptides (*Pane et al., 2016*).The sharp intense signal centred around 56 ppm (**Figure 1**) combine the significant inclusion of both methoxyl substituent on the aromatic rings of guaiacyl and syringyl units in lignin components, as well as the C-N bonds in amino acid moieties (*Monda et al., 2018; Spaccini et al., 2019*). The different peaks in the O-alkyl-C chemical shift region (60-110 ppm) derive mainly from the plant carbohydrates and are conventionally assigned to monomeric units of oligo and polysaccharide chains of cellulose and hemicellulose components of plant tissues (*Spaccini and Piccolo, 2009*). The signals at 61/62 ppm represent the out-of-plane carbon nucleus in position 6, followed by the intense coalescence around 73 ppm formed by the overlapping resonances of carbon 2, 3, and 5, in pyranoside structure. The shoulders at 82 ppm derive from the carbon 4 involved in the glycosidic bond with the most deshielded di-O-alkyl anomeric carbon at 103/5 ppm corresponding to the position 1 of glucose units. The broad bands extended along the aryl-C interval (116-140 ppm) involve the un-substituted and C-substituted phenyl units of different aromatic components, while the signals shown in the phenolic region (140-160 ppm) are indicative of O-bearing hydroxy and methoxy ring C in polyphenol compounds and lignin molecules, which carbon in position 3 and 5 in the aromatic ring of lignin derivatives, are coupled to the signal related to methoxyl substituents (*Spaccini et al., 2016; Monda et al., 2018*). Finally, the sharp signal at 174 ppm include all carbonyl and carboxyl groups of different components (aliphatic acids, amino acid moieties, etc). Aromatic region between 110–145 ppm is related to the contributions of non-

substituted and C-substituted phenyl carbons. The contribution of specific organic components in the molecular composition of HS may be highlighted by the calculation of the dimensionless structural parameters, such as aromaticity (ARM) and hydrophobic (HB/HI) indexes and alkyl (A/OA), and lignin (LigR) ratios (**Table 1**). The value of HB/HI index, >1, found in HS-artichoke, revealed a significant incorporation in the humic supramolecular structure of hydrophobic molecules; the corresponding significant relative abundance of aromatic compounds, shown by the ARM parameter, suggested the prevalent contribution of aromatic molecules (**Table 1**). Conversely, the lower value of A/OA ratio indicated the predominance of polar O-alkyl groups as compared to apolar alkyl components. The presence of aromatic units was further elucidated by the calculation of the LigR ratio (**Table 1**). In fact, the average value found in HS may be currently related to the prevalent incorporation of lignin units in the spectral regions associated with both the methoxyl groups and O-aryl-C (*Martinez-Balmori et al., 2014; de Aquino et al., 2019*).

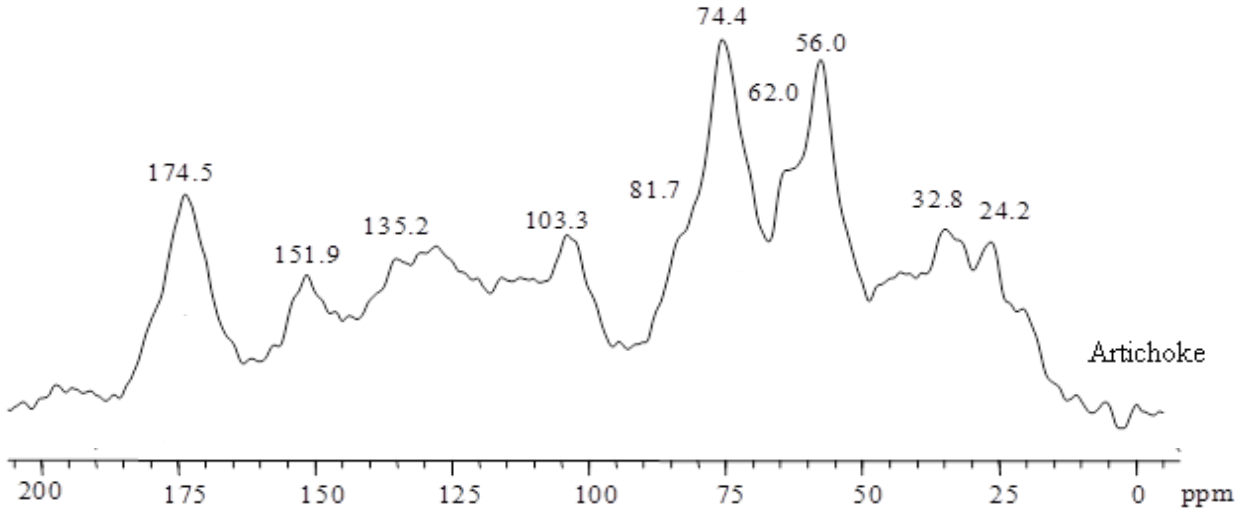

Figure 1: ^{13}C-CPMAS-NMR spectrum of humic substances.

Table 1: Relative distribution (%) of main C structures over chemical shift regions (ppm) and structural indexes[a] of different green compost teas as measured using ^{13}C-CPMAS-NMR spectrum of HS-artichoke**.**

	Carbonyl (190-160)	**O-Aryl-C (160-145)**	**Aromatic-C (145-110)**	**O-Alkyl-C (110-60)**	**CH_3O/CN (60-45)**	**Alkyl-C (45-0)**	**[a] A/OA**	**[b] ARM**	**[c] HB/HI**	**[d] LigR**
HS Artichoke	10.6	5.6	28.9	24.7	13.8	16.3	0.65	0.83	1.03	2.5

[a] HB/HI=hydrophobicity index=[Σ(0–45) +(45–60)/2 + (110–160)/ Σ(45–60)/2 + (60–110) + (160–190)]; [b] ARM = aromaticity index [(110–160)/Σ(0–45) + (60–110)]; [c] A/OA=alkyl/O-alkylratio (0–45)/(60–110); [d] LigR = Lignin ratio (45–60)/(145–160).

The total ion chromatograms (TIC) derived from the thermochemolysis of HS-artichoke is shown in **Figure 2** while the list of most relevant detected compounds and their quantitative estimation are reported in **Tables 2** and **3**, respectively. The thermochemolysis of HS sample released a large range of alkyl and aryl molecules which were primarily identified as methyl ethers and esters of natural compounds of main plant and microbial origin (**Table 2**). Compared to the results of NMR analyses, reduced levels of carbohydrates were observed among HS pyrolysis results. The lack of mono, oligo and polysaccharide components has previously been reported in pyrolytic products of plant woody tissues, soil organic matter and organic biomass (*Spaccini et al.,2013; de Aquino et al., 2019*). This lower performance is explained by the limited efficiency of thermochemolysis in the feasible detection of saccharide components in complex matrices. The thermal behaviour and pyrolytic rearrangement of polyhydroxy compounds combined with TMAH reaction conditions are estimated to influence negatively the determination of carbohydrates (*Spaccini et al., 2019*). The most common compounds were lignin components, followed by straight-chain fatty acids and lipid metabolites of microbial cells and by-products (**Tables 2, 3**). The most abundant lignin monomers were the oxidized forms of di- and tri-methoxyphenyl-propane molecules such as benzaldehydes (G4, S4) and benzoic acids (G6, S6) derivatives (**Table 2**). Other important and conventional products found in the thermochemolysis analyses of lignin were the cis and trans isomers of 1-(3,4-dimethoxyphenyl)-2-methoxyethylene (G7, G8) and 1-(3,4,5-trimethoxyphenyl)-2-methoxyethylene (S7, S8), as well as the enantiomers of 1-(3,4-dimethoxyphenyl)-1,2,3-trimethoxypropane (G14 and G15), and 1-(3,4,5-trimethoxyphenyl)-1,2,3-trimethoxypropane (S14 and S15). The 3-(4-methoxyphenyl)-2-propenoic acid (P18) was the most abundant P product, which may have resulted from both the oxidation of p-hydroxyphenyl units in lignin as well as from the transesterification of aromatic domains of suberin biopolymers in plant woody tissues. The presence of oxidized lignin units allows to determine dimensionless structural indices currently associated with the decomposition of lignin residues (*Spaccini et al., 2013; de Aquino er al., 2019*). In fact, the aldehyde and acid forms of guaiacyl and syringyl structures are related to the progressive oxidation of corresponding original monomers.

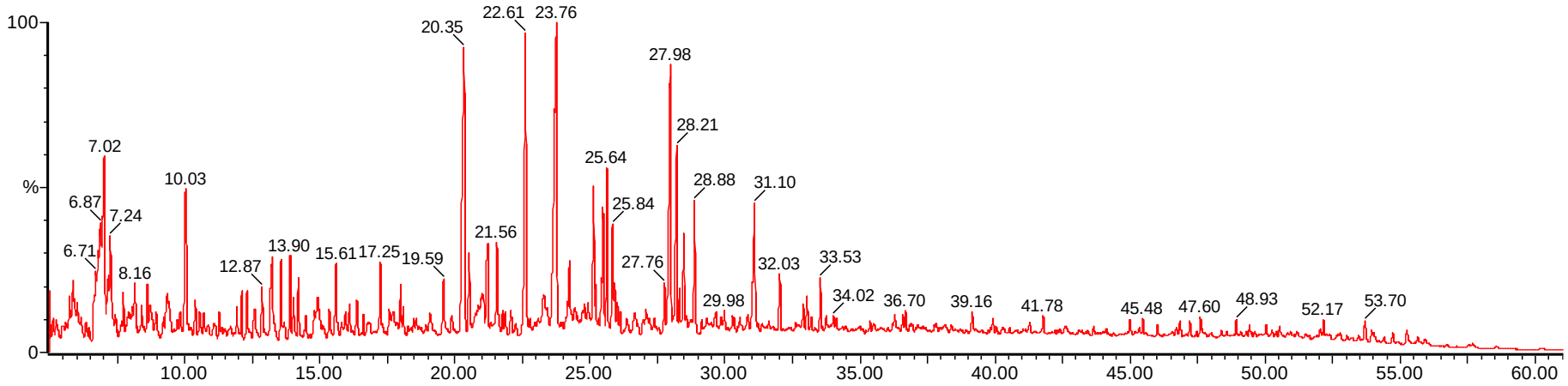

Figure 2: Total ion chromatograms of thermochemolysis products respectively of HS-artichoke.

Table 2: List of the main products released by HS-artichoke thermochemolysis.

RT	Compound	Origin
7.01	Phenol	phenol
7.24	Methoxy Benzene	Lig P1
7.25	Benzene, 1-Ethenyl-4-Methoxy-	Lig P3
7.26	Benzene, 1,2-Dimethoxy-	Lig G1
7.27	Dimethoxy Benzene	Carb
7.28	m/z 128	N
7.29	N derivative	N
7.30	N derivative	N
7.31	3,4-Dimethoxytoluene	Lig G2
7.32	Phenol, 2,6-Dimethoxy-	Carb
7.33	Benzaldehyde 4 Methoxy	Lig P4
7.34	1H-Indole, 1-Methyl-	N
7.35	1,2,3-Trimethoxybenzene	Lig S1
7.36	Ethanone, 1 (4 Methoxy Phenyl)	Lig P5
7.37	m/z 128	N
7.38	m/z 98	N
7.39	Dimethoxy Phenol	Carb
7.40	Benzene, 4-Ethenyl-1,2-Dimethoxy-	Lig G3
7.41	1,2,4-Trimethoxybenzene	Carb
7.42	Benzoic Acid, 4-Methoxy-, Methyl Ester	Lig P6
7.24	2-Propenoic Acid, 3-Phenyl-, Methyl Ester, (E)-	lip-biop
7.25	Benzene, 1,2,3-Trimethoxy-5-Methyl-	Lig S2
7.26	m/z 128	N
7.27	m/z 128	N
7.28	2H-Indol-2-One, 1,3-Dihydro-1-Methyl-	N
7.29	1H-Indole, 5-Methoxy-2-Methyl-	N
7.30	N derivative	N
7.31	Benzaldehyde, 3,4-Dimethoxy-	Lig G4
7.32	Benzene, 1,2-Dimethoxy-4-(1-Propenyl)-	Lig G21
7.33	Isomer Of G7/8	Lig G7 -G8
7.34	Benzenepropanoic Acid, 4-Methoxy-, Methyl Ester	Lig P12
7.35	1,2,3,4-Tetramethoxybenzene	Carb
7.37	Ethanone, 1-(3,4-Dimethoxyphenyl)-	Lig G5
7.38	Benzoic Acid, 3,4-Dimethoxy-, Methyl Ester	Lig G6
7.39	Benzaldehyde, 3,4,5-Trimethoxy-	Lig S4
7.40	m/z 98	N
7.41	Cis-1-Methoxy-2-(3,4-Dimethoxyphenyl)Ethylene	Lig G7
7.42	Trans-1-Methoxy-2-(3,4-Dimethoxyphenyl)Ethylene	Lig G8
7.24	Trans-1-Methoxy-1-(3,4-Dimethoxyphenyl)-1-Propene	Lig G11
7.25	2-Propenoic Acid, 3-(4-Methoxyphenyl)-, Methyl Ester	Lig P18/biop
7.26	Ethanone, 1-(3,4,5-Trimethoxyphenyl)-	Lig S5
7.27	Benzoic Acid, 3,4,5-Trimethoxy-, Methyl Ester	Lig S6
7.28	trans-3-methoxy-1-(3,4-dimethoxyphenyl)-1-propene	Lig G13
7.29	cis-1-(3,4,5-Trimethoxyphenyl)-2-methoxyethylene	Lig S7
7.30	cis 2-Propenoic Acid, 3-(3,4-Dimethoxyphenyl)-, Methyl Ester	Lig G18/biop

7.31	C15 Iso Fame (C14, 13 Methyl, Fame)	mic
7.32	1,2,3-Trimethoxy-1-(3,4-Dimethoxyphenyl)Propane (threo/erythro)	Lig G14
7.33	C15 Ante Iso Fame (C14, 12 Methyl, Fame)	mic
7.34	trans-1-(3,4,5-Trimethoxyphenyl)-2-methoxyethylene	Lig S8
7.35	1,2,3-Trimethoxy-1-(3,4-Dimethoxyphenyl)Propane (Threo/Erythro)	Lig G15
7.36	Cis-1-(3,4,5-Trimethoxyphenyl)-1-Methoxy-1-Propene	Lig S10
7.37	Cis-1-(3,4-Dimethoxyphenyl)-1,3-Dimethoxy-1-Propene	Lig G16
7.38	Trans 2-Propenoic Acid, 3-(3,4-Dimethoxyphenyl)-, Methyl Ester	LigG18/biop
7.39	1,2,3-Trimethoxy-1-(3,4,5-Trimethoxyphenyl)Propane (Threo/Erythro)	Lig S14
7.40	C16:1 Fame	mic
7.41	1,2,3-Trimethoxy-1-(3,4,5-Trimethoxyphenyl)Propane (Threo/Erythro)	Lig S15
7.42	C16 Fame	lip
7.24	Cis-1-(3,4,5-Trimethoxyphenyl)-1,3-Dimethoxyprop-1-Ene	Lig S16
7.25	Trans 2-PROPENOIC ACID, 3-(3,4,5-Trimethoxyphenyl)-, METHYL ESTER	Lig S18
7.26	C17 Iso Fame (C16, 15 Methyl, Fame)	mic
7.27	C17 Ante Iso Fame (C16, 14 Methyl, Fame)	mic
7.28	Trans(3,4,5-Trimethoxyphenyl)-1,3-Dimethoxyprop-1-Ene	Lig S16
7.29	C18:1 Fame	lip
7.30	C18:1 Fame	lip
7.31	C18 Fame	lip
7.32	Isomer PODOCARP-7-EN 3 ONE Dimethyl (Resin Acid)	lip/terpenoid
7.33	Isomer PODOCARP-7-EN 3 ONE Dimethyl (Resin Acid)	lip/terpenoid
7.34	2-Acetyl-6-(Adamantyl-1)Naphthalene	sterol
7.35	C20 Fame	lip
7.36	N Derivative	N
7.37	N Derivative	N
7.38	N Derivative	N
7.39	Sterol	sterol
7.40	C22 Fame	lip
7.41	C23 fame	lip
7.42	3,3',4,4'-Tetramethoxystilbene	aromatic
7.24	C24 Fame	lip
7.25	Squalene	sterol
7.26	C26-Ome	alcohol
7.27	Aromatic	aromatic
7.28	Alkane	alkane
7.29	C26 Fame	lip
7.30	C24, 24-CH3O, Fame	lip
7.31	C28-CH3O	alcohol
7.32	C28 Fame	lip
7.33	Sterol	sterol
7.34	C30 Fame	lip
7.35	Triterpenol (Pentacyclcic)	sterol
7.36	Sterol	sterol

FAME fatty acid methyl ester, Lg lignin, P phydroxyphenyl, G guayacil, S syringyl, ME methyl ester, Mic microbial origin, RT retention time (min).

Table 3: Composition (%) of main thermochemolysis products released from HS

Compound	%
FAME	10.1
Lignin	65.1
Sterol	1.9
Carbohidrates	3.0
N derivatives	12.7
Ad/Al$_G$	5.77
Ad/Al$_S$	8.93

FAME fatty acid methyl ester, Mic microbial origin fatty acids, AD/AL$_G$ [G6/G4], AD/AL$_S$ [S6/S4**].**

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Therefore, the ratios of acid structures to the corresponding aldehydes (G6/G4, S6/S4) are useful indicators of the degree of lignin bioxidative transformation during composting process (*Martinez-Balmori et al., 2013; de Aquino et al., 2019*).The linear fatty acids were composed by the even chain carbon homologues, thereby suggesting the prevalent plant origin of these alkyl fraction (*Martinez-Balmory et al., 2013, 2014*) In line with the NMR data, the results of thermochemolysis showed larger content of aromatic molecules pertaining almost exclusively to the various monomers of lignin derivatives, whereas significant lower abundance was found for the linear fatty acids (**Table 3**). Moreover, the values obtained for the structural indexes clearly highlight the prevalence of oxidized forms, thus suggesting an intense degradation process of lignin biopolymer (**Table 3**). Notwithstanding with the thermal lability of polar nitrogen components a significant contribution of in the thermochemolysis product was shown by nitrogen derivatives (**Table 3**), which detection also confirm the indication obtained from NMR analyses of a suitable incorporation of peptide fragments and/or aminoacids in the compost extract (*Martinez-Balmori et al., 2013*).

3.2. Bioactivity of HS-artichoke on growth of Basil plants

The phenological effects on Basil of HS application of at different concentration on the plant growth are summarized in **Figures 3** and **4**. The results of pot experiments, showed a marked increase of plant fresh weight biomasses in soil treatments with HS-artichoke as compared to control samples, while less influence of HS addition was revealed by the analysis of clorophyll content (**Figure 4**). An almost linear dose-response effect was observed for the plant biomass with an increase in fresh biomass weight of 8%, 16% and 25% respectively following the application of increasing concentrations (10-50-100 g L^{-1}) of HS, respectively with the best performance at the maximum concentration of 100 g L^{-1}. A similar trend was also observed in the analyses of chlorophyll content which larger variability resulted in a statistically significant effect only for the total chlorophyll content of plants treated with HS-artichoke at 50 g L^{-1} (**Figure 4**). Although the mechanisms involved in the stimulatory effect of HS on plant growth have not been yet clearly elucidated the bioactive properties may be correlated to both their chemical composition and physical properties. Recent studies indicated the combined positive role of hydrophobic characteristic and conformational behavior of humic assembly associated to the aromatic and phenolic components of humic fractions as potential molecular bio-effectors (*Canellas et al., 2012; Vaccaro et al., 2015; de Aquino et al., 2019; Spaccini et al., 2019*). Humic substances can be depicted as supramolecular association of relatively small heterogeneous molecules self-assembled by dispersive forces into high pliable structures made up by hydrophobic and hydrophilic microenvironments in fluid dynamic equilibrium (*Piccolo et al., 2002, 2019*). In water solutions, the humic colloids may hence adopt variable conformational arrangements thereby displaying a wide array of molecular interactions and exchange reactions between dissolved humic structures and root-plant system. In fact, it is the release of bioactive molecules from the humic supramolecular associations in the rhizosphere system that confers the plant bio-stimulation capacity to the humic substances from composts (*Canellas and*

Olivares, 2014; Canellas et al., 2015; Olaetxea et al., 2018). The dynamic equilibrium of contiguous hydrophilic and hydrophobic domains of HS conformations, as controlled by the soil/plant conditions.

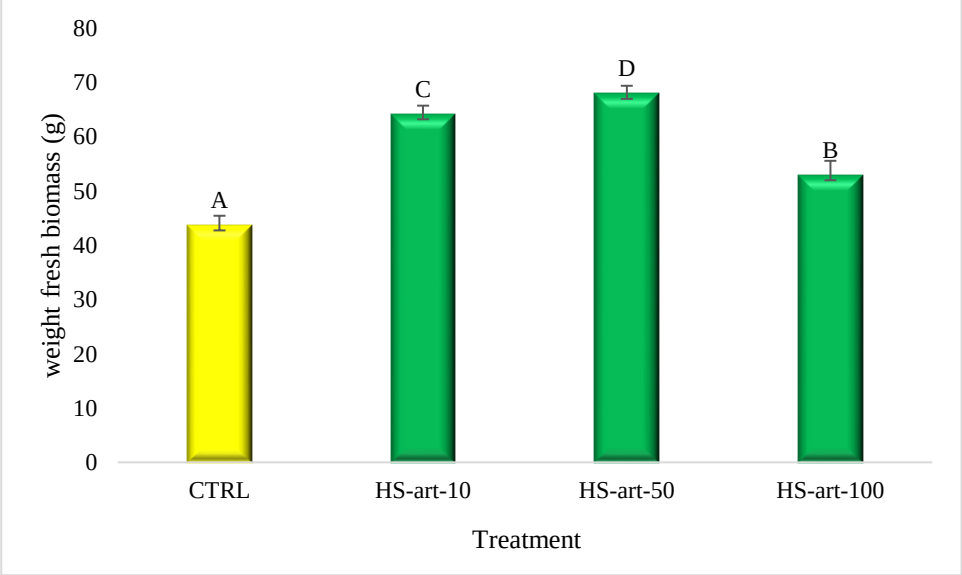

Figure 3: Effect of humic substances (artichoke) at different concentration (g L^{-1}) on weight fresh biomass of *Basil* plants. Vertical bars represent the standard deviation of the mean. Columns (mean ± S.D.) followed by different letters indicate significant difference according to LSD test ($p \leq 0.05$).

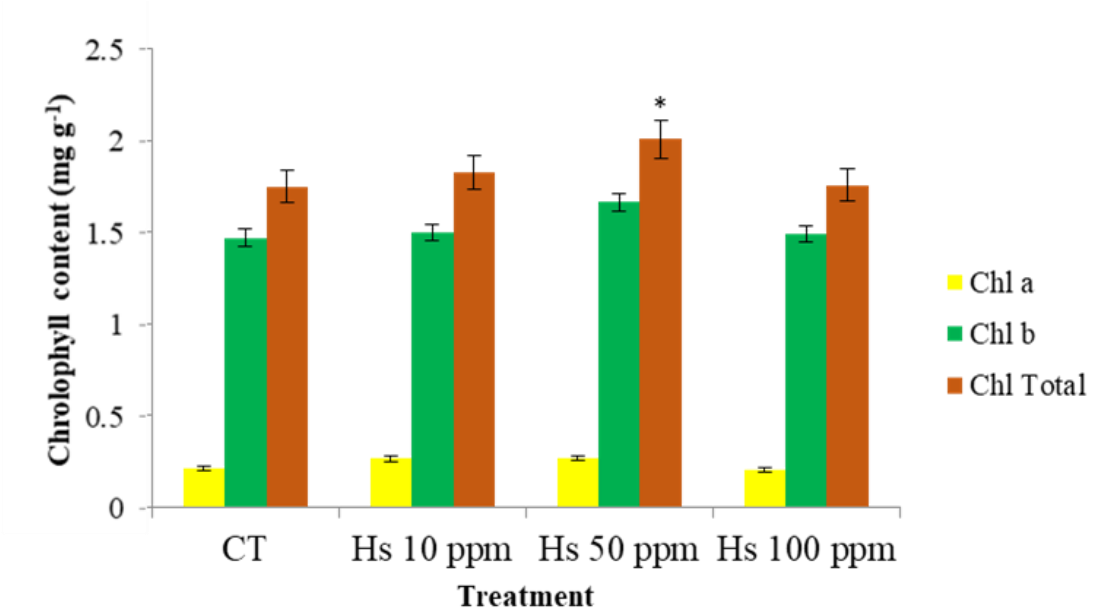

Figure 4: Effects of HS artichoke at different concentration on chlorophyll content of *Basil* plants. Columns (mean ± S.D.) followed by * indicate significant difference related to total choroplyll content according to LSD test ($p \leq 0.05$).

(pH, moisture, ionic strength, root exudates, active mineral surfaces, etc.), regulates the release and mobility of small bioactive molecules (*Monda et al., 2018; de Aquino et al., 2019*).

In the rhizosphere environments, the low aqueous solubility and the large surface tension triggered by the hydrophobic humic components, foster the interaction with the solid root systems. In this rhizo-plane the large concentration of root exudates (inorganic ions, organic acids, siderophores, amino acids, etc.) increase the chance of physical-chemical interactions with the weakly bound supramolecular structures (*Nardi et al., 2017*).The dynamic and flexible humic conformation, may hence undergo a favorable thermodynamic rearrangement with the subsequent possible release of retained polar bioavailable molecules that can unfold the bio-active properties in the close proximity of root membranes (*Nardi et al., 2017; Olaetxea et al., 2018*). In this respect, besides the hydrophobic character, specific biostimulation activities of HA from vermicompost have been correlated with the delivery of either soluble aromatic lignin components or peptidic moieties which are believed to promote hormone-like activities (*Martinez-Balmori et al., 2014; Scaglia et al., 2016; Canellas and Olivares 2017; Nardi et al., 2017).* The results HS characterization and of the pot experiments with addition of HS confirmed the role played by both the hydrophobic character and the presence of easily releasable oxidized lignin derivatives and nitrogen moieties in stimulating plant growth.

3.3. Effect of HS on Basil Essential oils (EOs) production

The Essential oils are a complex mixture of terpenes, sesquiterpenes, oxygenated derivatives and other aromatic compounds. Essential oils (EOs) isolated from *O. basilicum* may be classified in four major EO chemotypes of basil: (1) methyl chavicol-rich, (2) linalool-rich, (3) methyl eugenol-rich, (4) methyl cinnamate-rich, and also numerous subtypes (*Joshi et al., 2014*). Commonly, these substances are volatile and present at low concentrations (*Klimánkova et al., 2008*); moreover, they are extremely sensitive to heat and chemical conditions, and may thus undergo losses for thermal desorption or hydrolytic effect as degradation of esters and of unsaturated compounds (*Lucchesi et*

al., 2004). Several approaches have hence been used to improve the extraction yields and the recovery of essential oils, such as conventional methods (hydrodistillation, steam distillation, Soxhlet solvent extraction) and specific methodologies like supercritical fluid extraction (*Mahadagde et al., 2018*). In this respect the steam distillation is increasingly regarded as valuable methodology for the isolation of EOs (*Rassem et al., 2016*). Although specific organic molecules, such as monoterpenes may be more vulnerable to steam distillation conditions (*Diaz-Maroto et al., 2005*) this technique present suitable economic and environmental sustainability features, associated to high efficiency and good quality products, and thus become a reliable methodology for the modern industrial development of EOs in accordance to the principles of green extraction (*Li et al., 2014*).

The chromatogram related to the chemical characterization of EOs extracts obtained by steam distillation is shown in **Figure 5**. The twenty-nine compounds characterized and identified by GC–MS analyses, represented the 98.6% of the total oil components. As shown in **Table 4**, the identified extractives were associated to seven main groups based on chemical functional groups: aliphatic aldehydes, monoterpene hydrocarbons, aliphatic alcohols, oxygenated monoterpenes, monoterpenes esters, sesquiterpene hydrocarbons and oxygenated sesquiterpenes. The major constituents were eugenol (42.4%) and methyl eugenol (22.4%) followed by eucalyptol (6%), geranyl acetate (6.3%), methyl chavicol (5.4%), and trans-α-bergamotene (3%). *Avetisyan et al.* (*2017*) also found linalool (68%) and estragole (57.3%) as major compounds of the basil EOs. The application of HS did not produce appreciable qualitative changes in the composition of the EOs. Conversely, the application of humic substances exhibited a positive effect on the total EOs extraction yield. In particular the amount of EOs released from control was 0.34 mg g^{-1}, whereas the yield increased to 0.54 mg g^{-1} and 0.61 mg g^{-1} respectively, for HS treatments at 50 and 100 g/L concentration. The data of previous studies on basil plants showed that EO yields ranged from 0.2 to 0.4% (*Barcelos et al., 2013; Hazooumi et al., 2015; Avetisyan et al., 2017; Shiwakoti et al., 2017)*, which approximate the values found for control sample. As detailed by the semi-quantitative analysis reported in **Table 5,** the larger increase was found for the most representatives compounds, with a progressive higher increase of

relative abundance for eugenol, eucalyptol and geranyl acetate, following the application of HS at the concentration of 50 and 100 g L^{-1} compared to the control samples, while no effects were observed for the treatment 10 g L^{-1} (**Table 5**).

Previous studies have shown that the addition of humic acids and compost influences the production of essential oils from plants such as coriander (*Ram et al., 2003; Moslemi et al., 2012*). The specific method of action for compost and humic acid on the production of phenolic compound such as eugenol could be explained according to different primary metabolic pathways of carbon. Monoterpenes are generally synthesized from acetyl-COa through the mevalonate pathway. In contrast, eugenol is a constituent of the phenylpropanoid pathway bio-synthetized by phosphoenolpyruvate (PEP) and d-erythrosis-4-phosphate via the shikimate pathway. Indeed, the precursor necessary for the production of monoterpenes is diverted into the biochemical process of the shikimate pathway (*Dewick et al. 2009*). Therefore, the preferential synthesis of phenylpropanoids occurs at the expense of the biosynthesis of monoterpenes. The results on EOs extraction suggested that the treatments with HS had significant effects on phenylpropanoid derivates such as eugenol. According to *Verma et al. (2016),* HS can cause a partial oxidative phosphorylation in mitochondria, acting as plant growth regulators by increasing the biomass production and, consequently, the content of secondary metabolites, including EOs. Moreover, the application of humic materials revealed bioactive effects on the production of EOs from aromatic plants such as *Thymus vulgaris L. and* on essential oil percentage and oil constituents of Mint plant *(Hendawy et al. 2015; Noroozisharaf et al., 2018).* It has been claimed that the s increasing nutrient uptakes promoted by HS can induce a stimulation of enzyme activity and metabolism of essential oil production. Furthermore, the humic acid may activate the expression of the phenylalanine ammonialyase that catalyzes the first phase in the biosynthesis of hydroxycinnamic acids, by transforming tyrosine to *p*-coumaric acid and phenylalanine to trans-cinnamic acid. Also, the intermediates related to the shikimic pathway (flavonoids, some alkaloids such as isoquinoline alkaloids, tocopherols, and phenols) are influenced

by plant treatment with humic acid (*Schiavon et al., 2010*). In addition, an enzymatic system such as peroxidase and non-enzymatic antioxidant systems such as glutathione, ascorbate, phenols, alkaloids, carotenoids and tocopherols have been affected by treatments by humic materials (*Canellas et al., 2015; Pizzeghello et al., 2001*). A univocal structural-activity relationship for the bioactive properties of humic substances has not yet been clearly identified and different mechanism may be involved in the biostimulant activity (*Olaetxea et al., 2018; Piccolo et al., 2019*).

Although no specific studies are available on the relation between molecular composition of HS and EOs synthesis in aromatic plants, the chemical characterization of HS performed in this study obtained using advanced technique such as ^{13}C-CPMAS- NMR and THM-GC-MS, suggests that the positive effect carried out by HS on the yield and chemical composition of Basil EOs may be related to the hydrophobic wrapping system combined with the large content of of depolymerized and bio-available lignin derivatives and O-alkyl and nitrogen hydrophilic molecules

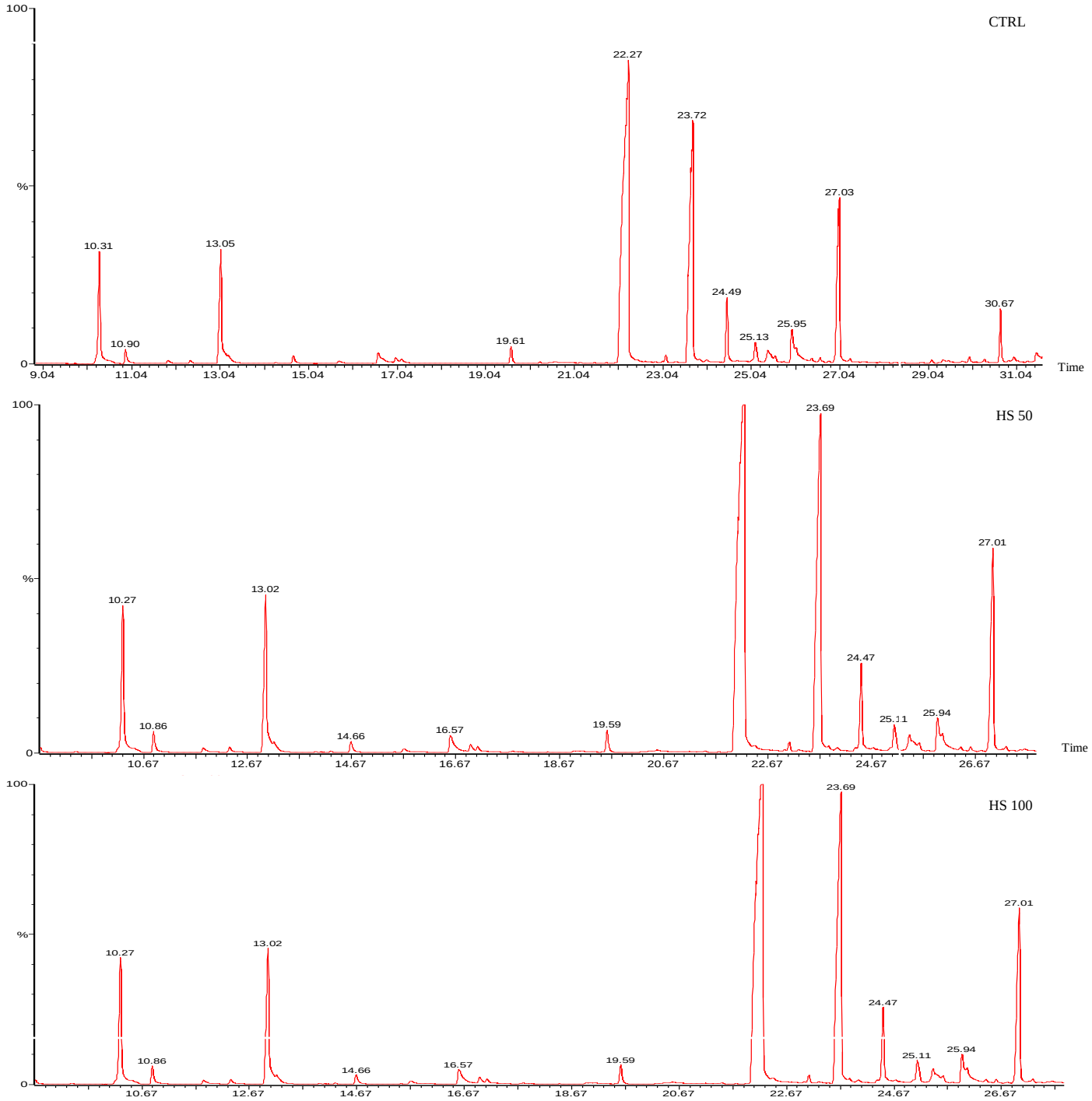

Figure 5: Gas chromatography-total ion chromatogram of Basil EOs extracted from plant treated with HS-artichoke at different concentration (0, 50, 100 g L^{-1}).

Table 4 : Chemical composition of *Ocimum basilicum* essential oils by GC-MS.

Retention time (Rt)	Compound	%
8.02	β-Phellandrene	0.01
8.21	Methyl-Cinnamate	0.15
8.56	1-Octenl-3-ol	0.2
8.74	β-Myrcene	0.23
9.42	Limonene	0.32
10.31	Eucalyptol	5.95
10.93	3-Carene	0.79
11.86	Estragole	0.13
13.05	methyl-Chavicol	0.1
14.69	Camphor	0.51
15.73	γ-Terpinene	0.27
14.71	β-Cubebene	0.51
16.61	β-Elemene	1.21
17.01	Cymene	0.17
17.02	α-Terpineol	0.02
19.61	Bornyl acetate	0.9
20.28	Geranic acid	0.07
21.18	Terpinen-4-ol	0.02
22.27	Eugenol	42.35
23.1	1,8-Cineole	0.35
23.67	Linalool	0.03
23.72	methyl-Eugenol	22.43
24.49	trans-α-Bergamotene	2.91
25.13	Caryophyllene	12.87
25.95	trans-α-Bergamotene	0.84
26.59	Azulene	0.12
27.03	Geranyl acetate	6.29
29.97	α-Cubebene	0.27
30.67	α-Cedrene	3.8

Table 5: Relative abundance (%) of main component EOs extracted from plants treated with different concentration of HS-artichoke (CTRL, 10, 50, 100 g L^{-1}).

Retention time (Rt)	Compound	% CTRL	% HS 10	% HS 50	% HS 100
10.31	Eucalyptol	5.95	6.01	6.53	7.24
13.05	methyl-Chavicol	5.40	5.35	5.76	6.98
22.27	Eugenol	42.35	42.40	43.01	45.09
23.72	methyl-Eugenol	22.43	22.57	22.98	24.01
24.49	trans-α-Bergamotene	2.91	2.89	3.01	4.09
27.03	Geranyl acetate	6.29	6.31	6.89	8.03

3.4. Influence of humic substances on Basil EOs antibacterial activity

The antibacterial activity of EOs obtained using steam distillation from plant treated with HS-artichoke and relative MIC values are reported in **Table 6**. The results indicate that the essential oils obtained from control plants showed moderate antibacterial activities, while the treatment with HS induced a progressive positive effect on antimicrobial propriety of these plant extracts in close relation with applied HS concentration. The comparison of EOs MIC value exhibited better performance against Gram-negative bacterial strains such as *Klebisella Pneumoniae* followed by *Escherichia coli* and *Pseudomonas aeruginosa*. Conversely, the analysis of Gram-positive bacteria response to application of EOs, revealed better results against *Enterococcus Faecalis* with a inhibition MIC value of 10 μg ml $^{-1}$ MIC in the case of treatment with HS. Moreover, the addition of HS at different concentrations induces a decrease of MIC values for all the bacterial strains examined, although the best antimicrobial proprieties was found for Gram negative bacteria cells with a MIC value of 1 and 4 μg ml $^{-1}$ for *Klebisella pneumoniae* and *Pseudomonas aeuruginosa*, respectively. In

the present study, we used Gram-negative bacteria which are considered, to be more resistant to essential oils than the Gram-positives (*Nazzaro et. al.. 2003*). This is largely attributed to the different structure of their cell wall which is more complex in Gram-negatives, not allowing the easy penetration of antibiotics and drugs, including the phenolic compounds such as eugenol present in the essential oils (*Trombetta et al., 2005; Tiwari et al., 2009*). The possible mechanism of action of the essential oils and their compounds is based on their ability to disrupt the bacterial cell wall and the cytoplasmic membrane that produces cell lysis and loss of intracellular compounds (*Lopez-Romero et al., 2015*).

Therefore, the subsequent changes in cell wall permeability and cytoplasmic membrane may affect the bacterial cell growth. Furthermore, essential oils exhibit the potential to change the permeability and the function of membrane proteins. Moreover, EOs can penetrate the phospholipid layer of the bacterial cell wall, bind to proteins and stop their normal functions due their phenolic nature (*Sakkas et al., 2016*)**.** In contrast with the results of *Morelli et al., (2017)*, which did not found a detectable effect of soil HS application nor in plant growth neither in the antibacterial activity of EOs, the results shown in this work suggest that humic substances influence the secondary metabolism of aromatic plants increasing the production of phenolic compounds, such as eugenol, which are involved in the antimicrobial activity of EOs. The different bioactive properties of humic substances may be hence related to the specific chemical composition and, as stressed in this study, depend on the structural properties combined with the possible release of aromatic compounds and polar molecules.

Table 6: Antimicrobial activity (MIC value) of EOs extracted from plants treated with different concentration of HS-artichoke (CTRL, 10, 50, 100 g L^{-1}). Assays were carried out by broth diluition method in Nutrient Broth. Replicates were from three independent experiments.

	MIC EOs (µg ml^{-1})											
Microrganisms tested	**CTRL**			HS 10 g L^{-1}			HS 50 g L^{-1}			HS 100 g L^{-1}		
	Replicates			Replicates			Replicates			Replicates		
Klebisella pneumonie ATCC700503	*3*	*3*	3	3	3	3	2	2	2	1	2	1
Escherichia coli ATCC35218	*9*	*7*	9	9	9	9	8	8	6	6	6	6
Pseudomonas aeruginosa ATCC27355	*9*	*9*	8	8	8	9	7	5	7	4	4	4
Staphylococcus aureus ATCC6538P	*18*	*18*	17	18	17	18	12	12	12	10	10	10
Enterococcus Faecalis ATCC29212	*10*	*10*	9	10	10	10	9	10	9	8	7	7

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3.5. Effect of humic substances on Antioxidant proprieties of Basil EOs.

The antioxidant activity of Basil EOs obtained from plant treated with different concentration of Humic substances is shown in **Figure 6**. Free radicals may cause many disease conditions such as heart diseases and cancer (*Pham-Huy et al., 2008*). Antioxidant activity is usually evaluated by the stable free radical DPPH assay that is an easy, rapid, and sensitive way to survey the scavenger activity of specific compounds or plant extracts. Commonly, antioxidants play an important role in scavenging reactive oxygen species (ROS) that appear during storage-related senescence of vegetables (*Hounsome et al., 2008*). Antioxidant capacity is directly correlated with phenolic compounds (*Koh et al., 2009*). In agreement with the results of antibacterial activity, EOs obtained from HS treated plants exhibited a higher antioxidant property, in respect to the extracts from control with an increase in inhibition percentage of 12 and 22 % for EOs obtained from plants treated with HS at 50 and 100 g L^{-1} respectively. The antioxidant activity of EOs cannot be attributed to a single chemical component but may be related to the quantity of phenolic components. (*Bhavaniramya et al., 2019*). To our knowledge the effect of humic substances on the antioxidant activity of essential oils extracted from basil plants has never been evaluated. In this study, this biological activity is apparently related to the chemical composition of the humic material and particularly to the aromatic component, as well as biostimulant effect and antibacterial performance.

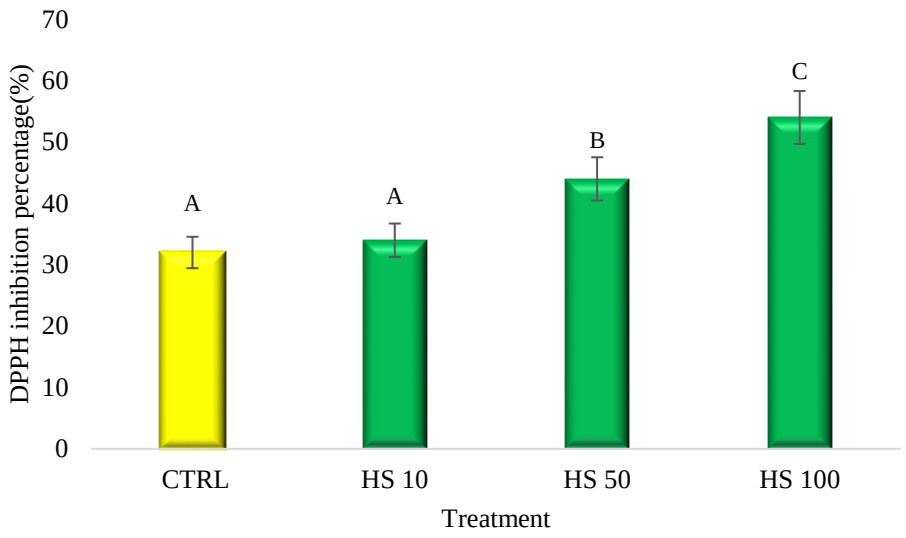

Figure 6: DPPH assay to determinate antioxidant activity of EOs obtained plants treated using different concentration of HS (CTRL, 10, 50 e 100 g L^{-1}). Vertical bars represent the standard deviation of the mean. Statistical analysis are performed using LSD test ($p \leq 0.05$). Different letters indicate significant differences between groups

Conclusions

Basil essential oils (EOs) are extensively used in nutraceutical and cosmetic fields due to their anti-inflammatory, antioxidant and antibacterial properties. In this study, humic substances obtained from on-farm composting of artichoke residues were used at different concentrations as treatment of Basil plants to evaluate the effects on the synthesis of essential oils (EOs) . The results showed that EOs obtained by steam distillation from Basil plants treated with increasing concentrations of HS contain a higher percentage of phenol compounds such as eugenol, one of the main constituents involved into nutraceutical activity of essential oils. In addition, essential oils obtained from plants treated with HS exhibit an increase of antibacterial performance against some bacterial strains

involved in to several common human diseases. This bio-stimulant activity may be related to chemical composition of humic substances and, particularly, to the content of aromatic compounds. The use of humic substances to treatment of aromatic plants to increase the production of essential oils opens the way for the application of these materials not only in the agro-food industry but also in the phytochemical industry.

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CHAPTER 7

Potential cytotoxic effect of different compost extracts on human cell cultures.

Abstract

Cancer is one of the most widespread diseases with a large impact on the worldwide sanitary services. Steady attention is devoted to find effective alternative treatments applying different plant-based therapeutic agents. Humic acid (HA) shows various pharmacological properties including anti-inflammatory and anti-proliferative effects. In our study, we examined the cytotoxic effects of natural organic derivatives from green compost (artichoke, coffee and pepper) such as humic substances and compost teas. These compounds were applied at increasing concentration at concentrations of 5, 10, 20, 50 and 100 μg ml $^{-1}$ on growth of HeLa (human cervical epithelial carcinoma cells) and CaCo-2 (human colorectal adenocarcinoma cells) cell cultures for 18, 24 and 48 hours. In detail, HS-coffee followed by HS-artichoke and CT-coffee at 100 μg ml $^{-1}$ exhibited a cytotoxic effect. The results of our study will shed light on the development of alternative therapeutic approaches in cancer treatment by evaluating the cytotoxic effect of compost extracts.

Keywords: compost extracts, HeLa cells, CaCo-2 cells, cytotoxic activity, MTT assay.

1. Introduction

Inflammation is a biological reaction of the immune system that can be activated by a combination of factors, including pathogens, damaged cells and toxic compounds (*Chen et al., 2018*). Autoimmune and cardiovascular diseases, neurodegenerative disorders as Alzheimer or cancer are some of the numerous conditions that provide a key role to the inflammatory process (*Van Rensburg, 2015*). A prophylactic control of inflammatory condition could be adopted as effective method to protect some predisposed candidates to prevent the evolution of these conditions (*Abou-Raya, and Abou-Raya, 2006*). Unfortunately, the use of non-steroidal anti-inflammatory agents may promote a side uncontrolled reaction and increase gastrointestinal toxicity risk as well as cardiovascular disease (*Varga et al., 2017*). Therefore, an increasing attention is devoted to the research of innovative products to reduce and mitigate the consequences of inflammatory processes. In this context, natural organic derivates such as humic substances (HS) and compost teas (CTs) found several applications in different pharmaceutical fields such as medicine and veterinary science due to the acknowledged antiviral, antimicrobial, profibrinolytic, anti-inflammatory and estrogenic activities (*Klöcking et al., 2005, Peña-Méndez et al., 2005*). Several studies on the medicinal properties of humic materials have already been reported (*van Rensburg et al., 2015*). The application of fulvic acids have shown the priorities to protect against cancer and related cancer–causing viruses (*Van Rensburg et al., 2002; Joone et al., 2003*) whereas humic acids, derived from peat extracts, have been used as a topical treatment of dermatitis and psoriasis, rheumatoid arthritis and eczema (*Wolina, 2009, Codish et al., 2005*). In addition, humic products showed a potential increase of T-cell immunity in patients infected with pulmonary tuberculosis and are effective in the treatment of hepatic diseases (*Schepetkinet al.,*

2002). The specific effect of humic materials on the mechanisms of inflammation has not yet been fully clarified. Previous investigation revealed that the humic derivates may act on either the release of cytokines or the activation of the superoxide dismutase mechanism, as well as support the migration and adhesion of inflammation-linked cells to damage sites (*Van Rensburg and Naude, 2009*). In particular, the release of tumour necrosis alpha factor (TNF-alpha) may be affected by the application of fulvic acid (*Junec et al., 2009*).

In addition, humic substances suppress the activation of the nuclear factor kappa B, stimulating the production of endothelial cells lipopolysaccharides (*Gau et al., 2000*). The activity of humic substances can also support the inhibition of complement system activation as well as the production of inflammation-related cytokines such as IL-1β, IL-6, IL-10 and TNF-α (*Van Rensburg & Naude, 2009; Joone et al., 2003*). The transition from the inflammatory to the proliferative phase represents a key step. Chronic inflammatory responses play an indispensable role in the development of inflammation-associated cancer, including *Helicobacter pylori* (*H. pylori*) associated gastric cancer, hepatitis virus-associated hepatocellular carcinoma, and colitis-associated colon cancer (*Chiba et al., 2002*). Some researchers have suggested that humic derivatives were able to reduce cell proliferation inducing apoptosis (*Yang et al., 2004*) and angiogenesis (*Aykac et al., 2018*). The use of the cell-based model is still the main method for in vitro drug efficacy screening to determine optimal drug dosage in cancer therapy (*Liang et al., 2017*).

Up to now, the most studied humic materials, as well as the most diffused commercial products, are those isolated from not renewable geochemical sources (e.g. peat, lignite, leonardite). However, the recent emphasis on sustainable approaches and on the development of circular economy should instead call for the application of bioactive humic components derived from renewable sources such as such as composts made of recycled biomasses from agro-food wastes, biorefinery residues, etc (*EC, 2017*). The aim of this study is the evaluation of cytotoxic effect of different organic derivates obtained from agricultural composted wastes (green composts) on human epithelial cells of colorectal

adeno-carcinoma (CaCo-2) and human cervical cancer cells (HeLa) by measuring cell viability with tetrazolium-based colorimetric MTT assay.

2. Materials and methods

2.1. Green composts and extraction of humic substances (HS) and compost teas (CTs)

The green composts used in this work were obtained in the on-farm composting facility of the Experimental Farm of University of Napoli Federico II at Castel-Volturno (CE). Three agricultural biomasses from vegetable crop residues, artichoke and pepper, and from industrial transformation, coffee pellets, were mixed with maize straw and woodchips from poplar trimming at 70/30 w/w ratio. The on-farm composting processes and the extraction methods for CTs and HS were performed as previously described in **Chapter 3 and Chapter 6**, respectively.

2.2. ^{13}C Cross-Polarization Magic-Angle-Spinning (CPMAS) NMR spectroscopy

The molecular composition of HS and CTs samples was performed by ^{13}C-CPMAS NMR analysis. The solid state NMR spectra of organic extracts were recorded on a Bruker AV-300 equipped with a 4 mm wide-bore MAS probe, with the following acquisition parameters: 13,000 Hz of rotor spin rate; 2 s of recycle time; 1H-power for CP 92.16 W: 1H 90° pulse 2.85 μs; 13C power for CP 150,4 W; 1 ms of contact time; 30 ms of acquisition time; 4000 scans. Samples were packed in 4 mm zirconium rotors with Kel-F caps. For the interpretation of ^{13}C-CPMAS-NMR spectra, the overall chemical shift range was divided into the following main resonance regions : alkyl-C (0–45 ppm); methoxyl-C and N-alkyl-C (45–60 ppm); O-alkyl-C (60–110 ppm); unsubstituted and alkyl-substituted aromatic-C (110–145 ppm); O- substituted aromatic-C (145-160 ppm); carboxyl- and carbonyl- C (160–200 ppm). The structural features of HS and CTs were summarized by the

following dimensionless structural parameters as described in previously **Chapters 3, 4 and 5**: Hydrophobic (HB) and Aromaticity (AR) index and Lignin Ratio (LR).

2.3. Human cells cultures

Two different types of human cells, HeLa (human cervical epithelioid cell carcinoma) and CaCo-2 (human colorectal adenocarcinoma cells) were obtained from the American Type Culture Collection (Manassas, VA, USA). Cells were cultivated in DMEM (Dulbecco's Modified Eagle Medium), supplemented with 10% FBS (Fetal bovine serum), 100 unit/mL of penicillin, and 100 unit/mL of streptomycin and 3.6 g/L $NaHCO_3$. Cells were routinely sub-cultured and propagated in a humidified CO_2 incubator (ShelLab, USA) at 5% CO2, 37°C and 95% humidity. The medium was replaced every 2–3 days.

2.4. Treatments and experimental design

Two different types of human cells HeLa and CaCo-2 were used to evaluate the cytotoxic effects of HS and CTs obtained from composted artichoke, coffee and pepper vegetable wastes. Cells were treated with different concentrations of humic derivates ranging from 5 to 100 μg ml $^{-1}$. After cell treatment, the cytotoxic activities of different compost exctracts were screened using 3-(4,5-dimethylthiazol-2-yl),5- biphenyl tetrazolium bromide (MTT). In addition, the morphological changes were assessed using phase contrast inverted microscope.

2.5. Cytotoxicity assay

The percentage of cell viability was evaluated using MTT (3-(4,5-dimethylthiazol-2-yl),5-biphenyl tetrazolium bromide) assay according to the method described by *Siddiqui et al. (2008)*. In detail, after trypsinization and washing, human cells were suspended and were used to inoculate 96 well culture plates at a final concentration of 10^4 cells/100 μl/ well. The plates were incubated at 5%

CO_2 and 37 °C to allow cell adherence. After 24h, compost extracts diluted in culture medium at five different concentration (5-10-20-50-100 μg ml $^{-1}$) were added to cells for MTT assay. Cultures cell without humic material was used as a positive control whereas DMEM Medium (Dulbecco's Modified Eagle Medium) without cells and compost extracts was a negative control. In addition, 10 μl of MTT reagent (5 mg ml $^{-1}$ in phosphate-buffered saline) were applied to each well, and the plates were further incubated for 4h. Then, 200 μl of Dimethyl sulfoxide (DMSO) were added to each well and mixed to dissolve the precipitated dark blue formazan crystals. The absorbance of formazan, directly proportional to the number of living cells, was measured at 550 nm using a microplate reader (Thermo Scientific, USA). The percentages of viability (%V) was calculated as following:

$$\%V=100(A_t/A_c)$$

where A_t and A_c are the absorbance for treated and control cells, respectively.

3. Results and discussion

In this study, two different lines cultural cells, such as HeLa and CaCo-2, have been treated with different green humic material at concentrations of 5, 10, 20, 50 and 100 μg ml $^{-1}$ for 18, 24 and 48 h. Cell viability was determined by MTT assay as described previously. All tested humic materials showed a clear dose-effect correlation on both cell lines used for cytotoxicity assay (**Figures 1, 2**). The results on CaCo-2 cells (**Figure 1A**) indicated a larger a cytotoxic activity for all HS at maximum dose (100 μg ml $^{-1}$) and longer incubation time (48h). The best response was found for the HS-coffee at 100 μg ml $^{-1}$) dose at 48h (**Figure 1A**) which exhibited a significant anti-proliferative effect (6%) also at lower concentration at different incubation times, 18h and 24h, but with lower efficiency (32 % and 24%). The lower cytotoxic effect for the HS treatment of CaCo-2 cells was shown by the HS-pepper, which however showed no significant decrease of cell growth in respect to control samples

(91%). The HS-artichoke revealed an intermediate suppressive activity at lower doses and progressive effectiveness (17 %), close to the data of HS-coffee with increasing concentration and maximum time incubation of 48 h (**Figure 1A**). Although an overall lower performance was shown for the treatments of CaCo-2 cells line with compost teas, all the applied extracts produced a significant decrease of proliferative cell development in respect to control (**Figure 1B**). The results of CTs samples showed a trend similar to the HS, highlighting a dose/time linear response and a larger anti-proliferative activity for coffee extract followed by CT-pepper and CT-artichoke at maximum dose applied (12%, 16 % and 19 % respectively) as reported in **Figure 1B**.

Conversely, in the case of HeLa cells the organic extract CT-pepper, CT-coffee and HS-artichoke showed no cytotoxic activity (**Figure 2A, 2B**) whereas HS-artichoke revealed a proliferation inhibition rate of 12% followed by HS-coffee and CT-coffee with 15 and 19%, respectively at same incubation condition (48h) and at maximum test concertation (**Figure 2**).

Our results indicate that the different cytotoxic activity of humic extracts is related to both the type of cell line used and the chemical characteristics of the organic materials. Previous studies, based on the use of synthetic humic acids, indicated a cytotoxic effect of HS on human breast adenocarcinoma MCF-7 cell line and HeLa cells (*Ting et al., 2010; Aykac et al., 2018*); however, the specific mechanisms of action of humic derivatives to suppress in vitro the cell proliferation, are still unknown. Recently, it has been reported that commercial humic acid can induce apoptosis in HL-60 (*Yang et al., 2004*) and HIT-T15 cells (*Yen et al., 2007*).

Apoptosis is a strongly regulated process that implies the activation of a series of molecular and biochemical events involved into the death of cells such as loss of cell viability, increase in the degree of DNA fragmentation and the activation of the caspase protein 3 (*Reed, 2000*). Many chemotherapeutic agents exert their antitumor effects by inducing apoptosis in cancer cells (*Kaufmann and Earnshaw, 2000*). Previously studies found that neoplastic transformation processes and the progression of metastases induce an alteration of the normal apoptotic pathway (*Su et al., 2005*). The relationship between the mechanism of carcinogenesis and apoptosis may explain the

effect of humic extracts in vitro, however up to date no investigation have been carried out on the possible role of the chemical composition of applied humic materials no to develop a valid hypothesis. No data are hence currently available on the structural-activity relationship between the molecular features of natural organic compounds and the pharmacological activity in vitro. The data presented here represent an innovative preliminary approach to evaluate the effect of different organic extracts from composted biomasses on tumoral cells. In this study, a variable activity on the proliferation of tumoral cells was found for the compost extracts with different chemical features.

The data reported in **Table 1**, show the relative amount of main functional groups over chemical shift regions found in HS and CTs samples, thus revealing for all the compost extracts a classical distribution of natural organic components of main plant origin. These includes the lipid compounds (0-45 ppm), methoxyl substituent of lignin derivatives combined with peptide moieties (45-60 ppm), the O-alkyl-C functions of simple carbohydrates oligo and polysaccharides (60-110 ppm) derived from cellulose and hemicellulose, the unsubstituted aromatic compounds (110-145 ppm) followed by the O-aryl-C molecules, such as lignin and polyphenols, and finally the carboxylic groups (160-190 ppm) of aliphatic acids, amino-acids and minor hemicellulose components. A possible attempt to relate the molecular characteristic of organic extracts to the antiproliferative activity may be based on the ascertained bioactive properties of humic substances and compost teas. In fact, a raising range of scientific researches have pointed out the occurrence of a large array of biological interaction of humic and water extracts from composted biomasses towards either plant development and microorganisms at molecular, physiological and transcriptional levels (*Trevisan et al., 2010; Canellas et al., 2011; Pane et al., 2014; Vaccaro et al., 2015*).

The main shared working hypothesis on the bioactive effect of these organic materials have highlighted the concomitant combination of conformational features and chemical composition on the interaction with plant and microbial cells (*Zancani et al., 2009, 2011; Canellas and Olivares et al., 2017).* In particular it was suggested a positive role of either aromatic and phenolic components or nitrogen derivatives and monosaccharides of humic and water dissolved fractions as potential

molecular bio-effectors (*Scaglia et al., 2016; de Aquino et al., 2019*; *Spaccini et al., 2019*). Moreover, the overall hydrophobic characteristics of organic fractions seem support a preferential vehiculation and preservation of bioactive compounds and their subsequent release upon the interaction with the cell membrane favored by the adhesion of apolar structures (*Dobbss et al., 2010;Canellas et al., 2012; Martinez-Balmori et al., 2014; Canellas and Olivares, 2017, Olaetxea et al., 2018*). In the present experiment the better response in the inhibition of CaCO-2 and He-La cells was shown by the more hydrophobic HS-coffee and HS-artichoke which were characterized by a large content of potential active phenolic and aromatic moieties, as indicated by the HB and AR index (**Table 1**). Moreover, the higher values of LR ratio of HS-coffee suggested the larger inclusion of nitrogen derivatives in the humic extracts (*de Aquino et al., 2019; Spaccini et al., 2019*).

Conversely, notwithstanding the significant content of aryl and O-aryl compounds, a lower hydrophobic character was shown by the less effective HS-pepper (**Figures 1A and 2A; Table 1**); furthermore the larger amount of long chain and rigid polysaccharides components (60-100 ppm) in this humic material may have reduced the conformational mobility of HS molecules thus preventing the suitable unfold of humic structure and the prompt release of bioactive compounds (*de Aquino et al., 2019; Spaccini et al., 2019).* A similar combination of molecular features and reactivity may be claimed for the data of cell treatments with the CT extracts of CaCo-2 line. In fact, the slight increase of hydrophobic due to the larger content of aromatic units found in CT-pepper in respect to corresponding HS (**Table 1**) were associated with an improvement of the overall antiproliferative reaction (**Figure 1B**). On the other hand, a concomitant decrease of hydrophobicity and aromatic compounds and a slight mitigation of suppressive effect were recorded for the application of CT-artichoke compared with the HS treatment, while the CT-coffee maintained both a chemical composition and inhibition potential similar to those of humic fractions (**Table 1**, **Figures 1 and 2**).

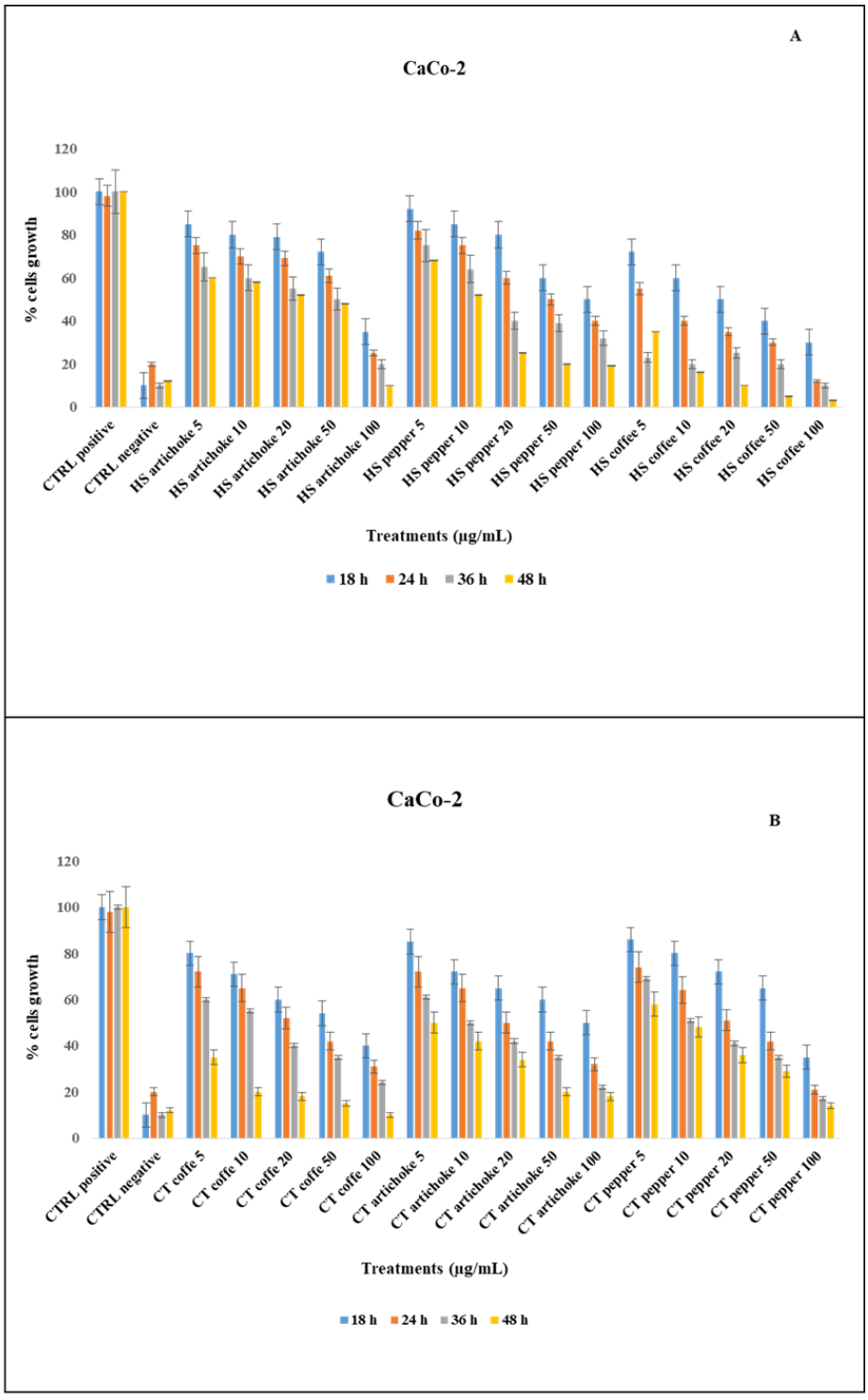

Figure 1: Effects of different humic derivates: HS (panel A) and CTs (panel B) on the viability of cervical cancer cells (HeLa). Cells viability was determined by the MTT assays. Vertical bars represent the standard deviation of the mean (n=2).The values (%) are expressed in relation to untreated control cells.

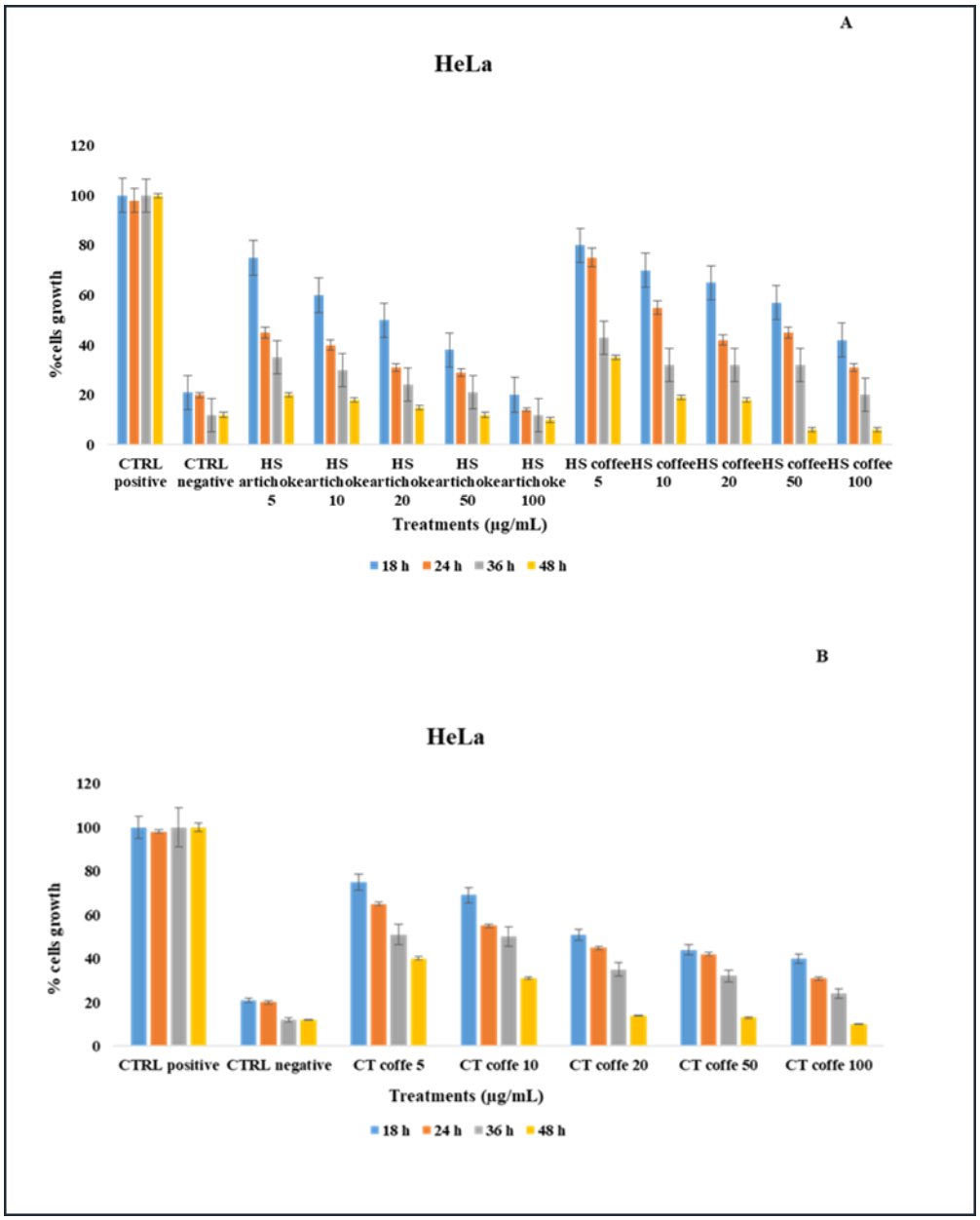

Figure 2 : Effects of different humic derivates: HS (panel A) and CTs (panel B) on the viability of human colorectal adenocarcinoma cells (CaCo-2). Cells viability was determined by the MTT assays. Vertical bars represent the standard deviation of the mean (n=2).The values (%) are expressed in relation to untreated control cells.

Table 1: Relative distribution (%) of signal area over chemical shift regions (ppm) in ^{13}C-CPMAS-NMR of artichoke, coffee and pepper humic erivates (HS and CTs).

	Carbonyl (190-160)	**O-Aryl-C (160-145)**	**Aromatic-C (145-110)**	**O-Alkyl-C (110-60)**	**CH_3O/CN (60-45)**	**Alkyl-C (45-0)**	**[a]HB**	**[b]ARM**	**[c]LigR**
HS Artichoke	10.6	5.6	28.9	24.7	13.8	16.3	1.4	34.5	2.5
HS Coffee	11.8	4.0	14.9	25.3	13.4	30.6	1.3	18.9	3.4
HS Pepper	11.0	5.0	15.7	33.5	15.0	20.8	0.9	20.7	3.0
CT Artichoke	13.9	4.2	15.0	27.3	12.1	27.6	1.1	19.2	2.9
CT coffee	13.9	4.3	16.6	26.4	11.9	26.9	1.2	20.9	2.8
CT pepper	12.3	5.8	19.2	31.1	14.3	17.4	1.0	25.0	2.5

a HB=hydrophobicity index=[Σ(0–45) + (45-60/2)+(110–160)/ Σ(45–60/2) + (60–110) + (160–190)]

b ARM = aromaticity index [(110–160)/Σ(0–190)] *100

c LigR = Lignin ratio (45–60)/(145–160)

Conclusions

Biomedical application of compost derivates concern their anti-viral, anti-inflammatory, anti-oxidant, anti-tumor and anti-toxin proprieties. In this work, we have measured cytotoxic activity of different compost extracts such as HS and CT on two different lines cells cultures: CaCO-2 and HeLa. In agreement with the available literature, our results indicated that the treatment of human cervical cancer HeLa or CaCO2 cells with HS or CTs, promoted the anti-proliferative action and tumor cell growth inhibition. Moreover, the effect was proportional to the applied concentration and time of incubation. Although the extension of bioactive properties on plant and microorganisms to biomedical application may be rather speculative, these novel approaches and initial results suggested that the different bioactivity of humic materials may be related to their structural and chemical composition. The present work provides a preliminary platform for further investigation of the possible mechanism and role of humic derivates on cancer cell lines. In this context, the further thoroughly investigation may hence promote the potential development of selected natural substances as a possible chemo-preventive or chemotherapeutic agent for the pharmaceutical industries.

GENERAL CONCLUSIONS

The use of recycled biomasses, residues and by-products, such as compost, is an effective way to conserve biological resources, and improve the productivity of agroecosystems and agri-food sectors. The use of green compost is one of the sustainable approaches in the development of the so-called circular economy as it involves an economic and environmental re-evaluation of agricultural biomass. Moreover, compost is a natural source of bioactive substances such as humic substances (HS) and compost teas (CTs). This thesis followed some different research lines that investigate the bio-activity of "green" compost extracts such as HS and CTs and its relation with chemical composition and molecular features. T

he first research lines focused on production, chemical characterization, evaluation of antioxidant and antimicrobial activity of Compost Teas extracted from three different vegetable biomasses such as artichoke, coffee and pepper.

The second research line involved the application of Humic substances and Compost teas extracted from artichoke and coffee composted biomasses on *Ocinum basilicum* plants to evaluate, using metabolomic and lipidomic approach, the effect on the biosynthesis of metabolites and lipids with nutraceutical application.

The third application consisted in the treatment on *Basil* plant with different concentration of HS-artichoke to evaluate the production of Essential oils (EOs) using steam distillation and their antioxidant and antibacterial activity.

Finally, the fourth research topic focused on the evaluation of cytotoxic activity of humic material on two different tumorigenic cells lines such as colorectal adenocarcinoma of the human epithelium (CaCo-2) and immortalized cells of cervical cancer cells (HeLa). In detail, chemical

characterization using advance technique such as^{13}C-CPMAS-NMR and THM-GC-MS showed overall hydrophobic features with larger content of bioavailable aromatic compounds in HS-artichoke and CT-coffee.

The combination of structural properties, molecular composition and conformational behavior of different organic fractions indicated the development of useful interactions with metabolic pathways and biochemical processes. A clear antibacterial effect against Gram-positive strains responsible for common human diseases such as *Staphylococcus Aureus* and *Enterococcus Faecalis,* was revealed by the applied CT samples. Although a best performance was observed against Gram-negative bacterial strains such as *Escherichia coli, Pseudomonas aeruginosa and Klebisella pneumonie*. These results suggest a possible induction of a conformational adaptation of CT components upon the interaction with the bacterial cell membrane that influence the CTs bioactivity. The cytotoxic effects of different compost extracts on two different tumorigenic cells lines (CaCo-2) and (HeLa) by MTT assay, showed an inhibition of cell proliferation with best performance in the case of HS-artichoke and CT-coffee. The application of humic and water extracts from artichoke and coffee based composts promoted a positive effect of Basil plant development, measured by phenological parameters such as fresh or dry biomass and chlorophyll content. Again, HS-artichoke and CT-Coffee have shown the larger positive effect at optimal concentration of 50 g L $^{-1}$. Metabolomic and lipidomic analyses carried out using Orbitrap technology on leaves of *Basil* plants treated with HS artichoke and CT-coffee at different concentrations (10-50-100 g L $^{-1}$), showed an increase of phenolic compounds content with nutraceutical activity including caffeic acid, rosmarinic acid and salicylic acid, p- coumaric acid, trans-cinnamic acid, naringenin, resveratrol and coenzyme Q-10. On the other hand, metabolomic and lipidomic approaches have shown that the treatment of aromatic plants with all compost materials affected plant metabolism increasing the production of secondary metabolites with nutraceutical, pharmacological and cosmetic activities suggesting a possible phytochemical application of these materials. Moreover, the encouraging results on the anti-

proliferative activity exhibited by humic materials on tested cell lines open the way for future applications of green materials in nutraceutical field.

REFERENCES

Abou-Raya, A., & Abou-Raya, S. (2006). Inflammation: A pivotal link between autoimmune diseases and atherosclerosis. *Autoimmunity Reviews*. https://doi.org/10.1016/j.autrev.2005.12.006

Akif Açıkgöz, M. (2019). Evaluation of Phytochemical Compositions and Biological Properties of Achillea gypsicola at Different Phenological Stages. Chemistry and Biodiversity. https://doi.org/10.1002/cbdv.201900373

Aeschbacher, M., Graf, C., Schwarzenbach, R. P., & Sander, M. (2012). Antioxidant properties of humic substances. *Environmental Science and Technology*. https://doi.org/10.1021/es300039h

Aguiar, N. O., Olivares, F. L., Novotny, E. H., Dobbss, L. B., Balmori, D. M., Santos-Júnior, L. G., … Canellas, L. P. (2013). Bioactivity of humic acids isolated from vermicomposts at different maturation stages. *Plant and Soil*. https://doi.org/10.1007/s11104-012-1277-5

Aguiar, N. O., Olivares, F. L., Novotny, E. H., & Canellas, L. P. (2018). Changes in metabolic profiling of sugarcane leaves induced by endophytic diazotrophic bacteria and humic acids. *PeerJ*. https://doi.org/10.7717/peerj.5445

Ameri, A., & Tehranifar, A. (2012). Effect of Humic Acid on Nutrient Uptake and Physiological Characteristic Fragaria ananassa var: Camarosa. *J. Biol. Environ. Sci*.

Aminifard, M. H., Aroiee, H., Azizi, M., Nemati, H., & Jaafar, H. Z. E. (2012). Effect of humic acid on antioxidant activities and fruit quality of hot pepper (Capsicum annuum L.). *Journal of Herbs, Spices and Medicinal Plants*. https://doi.org/10.1080/10496475.2012.713905

Andrade, R. P., & Casal, M. (2001). Expression of the lactate permease gene JEN1 from the yeast Saccharomyces cerevisiae. *Fungal Genetics and Biology*. https://doi.org/10.1006/fgbi.2001.1254

Anwar, J., Spanevello, R. M., Pimentel, V. C., Gutierres, J., Thomé, G., Cardoso, A., … Schetinger, M. R. C. (2013). Caffeic acid treatment alters the extracellular adenine nucleotide hydrolysis in platelets and lymphocytes of adult rats. *Food and Chemical Toxicology*. https://doi.org/10.1016/j.fct.2013.02.030

Avetisyan, A., Markosian, A., Petrosyan, M., Sahakyan, N., Babayan, A., Aloyan, S., & Trchounian, A. (2017). Chemical composition and some biological activities of the essential oils from basil Ocimum different cultivars. *BMC Complementary and Alternative Medicine*. https://doi.org/10.1186/s12906-017-1587-5

Avvakumova, N. P., Gerchikov, A. Y., Khairullina, V. R., & Zhdanova, A. V. (2011). Antioxidant properties of humic substances isolated from peloids. *Pharmaceutical Chemistry Journal*. https://doi.org/10.1007/s11094-011-0590-2

Aykac, A., Becer, E., Okcanoğlu, T. B., Güvenir, M., Süer, K., & Vatansever, S. (2018). The Cytotoxic Effects of Humic Acid on Human Breast Cancer Cells. *Proceedings*. https://doi.org/10.3390/proceedings2251565.

Babicki, S., Arndt, D., Marcu, A., Liang, Y., Grant, J. R., Maciejewski, A., & Wishart, D. S. (2016). Heatmapper: web-enabled heat mapping for all. *Nucleic Acids Research*. https://doi.org/10.1093/nar/gkw419

Bajda, A., Konopka-Postupolska, D., Krzymowska, M., Hennig, J., Skorupinska-Tudek, K., Surmacz, L., … Swiezewska, E. (2009). Role of polyisoprenoids in tobacco resistance against biotic stresses. Physiologia Plantarum. https://doi.org/10.1111/j.1399-3054.2009.01204.

Bampidis, V. A., Christodoulou, V., Florou-Paneri, P., Christaki, E., Chatzopoulou, P. S., Tsiligianni, T., & Spais, A. B. (2005). Effect of dietary dried oregano leaves on growth performance, carcase characteristics and serum cholesterol of female early maturing turkeys. *British Poultry Science*. https://doi.org/10.1080/00071660500256057

Barcelos, R. C., Jham, G. N., Dhingra, O. D., Mendonca, F. A., & M. Valente, V. M. (2013). Identification and Quantification of the Major Fungitoxic Components of the Brazilian Basil (Ocimum basilicum L.) Essential Oil. Journal of Food Research. https://doi.org/10.5539/jfr.v2n5p12.

Beatovic, D., Krstic-Miloševic, D., Trifunovic, S., Šiljegovic, J., Glamoclija, J., Ristic, M., & Jelacic, S. (2015). Chemical composition, antioxidant and antimicrobial activities of the essential oils of twelve Ocimum basilicum L. cultivars grown in Serbia. Records of Natural Products.

Bento, L.R., Melo, C.A., Ferreira, O.P., Moreira, A.B., Mounier, S., Piccolo, A., Spaccini, R., Bisinoti, M.C. (2020) Humic extracts of hydrochar and Amazonian Dark Earth: Molecular characteristics and effects on maize seed germination. Science of the Total Environment 708, Article number 135000 https://doi.org/10.1016/j.scitotenv.2019.135000

Bernards, M. A. (2010). Plant natural products: A primer. Canadian Journal of Zoology. https://doi.org/10.1139/Z10-035

Bernstein, N., Gorelick, J., Zerahia, R., Koch, S. 2019 Impact of N, P, K, and Humic Acid Supplementation on the Chemical Profile of Medical Cannabis (Cannabis sativa L). Frontiers in Plant Science Volume 10, Article number 736, doi.org/10.3389/fpls.2019.00736

Bhatt, R., Mishra, N., & Bansal, P. K. (2013). Phytochemical, Pharmacological And Pharmacokinetics Effects Of Rosmarinic Acid. Journal of Pharmaceutical and Scientific Innovation. https://doi.org/10.7897/2277-4572.02215

Bhavaniramya, S., Vishnupriya, S., Al-Aboody, M. S., Vijayakumar, R., & Baskaran, D. (2019). Role of essential oils in food safety: Antimicrobial and antioxidant applications. Grain & Oil Science and Technology. https://doi.org/10.1016/j.gaost.2019.03.001

Bino, R. J., Hall, R. D., Fiehn, O., Kopka, J., Saito, K., Draper, J., … Sumner, L. W. (2004). Potential of metabolomics as a functional genomics tool. *Trends in Plant Science*. https://doi.org/10.1016/j.tplants.2004.07.004

Bonanomi, G., Antignani, V., Pane, C., & Scala, F. (2007). Suppression of soilborne fungal diseases with organic amendments. *Journal of Plant Pathology*.

Botsoglou, N.A.; Taitzoglou, I.A.; Botsoglou, E.; Zervos, I.; Kokoli, A.; Christaki, E.; Nikolaidis, E. (2009) Effect of long-term dietary administration of oregano and rosemary on the antioxidant status of rat serum, liver, kidney and heart after carbon tetrachloride-induced oxidative stress. *J. Sci. Food Agric.* 89, 1397–1406.

Bourgaud, F., Gravot, A., Milesi, S., & Gontier, E. (2001). Production of plant secondary metabolites: A historical perspective. Plant Science. https://doi.org/10.1016/S0168-9452(01)00490-3

Bravo, L. (2009). Polyphenols: Chemistry, Dietary Sources, Metabolism, and Nutritional Significance. Nutrition Reviews. https://doi.org/10.1111/j.1753-4887.1998.tb01670.x

Brunow, G., (2001). Methods to reveal the structure of lignin. In: Hofrichter, M., Steinbüchel, A. (Eds.), Lignin. Humic Substances and Coal. Wiley-WCH, Weinheim,p. 89.

Canellas, L. P., Olivares, F. L., Okorokova-Façanha, A. L., Façanha, A. R. (2002). Humic acids isolated from earthworm compost enhance root elongation, lateral root emergence, and plasma membrane H+-ATPase activity in maize roots. Plant Physiology, 130(4), 1951-1957.

Canellas, L. P., Spaccini, R., Piccolo, A., Dobbss, L. B., Okorokova-Façanha, A. L., Santos, G. D. A., … Façanha, A. R. (2009). Relationships between chemical characteristics and root growth promotion of humic acids isolated from Brazilian oxisols. Soil Science. https://doi.org/10.1097/SS.0b013e3181bf1e03

Canellas, L. P., Piccolo, A., Dobbss, L. B., Spaccini, R., Olivares, F. L., Zandonadi, D. B., & Façanha, A. R. (2010). Chemical composition and bioactivity properties of size-fractions separated from a vermicompost humic acid. Chemosphere. https://doi.org/10.1016/j.chemosphere.2009.10.018

Canellas, L. P., Dantas, D. J., Aguiar, N. O., Peres, L. E. P., Zsögön, A., Olivares, F. L., … Piccolo, A. (2011). Probing the hormonal activity of fractionated molecular humic components in tomato auxin mutants. Annals of Applied Biology. https://doi.org/10.1111/j.1744-7348.2011.00487.x

Canellas, L. P., Dobbss, L. B., Oliveira, A. L., Chagas, J. G., Aguiar, N. O., Rumjanek, V. M., … Piccolo, A. (2012). Chemical properties of humic matter as related to induction of plant lateral roots. European Journal of Soil Science. https://doi.org/10.1111/j.1365-2389.2012.01439.x

Canellas, L. P., & Olivares, F. L. (2014). Physiological responses to humic substances as plant growth promoter. Chemical and Biological Technologies in Agriculture, 1(1), 1-11.

Canellas, L. P., Olivares, F. L., Aguiar, N. O., Jones, D. L., Nebbioso, A., Mazzei, P., & Piccolo, A. (2015). Humic and fulvic acids as biostimulants in horticulture. Scientia Horticulturae. https://doi.org/10.1016/j.scienta.2015.09.013

Canellas, L. P., & Olivares, F. L. (2017). Production of border cells and colonization of maize root tips by Herbaspirillum seropedicae are modulated by humic acid. *Plant and Soil*. https://doi.org/10.1007/s11104-017-3267-0

Canellas, L. P., Olivares, F. L., Canellas, N. O. A., Mazzei, P., & Piccolo, A. (2019). Humic acids increase the maize seedlings exudation yield. Chemical and Biological Technologies in Agriculture. https://doi.org/10.1186/s40538-018-0139-7

Caño-Delgado, A., Penfield, S., Smith, C., Catley, M., & Bevan, M. (2003). Reduced cellulose synthesis invokes lignification and defense responses in Arabidopsis thaliana. Plant Journal. https://doi.org/10.1046/j.1365-313X.2003.01729.x

Carballo, T., Gil, M. V., Gómez, X., González-Andrés, F., & Morán, A. (2008). Characterization of different compost extracts using Fourier-transform infrared spectroscopy (FTIR) and thermal analysis. Biodegradation, 19(6), 815–830. https://doi.org/10.1007/s10532-008-9184-4

Chemat, F., Vian, M. A., Ravi, H. K., Khadhraoui, B., Hilali, S., Perino, S., & Tixier, A. S. F. (2019). Review of alternative solvents for green extraction of food and natural products: Panorama, principles, applications and prospects. Molecules. https://doi.org/10.3390/molecules24163007

Chen, L., Deng, H., Cui, H., Fang, J., Zuo, Z., Deng, J., … Zhao, L. (2018). Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget*. https://doi.org/10.18632/oncotarget.23208

Chiba, T., Marusawa, H., & Ushijima, T. (2012). Inflammation-associated cancer development in digestive organs: Mechanisms and roles for genetic and epigenetic modulation. Gastroenterology. https://doi.org/10.1053/j.gastro.2012.07.009

Cho, A. S., Jeon, S. M., Kim, M. J., Yeo, J., Seo, K. Il, Choi, M. S., & Lee, M. K. (2010). Chlorogenic acid exhibits anti-obesity property and improves lipid metabolism in high-fat diet-induced-obese mice. Food and Chemical Toxicology. https://doi.org/10.1016/j.fct.2010.01.003

Chomel, M., Guittonny-Larchevêque, M., Fernandez, C., Gallet, C., DesRochers, A., Paré, D., … Baldy, V. (2016). Plant secondary metabolites: a key driver of litter decomposition and soil nutrient cycling. Journal of Ecology. https://doi.org/10.1111/1365-2745.12644

Chowdhary, K., Kumar, A., Sharma, S., Pathak, R., & Jangir, M. (2018). Ocimum sp.: Source of biorational pesticides. Industrial Crops and Products. https://doi.org/10.1016/j.indcrop.2018.05.068

Codish, S., Abu-Shakra, M., Flusser, D., Friger, M., & Sukenik, S. (2005). Mud compress therapy for the hands of patients with rheumatoid arthritis. Rheumatology International. https://doi.org/10.1007/s00296-003-0402-4

Cohen, J., (2001). World population in 2050: assessing the projections, Conference Series, Federal Reserve Bank of Boston, vol. 46.

Conte, P., Spaccini, R., & Piccolo, A. (2004). State of the art of CPMAS 13C-NMR spectroscopy applied to natural organic matter. *Progress in Nuclear Magnetic Resonance Spectroscopy*. https://doi.org/10.1016/j.pnmrs.2004.02.002

Cook, N. C.; Samman, S. (1996) Flavonoids-Chemistry, metabolism, cardioprotective effects, and dietary sources. *Nutr. Biochem.*, *7*, 66-76.

Costa, A., Drago, I., Behera, S., Zottini, M., Pizzo, P., Schroeder, J. I., … Schiavo, F. Lo. (2010). H2O2 in plant peroxisomes: An in vivo analysis uncovers a Ca2+-dependent scavenging system. *Plant Journal*. https://doi.org/10.1111/j.1365-313X.2010.04190.x

Couladis, M., Chinou, I. B., Tzakou, O., & Petrakis, P. V. (2003). Composition and antimicrobial activity of the essential oil of Hypericum rumeliacum subsp. apollinis (Boiss. & Heldr.). *Phytotherapy Research*. https://doi.org/10.1002/ptr.1093

Couladis, M.; Tzakou, O.; Verykokidou, E.; Harvala, C. (2003) Screening of some greek aromatic plants for antioxidant activity. *Phytother. Res*. 17, 194–195. 25.

Cozzolino, V., Di Meo, V., Monda, H., Spaccini, R., & Piccolo, A. (2016). The molecular characteristics of compost affect plant growth, arbuscular mycorrhizal fungi, and soil microbial community composition. Biology and Fertility of Soils. https://doi.org/10.1007/s00374-015-1046-8

Croteau, R., Kutchan, T. M., & Lewis, N. G. (2000). natural products (Secondary Metabolites). In biochemistry and molecular biology of plants. https://doi.org/10.1016/B978-0-08-088504-9.00018-0

Cueva, C., Mingo, S., Muñoz-González, I., Bustos, I., Requena, T., del Campo, R., … Moreno-Arribas, M. V. (2012). Antibacterial activity of wine phenolic compounds and oenological extracts against potential respiratory pathogens. Letters in Applied Microbiology. https://doi.org/10.1111/j.1472-765X.2012.03248.x

da Piedade Melo, A., Olivares, F. L., Médici, L. O., Torres-Neto, A., Dobbss, L. B., & Canellas, L. P. (2017). Mixed rhizobia and Herbaspirillum seropedicae inoculations with humic acid-like substances improve water-stress recovery in common beans. Chemical and Biological Technologies in Agriculture. https://doi.org/10.1186/s40538-017-0090-z

da Silva, J. D., Luz, A. I. R., da Silva, M. H. L., Andrade, E. H. A., Zoghbi, M. das G. B., & Maia, J. G. S. (2003). Essential oils of the leaves and stems of four Psidium spp. *Flavour and Fragrance Journal*. https://doi.org/10.1002/ffj.1219

de Aquino, A. M., Canellas, L. P., da Silva, A. P. S., Canellas, N. O., da S Lima, L., Olivares, F. L., … Spaccini, R. (2019). Evaluation of molecular properties of humic acids from vermicompost by 13 C-CPMAS-NMR spectroscopy and thermochemolysis–GC–MS. *Journal of Analytical and Applied Pyrolysis*. https://doi.org/10.1016/j.jaap.2019.104634

De Marco, A., Spaccini, R., Vittozzi, P., Esposito, F., Berg, B., De Santo, A.V. (2012) Decomposition of black locust and black pine leaf litter in two coeval forest stands on mount Vesuvius and dynamics of organic components assessed through proximate analysis and NMR spectroscopy. Soil Biol Biochem 51:1–15

Dewick, P. M. (2009). Medicinal Natural Products: A Biosynthetic Approach: Third Edition. In *Medicinal Natural Products: A Biosynthetic Approach: Third Edition*. https://doi.org/10.1002/9780470742761

Díaz-Maroto, M. C., Díaz-Maroto Hidalgo, I. J., Sánchez-Palomo, E., & Pérez-Coello, M. S. (2005). Volatile components and key odorants of fennel (Foeniculum vulgare Mill.) and thyme (Thymus vulgaris L.) oil extracts obtained by simultaneous distillation-extraction and supercritical fluid extraction. Journal of Agricultural and Food Chemistry. https://doi.org/10.1021/jf050340+

Dick, D. P.; Santos, J. H. Z. and Ferranti, E. M. (2003) Chemical characterization and infrared spectroscopy of soil organic matter from two southern brazilian soils. *Rev. Bras. Ciênc*. vol.27, n.1 pp.29-39.

Dionne, A., Tweddell, R. J., Antoun, H., & Avis, T. J. (2012). Effect of non-aerated compost teas on damping-off pathogens of tomato. Canadian Journal of Plant Pathology, 34(1), 51–57. https://doi.org/10.1080/07060661.2012.660195

Dobbss, L. B., Canellas, L. P., Olivares, F. L., Aguiar, N. O., Peres, L. E. P., Azevedo, M., … Façanha, A. R. (2010). Bioactivity of chemically transformed humic matter from vermicompost on plant root growth. *Journal of Agricultural and Food Chemistry*. https://doi.org/10.1021/jf904385c

du Jardin, P., (2012). The Science of Plant Biostimulants—A bibliographic analysis. Adhoc Study Report to the European Commission DG ENTR. 2012; http://ec.europa.eu/enterprise/sectors/chemicals/files/fertilizers/final report bio 2012en.pdf.

Dudonné, S., Vitrac, X., Coutiére, P., Woillez, M., & Mérillon, J. M. (2009). Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays. Journal of Agricultural and Food Chemistry. https://doi.org/10.1021/jf803011r

Duke, J. A. (2002). Handbook of Medicinal Herbs. In *Handbook of Medicinal Herbs*. https://doi.org/10.1201/9781420040463

Dunn, W. B., Bailey, N. J. C., & Johnson, H. E. (2005). Measuring the metabolome: Current analytical technologies. *Analyst*. https://doi.org/10.1039/b418288j

Efimova, I. V., Khil'ko, S. L., & Smirnova, O. V. (2012). Antioxidant activity of humic acids in radical-chain oxidation processes. Russian Journal of Applied Chemistry, 85(9), 1351–1354. https://doi.org/10.1134/S1070427212090091

El-Bassiouny, H. S. M., Bakry, B. A., El-Monem Attia, A. A., & Abd Allah, M. M. (2014). Physiological Role of Humic Acid and Nicotinamide on Improving Plant Growth, Yield, and Mineral Nutrient of Wheat (<i>Triticum durum</i>) Grown under Newly Reclaimed Sandy Soil. Agricultural Sciences. https://doi.org/10.4236/as.2014.58072

El-Gohary, A. E., El-Sherbeny, S. E., Ghazal, G. M. E. M., Khalid, K. A., & Hussein, M. S. (2014). Evaluation of Essential Oil and Monoterpenes of Peppermint (*Mentha piperita* L.) under Humic Acid with Foliar Nutrition. *Journal of Materials and Environmental Science, 5*, 1885-1890.

Elias, S. G., Copeland, L. O., McDonald, M. B., and Baalbaki, R. Z., (2012). Seed testing: principles and practices. Michigan State University Press: East Lansing.

Elsaiid, G., Abou zekry, S., Sarhan, E., El-Mokadem, M., & F., A.-W. (2018). In Vitro Suppressive Effect of Agriculture Residues and Municipal Solid Wastes Compost Tea on some Phytopathogenic Fungi. Journal of Scientific Research in Science. https://doi.org/10.21608/jsrs.2018.25679

Elsayed, A. I., Rafudeen, M. S., & Golldack, D. (2014). Physiological aspects of raffinose family oligosaccharides in plants: Protection against abiotic stress. Plant Biology. https://doi.org/10.1111/plb.12053

Ertani, A., Pizzeghello, D., Baglieri, A., Cadili, V., Tambone, F., Gennari, M., & Nardi, S. (2013). Humic-like substances from agro-industrial residues affect growth and nitrogen assimilation in maize (Zea mays L.) plantlets. Journal of Geochemical Exploration. https://doi.org/10.1016/j.gexplo.2012.10.001

Escarpa, A. and González, M.C. (2001) Approach to the Content of Total Extractable Phenolic Compounds from Different Food Samples by Comparison of Chromatographic and Spectrophotometric Methods. Analytica Chimica Acta, 427, 119-127.

Eudoxie, G., & Martin, M. (2019). Compost Tea Quality and Fertility. In *Organic Fertilizers - History, Production and Applications*. https://doi.org/10.5772/intechopen.86877

European Academy Science Advisory Council (EASAC policy report 36), (2018). Opportunities for soil sustainability in Europe. © German National Academy ISBN 978-3-8047-3898-0.

European Commission, (2010) (Turbé A., De Toni A., Benito P., Lavelle P., Lavelle P., Ruiz N., Van der Putten W.H., Labouze E., Mudgal S.) Soil biodiversity: functions, threats and tools for policy makers. Bio Intelligence Service, IRD, and NIOO, Report for European Commission (DG Environment)

European Commission, (2011) (Gobin A., Campling P., Janssen L., Desmet N., van Delden H., Hurkens J., Lavelle P., Berman S.) Soil organic matter management across the EU - best practices, constraints and trade-offs. Final Report for the European Commission's DG Environment

European Commission, (2017). Report from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions on the Implementation of the Circular Economy Action Plan – COM (2017) 33 final. http://ec.europa.eu/environment/circular-economy/implementation_report.pdf.

FAO WFP, IFAD (2012). The State of Food Insecurity in the World 2012. Economic growth is necessary but not sufficient to accelerate reduction of hunger and malnutrition. Rome, FAO.

FAO, (2017). Soil Organic Carbon: the hidden potential, Food and Agriculture Organization of the United Nations. Rome, Italy.Fernie.

Farag, M. A., Ezzat, S. M., Salama, M. M., & Tadros, M. G. (2016). Anti-acetylcholinesterase potential and metabolome classification of 4 Ocimum species as determined via UPLC/qTOF/MS and chemometric tools. Journal of Pharmaceutical and Biomedical Analysis. https://doi.org/10.1016/j.jpba.2016.03.037

Felgines, C., Texier, O., Morand, C., Manach, C., Scalbert, A., Régerat, F., & Rémésy, C. (2000). Bioavailability of the flavanone naringenin and its glycosides in rats. American Journal of Physiology - Gastrointestinal and Liver Physiology. https://doi.org/10.1152/ajpgi.2000.279.6.g1148

Fernie, A. R., Aharoni, A., Willmitzer, L., Stitt, M., Tohge, T., Kopka, J., … de Luca, V. (2011). Recommendations for reporting metabolite data. *Plant Cell*. https://doi.org/10.1105/tpc.111.086272

Fiehn, O. (2001). Combining genomics, metabolome analysis, and biochemical modelling to understand metabolic networks. Comparative and Functional Genomics. https://doi.org/10.1002/cfg.82

Fiehn, O. (2002). Metabolomics - The link between genotypes and phenotypes. *Plant Molecular Biology*. https://doi.org/10.1023/A:1013713905833

Fiehn O. (2003) Metabolic networks of Cucurbita maxima phloem. Phytochem.; 62: 875–886.

Filly, A., Fabiano-Tixier, A. S., Louis, C., Fernandez, X., & Chemat, F. (2016). Water as a green solvent combined with different techniques for extraction of essential oil from lavender flowers. Comptes Rendus Chimie. https://doi.org/10.1016/j.crci.2016.01.018

Flanigan, P. M., & Niemeyer, E. D. (2014). Effect of cultivar on phenolic levels, anthocyanin composition, and antioxidant properties in purple basil (Ocimum basilicum L.). Food Chemistry. https://doi.org/10.1016/j.foodchem.2014.05.061

Fouillen, L., Colsch, B., & Lessire, R. (2013). The lipid world concept of plant lipidomics. In Advances in Botanical Research. https://doi.org/10.1016/B978-0-12-397922-3.00007-1

Gabri, G., & Chialva, F. (1981). Qualitative evaluation of aromatic herbs by direct head space (GC)2 analysis. Methodology and some preliminary applications. Journal of High Resolution Chromatography. https://doi.org/10.1002/jhrc.1240040504

Gaio, I., Saggiorato, A. G., Treichel, H., Cichoski, A. J., Astolfi, V., Cardoso, R. I., … Cansian, R. L. (2015). Antibacterial activity of basil essential oil (Ocimum basilicum L.) in Italian-type sausage. Journal Fur Verbraucherschutz Und Lebensmittelsicherheit. https://doi.org/10.1007/s00003-015-0936-x

Gajula, D., Verghese, M., Boateng, J., Walker, L. T., Shackelford, L., Mentreddy, S. R., & Cedric, S. (2009). Determination of total phenolics, flavonoids and antioxidant and chemopreventive potential of basil (Ocimum basilicum L. and Ocimum tenuiflorum L.). International Journal of Cancer Research. https://doi.org/10.3923/ijcr.2009.130.143

García, A. C., Santos, L. A., Izquierdo, F. G., Rumjanek, V. M., Castro, R. N., dos Santos, F. S., … Berbara, R. L. L. (2014). Potentialities of vermicompost humic acids to alleviate water stress in rice plants (Oryza sativa L.). Journal of Geochemical Exploration. https://doi.org/10.1016/j.gexplo.2013.10.005

Garciá, A. C., De Souza, L. G. A., Pereira, M. G., Castro, R. N., Garciá-Mina, J. M., Zonta, E., … Berbara, R. L. L. (2016). Structure-Property-Function Relationship in Humic Substances to Explain the Biological Activity in Plants. Scientific Reports. https://doi.org/10.1038/srep20798

Ghasemi, K., Bolandnazar, S., Tabatabaei, S. J., Pirdashti, H., Arzanlou, M., Ebrahimzadeh, M. A., & Fathi, H. (2015). Antioxidant properties of garlic as affected by selenium and humic acid treatments. *New Zealand Journal of Crop and Horticultural Science*. https://doi.org/10.1080/01140671.2014.991743

Glauser, G., Boccard, J., Wolfender, J. L., & Rudaz, S. (2013). Metabolomics: Application in Plant Sciences. In *Metabolomics in Practice: Successful Strategies to Generate and Analyze Metabolic Data*. https://doi.org/10.1002/9783527655861.ch13

Gu, C., Howell, K., Dunshea, F. R., & Suleria, H. A. R. (2019). LC-ESI-QTOF/MS characterisation of phenolic acids and flavonoids in polyphenol-rich fruits and vegetables and their potential antioxidant activities. Antioxidants, 8(9), 1–15. https://doi.org/10.3390/antiox8090405

Haghighi, M., Mohmmadnia, S., & Pessarakli, M. (2016). Effects of mycorrhiza colonization on growth, root exudates, antioxidant activity and photosynthesis trait of cucumber grown in Johnson modified nutrient solution. *Journal of Plant Nutrition*. https://doi.org/10.1080/01904167.2016.1193604

Hargreaves, J., Adl, M. S., Warman, P. R., & Rupasinghe, H. P. V. (2008). The effects of organic amendments on mineral element uptake and fruit quality of raspberries. Plant and Soil. https://doi.org/10.1007/s11104-008-9621-5

Hargreaves, J. C., Adl, M. S., & Warman, P. R. (2009). Are compost teas an effective nutrient amendment in the cultivation of strawberries? Soil and plant tissue effects. Journal of the Science of Food and Agriculture. https://doi.org/10.1002/jsfa.3456

Hazzoumi, Z., Moustakime, Y., hassan Elharchli, E., & Joutei, K. A. (2015). Effect of arbuscular mycorrhizal fungi (AMF) and water stress on growth, phenolic compounds, glandular hairs, and yield of essential oil in basil (Ocimum gratissimum L). Chemical and Biological Technologies in Agriculture. https://doi.org/10.1186/s40538-015-0035-3

Hendawy, S. F., & Khalid, K. A. (2005). Response of sage (*Salvia officinalis* L.) plants to zinc application under different salinity levels. *Journal of Applied Sciences Research*. https://doi.org/10.1164/rccm.200406-788PP

Hendawy, S. F., Hussein, M. S., El-Gohary, A. E., & Ibrahim, M. E. (2015). Effect of Foliar Organic Fertilization on the Growth, Yield and Oil Content of Mentha Piperita Var. Citrata. Asian Journal of Agricultural Research, 9, 237-248. https://doi.org/10.3923/ajar.2015.237.248.

Hernandez, O. L., Calderín, A., Huelva, R., Martínez-Balmori, D., Guridi, F., Aguiar, N. O., … Canellas, L. P. (2014). Humic substances from vermicompost enhance urban lettuce production. Agronomy for Sustainable Development. https://doi.org/10.1007/s13593-014-0221-x

Hounsome, N., Hounsome, B., Tomos, D., & Edwards-Jones, G. (2008). Plant metabolites and nutritional quality of vegetables. Journal of Food Science. https://doi.org/10.1111/j.1750-3841.2008.00716.x

Huang, Z., Zeng, Z., Song, Z., Chen, A., Zeng, G., Xiao, R., He, K., Yuan, L., Li, H., Chen, G. (2020). Antimicrobial efficacy and mechanisms of silver nanoparticles against Phanerochaete chrysosporium in the presence of common electrolytes and humic acid. *Journal of Hazardous Materials 383*, Article number 121153

Ingham, E. (1999). Making a High Quality Compost Tea. BioCycle.

Jaiwal, P.K. (2006). Plant genetic engineering Vol. 7, Metabolic engineering and molecular farming-I. Houston, USA: Studium Press LLC, p. 1--361

Jansen Van Rensburg, C. E., & Naude, P. J. (2009). Potassium humate inhibits complement activation and the production of inflammatory cytokines in vitro. Inflammation. https://doi.org/10.1007/s10753-009-9130-6

Jayaprakasha, G.K. & Patil, B.S. (2007). In vitro evaluation of the antioxidant activities in fruit extracts from citron and blood orange. Food Chem., 101: 410-418.

Jindo, K., Soares, T. S., Peres, L. E. P., Azevedo, I. G., Aguiar, N. O., Mazzei, P., … Canellas, L. P. (2016). Phosphorus speciation and high-affinity transporters are influenced by humic substances. *Journal of Plant Nutrition and Soil Science*. https://doi.org/10.1002/jpln.201500228

Jooné, G. K., Dekker, J., & Van Rensburg, C. E. J. (2003). Investigation of the immunostimulatory properties of oxihumate. Zeitschrift Fur Naturforschung - Section C Journal of Biosciences. https://doi.org/10.1515/znc-2003-3-421

Jorge, T. F., Mata, A. T., & António, C. (2016). Mass spectrometry as a quantitative tool in plant metabolomics. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences*. https://doi.org/10.1098/rsta.2015.0370

Joshi, R. (2014). Chemical composition and antimicrobial activity of the essential oil of Ocimum basilicum L. (sweet basil) from Western Ghats of North West Karnataka, India. Ancient Science of Life. https://doi.org/10.4103/0257-7941.144618

Junek, R., Morrow, R., Schoenherr, J. I., Schubert, R., Kallmeyer, R., Phull, S., & Klöcking, R. (2009). Bimodal effect of humic acids on the LPS-induced TNF-α release from differentiated U937 cells. Phytomedicine. https://doi.org/10.1016/j.phymed.2008.10.003

Kabera, J. N., Semana, E., Mussa, A. R., & He, X. (2014). Plant Secondary Metabolites: Biosynthesis, Classification, Function and Pharmacological Properties. Journal of Pharmacy and Pharmacology. https://doi.org/10.1016/0300-9084(96)82199-7

Kadri, A., Gharsallah, N., Damak, M., & Gdoura, R. (2011). Chemical composition and in vitro antioxidant properties of essential oil of ricinus communis L. *Journal of Medicinal Plants Research*.

Kaufmann, S. H., & Earnshaw, W. C. (2000). Induction of apoptosis by cancer chemotherapy. Experimental Cell Research. https://doi.org/10.1006/excr.2000.4838

Kaurinovic, B., & Vastag, D. (2019). Flavonoids and Phenolic Acids as Potential Natural Antioxidants. In Antioxidants. https://doi.org/10.5772/intechopen.83731

Kavi Kishor, P. B., & Sreenivasulu, N. (2014). Is proline accumulation per se correlated with stress tolerance or is proline homeostasis a more critical issue? Plant, Cell and Environment. https://doi.org/10.1111/pce.12157

Khair-ul-Bariyah, S., Ahmed, D., & Ikram, M. (2012). Ocimum Basilicum: A Review on Phytochemical and Pharmacological Studies. *Pakistan Journal of Chemistry*. https://doi.org/10.15228/2012.v02.i02.p05

Khazaie, H. R., Rezaie, E. E., & Bannayan, M. (2011). Application times and concentration of humic acid impact on aboveground biomass and oil production of hyssop (*Hyssopus officinalis*). *Journal of Medicinal Plants Research, 5*, 5148-5154.

Kim, H. K., Choi, Y. H., & Verpoorte, R. (2010). NMR-based metabolomic analysis of plants. *Nature Protocols*. https://doi.org/10.1038/nprot.2009.237

Kim, M. J., Shim, C. K., Kim, Y. K., Hong, S. J., Park, J. H., Han, E. J., ….(2015). Effect of aerated compost tea on the growth promotion of lettuce, soybean, and sweet corn in organic cultivation. Plant Pathology Journal, 31(3), 259–268. https://doi.org/10.5423/PPJ.OA.02.2015.0024

Klessig, D. F., Tian, M., & Choi, H. W. (2016). Multiple targets of salicylic acid and its derivatives in plants and animals. Frontiers in Immunology. https://doi.org/10.3389/fimmu.2016.00206

Klimánková, E., Holadová, K., Hajšlová, J., Čajka, T., Poustka, J., & Koudela, M. (2008). Aroma profiles of five basil (Ocimum basilicum L.) cultivars grown under conventional and organic conditions. Food Chemistry. https://doi.org/10.1016/j.foodchem.2007.07.062

Klöcking, R., & Helbig, B. (2005). Humic Substances, Medical Aspects and Applications of. In Biopolymers Online. https://doi.org/10.1002/3527600035.bpol1013

Koca, N., & Karaman, Ş. (2015). The effects of plant growth regulators and l-phenylalanine on phenolic compounds of sweet basil. Food Chemistry. https://doi.org/10.1016/j.foodchem.2014.06.065

Koh, E., Wimalasiri, K. M. S., Chassy, A. W., & Mitchell, A. E. (2009). Content of ascorbic acid, quercetin, kaempferol and total phenolics in commercial broccoli. Journal of Food Composition and Analysis. https://doi.org/10.1016/j.jfca.2009.01.019

Kolodziej, E. P., Qu, S., Forsgren, K. L., Long, S. A., Gloer, J. B., Jones, G. D., … Cwiertny, D. M. (2013). Identification and environmental implications of photo-transformation products of trenbolone acetate metabolites. *Environmental Science and Technology*. https://doi.org/10.1021/es3052069

Kumar, J., & Gupta, P. K. (2008). Molecular approaches for improvement of medicinal and aromatic plants. Plant Biotechnology Reports. https://doi.org/10.1007/s11816-008-0059-2

Kwee, E. M., & Niemeyer, E. D. (2011). Variations in phenolic composition and antioxidant properties among 15 basil (Ocimum basilicum L.) cultivars. Food Chemistry. https://doi.org/10.1016/j.foodchem.2011.04.011

Lanthier, M., & Peters, S. (2012). Microbial content of actively aerated compost Tea after variations of ingredients or procedures. Acta Horticulturae. https://doi.org/10.17660/actahortic.2013.1009.26

Lawrence, B. M. (1988). A further examination of the variation of Ocimum basilicum L. *Developments in Food Science*.

Lee, S. J., Umano, K., Shibamoto, T., & Lee, K.-G. (2005). Identification of volatile components in basil (Ocimum basilicum L.) and thyme leaves (Thymus vulgaris L.) and their antioxidant properties. Food Chemistry, 91, 131–137.

Lee, J., & Scagel, C. F. (2009). Chicoric acid found in basil (Ocimum basilicum L.) leaves. Food Chemistry. https://doi.org/10.1016/j.foodchem.2008.12.075

Lermen, C., Morelli, F., Gazim, Z. C., da Silva, A. P., Gonçalves, J. E., Dragunski, D. C., & Alberton, O. (2015). Essential oil content and chemical composition of Cymbopogon citratus inoculated with arbuscular mycorrhizal fungi under different levels of lead. Industrial Crops and Products. https://doi.org/10.1016/j.indcrop.2015.07.009

Li, Y., Fabiano-Tixier, A.-S., & Chemat, F. (2014). Essential Oils as Reagents in Green Chemistry. *SpringerBriefs in Green Chemistry for Sustainability*. https://doi.org/10.1007/978-3-319-08449-7

Liang, J., Susan Sun, X., Yang, Z., & Cao, S. (2017). Anticancer Drug Camptothecin Test in 3D Hydrogel Networks with HeLa cells. Scientific Reports. https://doi.org/10.1038/srep37626

Lichtenthaler, H. K. (1987). Chlorophylls Carotenoids. Chlorophylls and Carotenoids: Pigments of Photosynthetic Biomembranes.

Loffredo, E., Berloco, M., & Senesi, N. (2008). The role of humic fractions from soil and compost in controlling the growth in vitro of phytopathogenic and antagonistic soil-borne fungi. Ecotoxicology and Environmental Safety. https://doi.org/10.1016/j.ecoenv.2007.11.005

López García de la Serrana, H., & López Martínez, M. C. (2007). Different radical scavenging tests in virgin olive oil and their relation to the total phenol content. Analytica Chimica Acta, 593(1), 103–107. https://doi.org/10.1016/j.aca.2007.04.037

Lopez-Romero, J. C., González-Ríos, H., Borges, A., & Simões, M. (2015). Antibacterial Effects and Mode of Action of Selected Essential Oils Components against Escherichia coli and Staphylococcus aureus. Evidence-Based Complementary and Alternative Medicine. https://doi.org/10.1155/2015/795435

Loughrin, J. H., & Kasperbauer, M. J. (2001). Light reflected from colored mulches affects aroma and phenol content of sweet basil (Ocimum basilicum L.) leaves. *Journal of Agricultural and Food Chemistry*. https://doi.org/10.1021/jf0012648

Lucchesi, M. E., Chemat, F., & Smadja, J. (2004). Solvent-free microwave extraction of essential oil from aromatic herbs: Comparison with conventional hydro-distillation. Journal of Chromatography A. https://doi.org/10.1016/j.chroma.2004.05.083

Lupton, D., Khan, M. M., Al-Yahyai, R. A., & Hanif, M. A. (2012). Basil: A natural source of antioxidants and neutraceuticals. Natural Products and Their Active Compounds on Disease Prevention.

Mahadagde, M. P. (2018). Techniques Available for the Extraction of Essential Oils from Plants: A Review. International Journal for Research in Applied Science and Engineering Technology. https://doi.org/10.22214/ijraset.2018.3643

Malik, A., Suryapani, S., & Ahmad, J. (2011). Chemical vs organic cultivation of medicinal and aromatic plants: the choice is clear. Int J Med Arom Plants.

Maloney, G. S., Kochevenko, A., Tieman, D. M., Tohge, T., Krieger, U., Zamir, D., … Klee, H. J. (2010). Characterization of the branched-chain amino acid aminotransferase enzyme family in tomato. *Plant Physiology*. https://doi.org/10.1104/pp.110.154922

Maniadakis, K., Lasaridi, K., Manios, Y., Kyriacou, M., & Manios, T. (2004). Integrated Waste Management Through Producers and Consumers Education: Composting of Vegetable Crop Residues for Reuse in Cultivation. Journal of Environmental Science and Health - Part B Pesticides, Food Contaminants, and Agricultural Wastes. https://doi.org/10.1081/PFC-120027447

Marotti, M., Piccaglia, R., & Giovanelli, E. (1996). Differences in Essential Oil Composition of Basil (Ocimum basilicum L.) Italian Cultivars Related to Morphological Characteristics. Journal of Agricultural and Food Chemistry. https://doi.org/10.1021/jf9601067

Martinez-Balmori, D., Olivares, F. L., Spaccini, R., Aguiar, K. P., Araújo, M. F., Aguiar, N. O., … Canellas, L. P. (2013). Molecular characteristics of vermicompost and their relationship to preservation of inoculated nitrogen-fixing bacteria. *Journal of Analytical and Applied Pyrolysis*. https://doi.org/10.1016/j.jaap.2013.05.015

Martinez-Balmori, D., Spaccini, R., Aguiar, N. O., Novotny, E. H., Olivares, F. L., & Canellas, L. P. (2014). Molecular characteristics of humic acids isolated from vermicomposts and their relationship to bioactivity. *Journal of Agricultural and Food Chemistry*. https://doi.org/10.1021/jf504629c

Martins, A. P., Salgueiro, L. R., Vila, R., Tomi, F., Canigueral, S., Casanova, J., … Adzet, T. (1999). Composition of the essential oils of Ocimum canum, O. gratissimum and O. minimum. Planta Medica. https://doi.org/10.1055/s-2006-960465

Mazzei P, Piccolo, A. (2015) Interactions between natural organic matter and organic pollutants as revealed by NMR spectroscopy. Magn Reson Chem 53:667–678

Mehta, R. A., Cassol, T., Li, N., Ali, N., Handa, A. K., & Mattoo, A. K. (2002). Engineered polyamine accumulation in tomato enhances phytonutrient content, juice quality and vine life. Nature Biotechnology, 20, 613–618

Michaeli, S., & Fromm, H. (2015). Closing the loop on the GABA shunt in plants: Are GABA metabolism and signaling entwined? Frontiers in Plant Science. https://doi.org/10.3389/fpls.2015.00419

Mohammadi, M., Majd, A., Nejadsattari, T., & Hashemi, M. (2014). Antioxidant and Anticancer Activities of Ocimum basilicum L. cv.Dark Opal (Lamiaceae). Pharmacognosy Communications.

Mohd Din, A.R.J., Hanapi, S.Z., Sarmidi, M.R. (2016) Suppressive effect of compost tea on fungal pathogen causing melanose on Melicope ptelefolia (Tenggek Burung) leaf. 6th International conference of biotechnology for wellness industries. Melaka, pp 5–8

Mohd Din, A. R. J., Hanapi, S. Z., Futamata, H., & Sarmidi, M. R. (2018). Utilization of compost tea for biochemical response assessment associated with resistance to phytopathogen causing leaf spot in Melicope ptelefolia. Organic Agriculture, 445–456. https://doi.org/10.1007/s13165-018-0235-9

Monda, H., Cozzolino, V., Vinci, G., Spaccini, R., & Piccolo, A. (2017). Molecular characteristics of water-extractable organic matter from different composted biomasses and their effects on seed germination and early growth of maize. *Science of the Total Environment*. https://doi.org/10.1016/j.scitotenv.2017.03.026

Monda, H., Cozzolino, V., Vinci, G., Drosos, M., Savy, D., & Piccolo, A. (2018). Molecular composition of the Humeome extracted from different green composts and their biostimulation on early growth of maize. *Plant and Soil*. https://doi.org/10.1007/s11104-018-3642-5

Morales-Corts, M. R., Pérez-Sánchez, R., & Gómez-Sánchez, M. Á. (2018). Efficiency of garden waste compost teas on tomato growth and its suppressiveness against soilborne pathogens. Scientia Agricola, 75(5), 400–409. https://doi.org/10.1590/1678-992x-2016-0439

Morelli, F., Ferarrese, L., Munhoz, C. L., & Alberton, O. (2017). Antimicrobial activity of essential oil and growth of Ocimum basilicum (L.) inoculated with mycorrhiza and humic substances applied to soil. Genetics and Molecular Research. https://doi.org/10.4238/gmr16039710

Moretti, S. M. L., Bertoncini, E. I., & Abreu-Junior, C. H. (2015). Composting sewage sludge with green waste from tree pruning. Scientia Agricola, 72(5), 432–439. https://doi.org/10.1590/0103-9016-2014-0341

Moslemi, M., Aboutalebi, A., Hasanzadeh, H. and Farahi, M. H. 2012. Evaluation the effects of different levels of phosphorous on yield and yield components of coriander (Coriandrum sativum L.). World of Applied Science Journal 19(11): 1621-1624.

Mousavi, L., Salleh, R. M., & Murugaiyah, V. (2018). Phytochemical and bioactive compounds identification of Ocimum tenuiflorum leaves of methanol extract and its fraction with an antidiabetic potential. International Journal of Food Properties. https://doi.org/10.1080/10942912.2018.1508161

Muscolo, A., Sidari, M., Francioso, O., Tugnoli, V., Nardi, S. (2007) The auxin-like activity of humic substances is related to membrane interactions in carrot cell cultures. J. Chem. Ecol. 33, 115–129.

Muscolo, A., Sidari, M., & Nardi, S. (2013). Humic substance: Relationship between structure and activity. deeper information suggests univocal findings. Journal of Geochemical Exploration. https://doi.org/10.1016/j.gexplo.2012.10.012

Naguib, N. Y. M. (2011). Organic vs chemical fertilization of medicinal plants: A concise review of researches. Advances in Environmental Biology.

Naidu, Y., Meon, S., Kadir, J., & Siddiqui, Y. (2010). Microbial starter for the enhancement of biological activity of compost tea. International Journal of Agriculture and Biology, 12(1), 51–56.

Naidu, Y., Meon, S., & Siddiqui, Y. (2013). Foliar application of microbial-enriched compost tea enhances growth, yield and quality of muskmelon (Cucumis melo L.) cultivated under fertigation system. Scientia Horticulturae. https://doi.org/10.1016/j.scienta.2013.04.024

Nardi, S., Pizzeghello, D., Muscolo, A., & Vianello, A. (2002). Physiological effects of humic substances on higher plants. Soil Biology and Biochemistry. https://doi.org/10.1016/S0038-0717(02)00174-8

Nardi, S., Muscolo, A., Vaccaro, S., Baiano, S., Spaccini, R., & Piccolo, A. (2007). Relationship between molecular characteristics of soil humic fractions and glycolytic pathway and krebs cycle in maize seedlings. Soil Biology and Biochemistry. https://doi.org/10.1016/j.soilbio.2007.07.006

Nardi, S., Pizzeghello, D., Schiavon, M., Ertani, A. (2016): Plant biostimulants : physiological responses induced by protein hydrolyzed-based products and humic substances in plant metabolism.Sci. Agric. 73, 18–23.

Nardi, S., Ertani, A., & Francioso, O. (2017). Soil–root cross-talking: The role of humic substances. Journal of Plant Nutrition and Soil Science. https://doi.org/10.1002/jpln.201600348

Nazzaro, F., Fratianni, F., De Martino, L., Coppola, R., & De Feo, V. (2013). Effect of essential oils on pathogenic bacteria. Pharmaceuticals. https://doi.org/10.3390/ph6121451

Nchu, F., Matanzima, Y., & Laubscher, C. P. (2018). Prospects of N Fertilization in Medicinal Plants Cultivation. In Nitrogen in Agriculture - Updates. https://doi.org/10.5772/intechopen.68165

Ncube, B., Finnie, J. F., & Van Staden, J. (2012). Quality from the field: The impact of environmental factors as quality determinants in medicinal plants. South African Journal of Botany. https://doi.org/10.1016/j.sajb.2012.05.009

Niemeyer, J., Chen, Y., & Bollag, J. M. (1992). Characterization of humic acids, composts, and peat by diffuse reflectance Fourier-transform infrared spectroscopy. *Soil Science Society of America Journal*. https://doi.org/10.2136/sssaj1992.03615995005600010021x

Noroozisharaf, A., & Kaviani, M. (2018). Effect of soil application of humic acid on nutrients uptake, essential oil and chemical compositions of garden thyme (Thymus vulgaris L.) under greenhouse conditions. Physiology and Molecular Biology of Plants. https://doi.org/10.1007/s12298-018-0510-y

Ohara, K., Kokado, Y., Yamamoto, H., Sato, F., & Yazaki, K. (2004). Engineering of ubiquinone biosynthesis using the yeast coq2 gene confers oxidative stress tolerance in transgenic tobacco. Plant Journal. https://doi.org/10.1111/j.1365-313X.2004.02246.x

Okada, K., Ohara, K., Yazaki, K., Nozaki, K., Uchida, N., Kawamukai, M., … Yamane, H. (2004). The AtPPT1 gene encoding 4-hydroxybenzoate polyprenyl diphosphate transferase in ubiquinone biosynthesis is required for embryo development in Arabidopsis thaliana. Plant Molecular Biology. https://doi.org/10.1007/s11103-004-1298-4

Okazaki, Y., Kamide, Y., Hirai, M. Y., & Saito, K. (2013). Plant lipidomics based on hydrophilic interaction chromatography coupled to ion trap time-of-flight mass spectrometry. Metabolomics. https://doi.org/10.1007/s11306-011-0318-z

Olaetxea, M., Mora, V., Bacaicoa, E., Garnica, M., Fuentes, M., Casanova, E., … García-Mina, J. M. (2015). Abscisic acid regulation of root hydraulic conductivity and aquaporin gene expression is crucial to the plant shoot growth enhancement caused by rhizosphere humic acids. Plant Physiology. https://doi.org/10.1104/pp.15.00596

Olaetxea, M., De Hita, D., Garcia, C. A., Fuentes, M., Baigorri, R., Mora, V., … Garcia-Mina, J. M. (2018). Hypothetical framework integrating the main mechanisms involved in the promoting action of rhizospheric humic substances on plant root- and shoot- growth. Applied Soil Ecology. https://doi.org/10.1016/j.apsoil.2017.06.007

Olivares, F. L., Busato, J. G., de Paula, A. M., da Silva Lima, L., Aguiar, N. O., & Canellas, L. P. (2017). Plant growth promoting bacteria and humic substances: crop promotion and mechanisms of action. *Chemical and Biological Technologies in Agriculture*. https://doi.org/10.1186/s40538-017-0112-x

Oliver, S. G., Winson, M. K., Kell, D. B., & Baganz, F. (1998). Systematic functional analysis of the yeast genome. *Trends in Biotechnology*. https://doi.org/10.1016/S0167-7799(98)01214-1

On, A., Wong, F., Ko, Q., Tweddell, R. J., Antoun, H., & Avis, T. J. (2015). Antifungal effects of compost tea microorganisms on tomato pathogens. Biological Control. https://doi.org/10.1016/j.biocontrol.2014.09.017

Özcan, M., & Chalchat, J.-C. (2018). Essential oil composition of Ocimum basilicum L. and Ocimum minimum L. in Turkey. Czech Journal of Food Sciences. https://doi.org/10.17221/3536-cjfs

Padalia, R. C., Verma, R. S., Chauhan, A., & Chanotiya, C. S. (2013). Changes in aroma profiles of 11 Indian Ocimum taxa during plant ontogeny. Acta Physiologiae Plantarum. https://doi.org/10.1007/s11738-013-1293-y

Paine, J. A., Shipton, C. A., Chaggar, S., Howells, R. M., Kennedy, M. J., Vernon, G., … Drake, R. (2005). Improving the nutritional value of Golden Rice through increased pro-vitamin A content. *Nature Biotechnology*. https://doi.org/10.1038/nbt1082

Palaniappan, K., & Holley, R. A. (2010). Use of natural antimicrobials to increase antibiotic susceptibility of drug resistant bacteria. International Journal of Food Microbiology, 140(2–3), 164–168. https://doi.org/10.1016/j.ijfoodmicro.2010.04.001

Pane, C., Parisi, M., Zaccardelli, M., Graziani, G., & Fogliano, V. (2011). Putative role of antioxidant activity of high pigment tomato cultivars in resistance against Botrytis cinerea post-harvest infection. Acta Horticulturae. https://doi.org/10.17660/ActaHortic.2011.914.82

Pane, C., Villecco, D., Campanile, F., & Zaccardelli, M. (2012). Novel strains of Bacillus, isolated from compost and compost-amended soils, as biological control agents against soil-borne phytopathogenic fungi. Biocontrol Science and Technology, 22(12), 1373–1388. https://doi.org/10.1080/09583157.2012.729143

Pane, C., Piccolo, A., Spaccini, R., Celano, G., Villecco, D., Zaccardelli, M. (2013). Agricultural waste-based composts exhibiting suppressivity to diseases caused by the phytopathogenic soil-borne fungi Rhizoctonia solani and Sclerotinia minor. *Applied Soil Ecology*, 65, 43-51.

Pane, C., Palese, A. M., Celano, G., & Zaccardelli, M. (2014). Effects of compost tea treatments on productivity of lettuce and kohlrabi systems under organic cropping management. Italian Journal of Agronomy, 9(3), 153–156. https://doi.org/10.4081/ija.2014.596

Pane, C., Palese, A. M., Spaccini, R., Piccolo, A., Celano, G., & Zaccardelli, M. (2016). Enhancing sustainability of a processing tomato cultivation system by using bioactive compost teas. Scientia Horticulturae, 202, 117–124. https://doi.org/10.1016/j.scienta.2016.02.034

Pane, K., Durante, L., Pizzo, E., Varcamonti, M., Zanfardino, A., Sgambati, V., … & Notomista, E. (2016). Rational design of a carrier protein for the production of recombinant toxic peptides in Escherichia coli. PLoS ONE, 11(1), 1–23. https://doi.org/10.1371/journal.pone.0146552

Pane, K., Verrillo, M., Avitabile, A., Pizzo, E., Varcamonti, M., Zanfardino, A., …. & Notomista, E. (2018). Chemical Cleavage of an Asp-Cys Sequence Allows Efficient Production of Recombinant Peptides with an N-Terminal Cysteine Residue. Bioconjugate Chemistry. https://doi.org/10.1021/acs.bioconjchem.8b00083

Pant, A., Radovich, T. J. K., Hue, N. V., & Arancon, N. Q. (2011). Effects of vermicompost tea (aqueous extract) on pak choi yield, quality, and on soil biological properties. Compost Science and Utilization. https://doi.org/10.1080/1065657X.2011.10737010

Parmar, S. S., Jaiwal, A., Dhankher, O. P., & Jaiwal, P. K. (2015). Coenzyme Q10 production in plants: Current status and future prospects. Critical Reviews in Biotechnology. https://doi.org/10.3109/07388551.2013.823594

Peña-Méndez, E. M., Havel, J., & Patočka, J. (2005). Humic substance - Compounds of still unknown structure: Applications in agriculture, industry, environment, and biomedicine. Journal of Applied Biomedicine. https://doi.org/10.32725/jab.2005.002

Peng, B., & Ahrends, R. (2016). Adaptation of Skyline for Targeted Lipidomics. Journal of Proteome Research. https://doi.org/10.1021/acs.jproteome.5b00841

Pereira, H. M., Navarro, L. M., & Martins, I. S. (2012). Global biodiversity change: The bad, the good, and the unknown. *Annual Review of Environment and Resources*, *37*, 25–50.CrossRefGoogle Scholar

Pereira, M. M. A., Morais, L. C., Marques, E. A., Martins, A. D., Cavalcanti, V. P., Rodrigues, F. A., … Dória, J. (2019). Humic Substances and Efficient Microorganisms: Elicitation of Medicinal Plants—A Review. Journal of Agricultural Science. https://doi.org/10.5539/jas.v11n7p268

Pergola, M., Piccolo, A., Palese, A. M., Ingrao, C., Di Meo, V., & Celano, G. (2018). A combined assessment of the energy, economic and environmental issues associated with on-farm manure composting processes: Two case studies in South of Italy. Journal of Cleaner Production. https://doi.org/10.1016/j.jclepro.2017.04.111

Perricone, M., Arace, E., Corbo, M. R., Sinigaglia, M., & Bevilacqua, A. (2015). Bioactivity of essential oils: A review on their interaction with food components. Frontiers in Microbiology. https://doi.org/10.3389/fmicb.2015.00076

Petrovska, B. B. (2012). Historical review of medicinal plants' usage. Pharmacognosy Reviews. https://doi.org/10.4103/0973-7847.95849

Pham-Huy, L. A., He, H., & Pham-Huy, C. (2008). Free radicals, antioxidants in disease and health. International Journal of Biomedical Science.

Piazzon, A., Vrhovsek, U., Masuero, D., Mattivi, F., Mandoj, F., & Nardini, M. (2012). Antioxidant activity of phenolic acids and their metabolites: Synthesis and antioxidant properties of the sulfate derivatives of ferulic and caffeic acids and of the acyl glucuronide of ferulic acid. Journal of Agricultural and Food Chemistry. https://doi.org/10.1021/jf304076z

Piccolo, A., Zena, A., & Conte, P. (1996). A comparison of acid hydrolyses for the determination of carbohydrate content in soils. *Communications in Soil Science and Plant Analysis*. https://doi.org/10.1080/00103629609369749

Piccolo, A., Conte, P. (1998). Advances in nuclear magnetic resonance and infrared spectroscopies of soil organic particles, in: P.M. Huang, N. Senesi, J. Bufflea (Eds.), Structure and Surface Reactions of Soil Particles, Wiley, New York, pp. 183–250.

Piccolo, A., Cozzolino, A., Conte, P., & Spaccini, R. (2000). Polymerization of humic substances by an enzyme-catalyzed oxidative coupling. *Naturwissenschaften*. https://doi.org/10.1007/s001140050747

Piccolo, A. (2001). The supramolecular structure of humic substances. Soil Science. https://doi.org/10.1097/00010694-200111000-00007

Piccolo, A., Conte, P., & Cozzolino, A. (2001). Chromatographic and spectrophotometric properties of dissolved humic substances compared with macromolecular polymers. *Soil Science*. https://doi.org/10.1097/00010694-200103000-00003

Piccolo, A. (2002). The supramolecular structure of humic substances: A novel understanding of humus chemistry and implications in soil science. Advances in Agronomy. https://doi.org/10.1016/s0065-2113(02)75003-7

Piccolo, A., Conte, P., Trivellone, E., Van Lagen, B., & Buurman, P. (2002). Reduced heterogeneity of a lignite humic acid by preparative HPSEC following interaction with an organic acid. Characterization of size-separates by Pyr-GC-MS and 1H-NMR spectroscopy. Environmental Science and Technology. https://doi.org/10.1021/es010981v

Piccolo, A., & Spiteller, M. (2003). Electrospray ionization mass spectrometry of terrestrial humic substances and their size fractions. Analytical and Bioanalytical Chemistry. https://doi.org/10.1007/s00216-003-2186-5

Piccolo, A., Façanha, A.R., (2010). Bioactivity of chemically transformed humic matter from vermicompost on plant root growth. J. Agric. Food Chem. 58, 3681–3688

Piccolo, A., Spaccini, R., Drosos, M., Vinci, G., & Cozzolino, V. (2018). The molecular composition of humus carbon: Recalcitrance and reactivity in soils. In *The Future of Soil Carbon: Its Conservation and Formation*. https://doi.org/10.1016/B978-0-12-811687-6.00004-3

Piccolo, A., Spaccini, R., Savy, D., Drosos, M., & Cozzolino, V. (2019). The Soil Humeome: Chemical Structure, Functions and Technological Perspectives. In *Sustainable Agrochemistry*. https://doi.org/10.1007/978-3-030-17891-8_7

Pietschmann, A., Mehdipour, P., Atri, M., Hofmann, W., Hosseini-Asl, S.S., Scherneck, S. and Peters, H. (2005). Mutation analysis of BRCA1 and BRCA2 genes in Iranian high risk breast cancer families. *Journal of Cancer Research and Clinical Oncology* 131(8): 552- 558.

Pizzeghello, D., Nicolini, G., & Nardi, S. (2001). Hormone-like activity of humic substances in Fagus sylvaticae forests. New Phytologist. https://doi.org/10.1046/j.0028-646x.2001.00223.x

Politeo, O., Jukic, M., & Milos, M. (2007). Chemical composition and antioxidant capacity of free volatile aglycones from basil (Ocimum basilicum L.) compared with its essential oil. *Food Chemistry*. https://doi.org/10.1016/j.foodchem.2006.01.045

Pragasam, S. J., Venkatesan, V., & Rasool, M. (2013). Immunomodulatory and anti-inflammatory effect of p-coumaric acid, a common dietary polyphenol on experimental inflammation in rats. Inflammation. https://doi.org/10.1007/s10753-012-9532-8

Pramod, K., Ansari, S. H., & Ali, J. (2010). Eugenol: A natural compound with versatile pharmacological actions. Natural Product Communications. https://doi.org/10.1177/1934578x1000501236

Pravst, I., Prošek, M., Wondra, A. G., Žmitek, K., & Žmitek, J. (2009). The stability of Coenzyme Q10 in fortified foods. Acta Chimica Slovenica.

Pravst, I., Žmitek, K., & Žmitek, J. (2010). Coenzyme Q10 contents in foods and fortification strategies. Critical Reviews in Food Science and Nutrition. https://doi.org/10.1080/10408390902773037

Rahimi, A. R., Mashayekhi, K., Ghasemnezhad, A., Ebrahimi, P., & Hemmati, K. (2009). Salicylic acid plus nutritional minerals influence the essential oil production and linalool accumulation of coriander seeds (Coriandrum sativum L.). Zeitschrift Fur Arznei- Und Gewurzpflanzen.

Ram, M., Singh, R., Ram, D. and Sangwan, R. S. (2003). Foliar application of phosphate increased the yield of essential oil in menthol mint (Mentha arvensis). Australian Journal Agriculture Experiments 12: 1263 - 1268

Ramakrishna, A., & Ravishankar, G. A. (2011). Influence of abiotic stress signals on secondary metabolites in plants. Plant Signaling and Behavior. https://doi.org/10.4161/psb.6.11.17613

Ramos, A. C., Dobbss, L. B., Santos, L. A., Fernandes, M. S., Olivares, F. L., Aguiar, N. O., & Canellas, L. P. (2015). Humic matter elicits proton and calcium fluxes and signaling dependent on Ca2+-dependent protein kinase (CDPK) at early stages of lateral plant root development. *Chemical and Biological Technologies in Agriculture*. https://doi.org/10.1186/s40538-014-0030-0

Rampler, E., Schoeny, H., Mitic, B. M., El Abiead, Y., Schwaiger, M., & Koellensperger, G. (2018). Simultaneous non-polar and polar lipid analysis by on-line combination of HILIC, RP and high resolution MS. Analyst. https://doi.org/10.1039/c7an01984j

Rassem, H., Nour, A., & R. M., Y. (2016). Techniques For Extraction of Essential Oils From Plants: A Review. Australian Journal of Basic and Applied Sciences.

Ray, DK., Ramankutty, N., Mueller, N.D., West, P.C., Foley, J.A. (2012). Recent patterns of crop yield growth and stagnation. *Nature Communications* 3:1293. doi:10.1038/ncomms2296.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine. https://doi.org/10.1016/S0891-5849(98)00315-3

Reed, J. C. (2000). Mechanisms of Apoptosis. The American Journal of Pathology. https://doi.org/10.1016/s0002-9440(10)64779-7

Regulation (EU) 2019/1009 of the European Parliament and of the Council of 5 June 2019 laying down rules on the making available on the market of EU fertilising products and amending Regulations (EC) No 1069/2009 and (EC) No 1107/2009 and repealing Regulation (EC) No 2003/2003 (Text with EEA relevance). http://data.europa.eu/eli/reg/2019/1009/oj

Rice-Evans, C. A., Miller, N. J., & Paganga, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radical Biology and Medicine. https://doi.org/10.1016/0891-5849(95)02227-9

Rupasinghe, T. W. T., & Roessner, U. (2018). Extraction of plant lipids for LC-MS-Based untargeted plant lipidomics. In *Methods in Molecular Biology*. https://doi.org/10.1007/978-1-4939-7819-9_9

Sacchetti, G., Maietti, S., Muzzoli, M., Scaglianti, M., Manfredini, S., Radice, M., & Bruni, R. (2005). Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. *Food Chemistry*. https://doi.org/10.1016/j.foodchem.2004.06.031

Said-Al Ahl, H. A. H., & Hussein, M. S. (2010). Effect of water stress and potassium humate on the productivity of oregano plant using saline and fresh water irrigation. *Ozean Journal of Applied Sciences*.

Said-Al Ahl, H., Gendy, A. G., & El, E. A. O. (2016). Humic acid and indole acetic acid affect yield and essential oil of dill grown under two different locations in Egypt. *International Journal of Pharmacy and Pharmaceutical Sciences, 8*, 146-157.

Saito, K., & Matsuda, F. (2010). Metabolomics for Functional Genomics, Systems Biology, and Biotechnology. *Annual Review of Plant Biology*. https://doi.org/10.1146/annurev.arplant.043008.092035

Sajjadi, S. E. (2006). Analysis of the essential oils of two cultivated basil (Ocimum basilicum L.) from Iran. *Daru*.

Sakkas, H., Gousia, P., Economou, V., Sakkas, V., Petsios, S., & Papadopoulou, C. (2016). In vitro antimicrobial activity of five essential oils on multidrug resistant Gram-negative clinical isolates. Journal of Intercultural Ethnopharmacology. https://doi.org/10.5455/jice.20160331064446

Salehi, B., Mishra, A. P., Nigam, M., Sener, B., Kilic, M., Sharifi-Rad, M., … Sharifi-Rad, J. (2018). Resveratrol: A double-edged sword in health benefits. Biomedicines. https://doi.org/10.3390/biomedicines6030091
Samaniego Sánchez, C., Troncoso González, A. M., García-Parrilla, M. C., Quesada Granados, J. J.,

Sartoratotto, A., Machado, A.L.M., Delarmelina, C., Figueira, G.M., Duarte, M.C.T., Rehder, V.L.G., (2004). Composition and antimicrobial activity of essential oils from aromatic plants used in Brazil. *Braz. J. Microbiol*., 35: 275-280.

Savy D, Cozzolino V, Vinci G, Nebbioso A, Piccolo A (2015) Water-soluble lignins from different bioenergy crops stimulate the early development of maize (Zea mays,L.).Molecules 20:19958–19970

Savy, D., Cozzolino, V., Nebbioso, A., Drosos, M., Nuzzo, A., Mazzei, P., & Piccolo, A. (2016). Humic-like bioactivity on emergence and early growth of maize (Zea mays L.) of water-soluble lignins isolated from biomass for energy. *Plant and Soil*. https://doi.org/10.1007/s11104-015-2780-2

Scaglia, B., Nunes, R. R., Rezende, M. O. O., Tambone, F., & Adani, F. (2016). Investigating organic molecules responsible of auxin-like activity of humic acid fraction extracted from vermicompost. Science of the Total Environment. https://doi.org/10.1016/j.scitotenv.2016.03.212

Schepetkin, I., Khlebnikov, A., & Kwon, B. S. (2002). Medical drugs from humus matter: Focus on mumie. Drug Development Research. https://doi.org/10.1002/ddr.10058

Scheuerell, S., & Mahaffee, W. (2002). Compost tea: Principles and prospects for plant disease control. Compost Science and Utilization. https://doi.org/10.1080/1065657X.2002.10702095

Scheuerell, S. J., & Mahaffee, W. F. (2004). Compost tea as a container medium drench for suppressing seedling damping-off caused by Pythium ultimum. *Phytopathology*. https://doi.org/10.1094/PHYTO.2004.94.11.1156

Schiavon, M., Pizzeghello, D., Muscolo, A., Vaccaro, S., Francioso, O., & Nardi, S. (2010). High molecular size humic substances enhance phenylpropanoid metabolism in maize (Zea mays L.). Journal of Chemical Ecology. https://doi.org/10.1007/s10886-010-9790-6

Schoch, G., Goepfert, S., Morant, M., Hehn, A., Meyer, D., Ullmann, P., & Werck-Reichhart, D. (2001). CYP98A3 from Arabidopsis thaliana Is a 3′-Hydroxylase of Phenolic Esters, a Missing Link in the Phenylpropanoid Pathway. Journal of Biological Chemistry. https://doi.org/10.1074/jbc.M104047200

Scotti, R., Pane, C., Spaccini, R., Palese, A. M., Piccolo, A., Celano, G., & Zaccardelli, M. (2016). On-farm compost: A useful tool to improve soil quality under intensive farming systems. Applied Soil Ecology, 107, 13–23. https://doi.org/10.1016/j.apsoil.2016.05.004

Sengupta, S., Mukherjee, S., Basak, P., & Majumder, A. L. (2015). Significance of galactinol and raffinose family oligosaccharide synthesis in plants. Frontiers in Plant Science. https://doi.org/10.3389/fpls.2015.00656

Sgherri, C., Cecconami, S., Pinzino, C., Navari-Izzo, F., & Izzo, R. (2010). Levels of antioxidants and nutraceuticals in basil grown in hydroponics and soil. Food Chemistry. https://doi.org/10.1016/j.foodchem.2010.04.058

Shaheen, A.M., Rizk, F.A., Sawan, O.M., Bakry, M.O. (2013). Sustaining the quality and quantity of onion productivity throughout complementrity treatments between compost tea and amino acids Middle East J. Agric. Res., 2: 108-15, 2013

Shang, E.X.; Li, Y.; Niu, J.F.; Zhou, Y.J.;Wang, T.Y.; Crittenden, J.C. Relative importance of humic and fulvic acid on ROS generation, dissolution, and toxicity of sulfide nanoparticles. Water Res. 2017, 124, 595–604.

Shelp, B. J., Bown, A. W., & McLean, M. D. (1999). Metabolism and functions of gamma-aminobutyric acid. Trends in Plant Science. https://doi.org/10.1016/S1360-1385(99)01486-7

Shiwakoti, S., Saleh, O., Poudyal, S., Barka, A., Qian, Y., & Zheljazkov, V. D. (2017). Yield, Composition and Antioxidant Capacity of the Essential Oil of Sweet Basil and Holy Basil as Influenced by Distillation Methods. Chemistry and Biodiversity. https://doi.org/10.1002/cbdv.201600417

Shulaev, V., & Chapman, K. D. (2017). Plant lipidomics at the crossroads: From technology to biology driven science. *Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids*. https://doi.org/10.1016/j.bbalip.2017.02.011

Siddiqui, B.S., Aslam, H. Ali, S.T., Begum, S. and Khatoon, N. 2007 (a). Two new triterpenoids and a steroidal glycoside from the aerial parts of *Ocimum basilicum.* Chemical and Pharmaceutical Bulletin (Tokyo) 55: 516-9.

Siddiqui, B.S., Aslam, H., Begum, S. and Ali, S.T. 2007 (b) . New cinnamic acid esters from *Ocimum basilicum. Natural Product Research* 21: 736-41.

Siddiqui, Y., Meon, S., Ismail, R., Rahmani, M., & Ali, A. (2008). Bio-efficiency of compost extracts on the wet rot incidence, morphological and physiological growth of okra (Abelmoschus esculentus [(L.) Moench]). Scientia Horticulturae. https://doi.org/10.1016/j.scienta.2008.03.008

Siddiqui, Y., Meon, S., Ismail, R., & Rahmani, M. (2009). Bio-potential of compost tea from agro-waste to suppress Choanephora cucurbitarum L. the causal pathogen of wet rot of okra. Biological Control. https://doi.org/10.1016/j.biocontrol.2008.11.008

Siddiqui, Y., Islam, T. M., Naidu, Y., & Meon, S. (2011). The conjunctive use of compost tea and inorganic fertiliser on the growth, yield and terpenoid content of Centella asiatica (L.) urban. Scientia Horticulturae. https://doi.org/10.1016/j.scienta.2011.05.043

Simirgiotis, M. J., Quispe, C., Mocan, A., Villatoro, J. M., Areche, C., Bórquez, J., … Echiburu-Chau, C. (2017). UHPLC high resolution orbitrap metabolomic fingerprinting of the unique species Ophryosporus triangularis meyen from the atacama desert, Northern Chile. Brazilian Journal of Pharmacognosy. https://doi.org/10.1016/j.bjp.2016.10.002

Simpson, A. J. (2002). Determining the molecular weight, aggregation, structures and interactions of natural organic matter using diffusion ordered spectroscopy. *Magnetic Resonance in Chemistry*. https://doi.org/10.1002/mrc.1106

Singh, D., & Chaudhuri, P. K. (2018). A review on phytochemical and pharmacological properties of Holy basil (Ocimum sanctum L.). Industrial Crops and Products. https://doi.org/10.1016/j.indcrop.2018.03.048

Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods in Enzymology. https://doi.org/10.1016/S0076-6879(99)99017-1

Šmejkalová, D., Spaccini, R., & Piccolo, A. (2008). Multivariate analysis of CPMAS 13C-NMR spectra of soils and humic matter as a tool to evaluate organic carbon quality in natural systems. *European Journal of Soil Science*. https://doi.org/10.1111/j.1365-2389.2007.01005.x

Song, X. Y., Spaccini, R., Pan, G., & Piccolo, A. (2013). Stabilization by hydrophobic protection as a molecular mechanism for organic carbon sequestration in maize-amended rice paddy soils. *Science of the Total Environment*. https://doi.org/10.1016/j.scitotenv.2013.04.052

Spaccini, R., & Piccolo, A. (2007) a. Molecular characterization of compost at increasing stages of maturity. 1. Chemical fractionation and infrared spectroscopy. *Journal of Agricultural and Food Chemistry*. https://doi.org/10.1021/jf0625398

Spaccini, R., & Piccolo, A. (2007) b. Molecular characterization of compost at increasing stages of maturity. 2. Thermochemolysis-GC-MS and 13C-CPMAS-NMR spectroscopy. *Journal of Agricultural and Food Chemistry*. https://doi.org/10.1021/jf0625407

Spaccini, R., & Piccolo, A. (2008). Spectroscopic characterization of compost at different maturity stages. *Clean - Soil, Air, Water*. https://doi.org/10.1002/clen.200720012

Spaccini, R., & Piccolo, A. (2009) Molecular characteristics of humic acids extracted from compost at increasing maturity stages. Soil Biol Biochem 41:1164–1172

Spaccini, R., Piccolo, A., Haberhauer, G., Stemmer, M., & Gerzabek, M. H. (2001). Decomposition of maize straw in three European soils as revealed by DRIFT spectra of soil particle fractions. *Geoderma*. https://doi.org/10.1016/S0016-7061(00)00073-2

Spaccini, R., Song, X. Y., Cozzolino, V., & Piccolo, A. (2013). Molecular evaluation of soil organic matter characteristics in three agricultural soils by improved off-line thermochemolysis: The effect of hydrofluoric acid demineralisation treatment. *Analytica Chimica Acta*. https://doi.org/10.1016/j.aca.2013.09.031

Spaccini, R., Todisco, D., Drosos, M., Nebbioso, A., & Piccolo, A. (2016). Decomposition of bio-degradable plastic polymer in a real on-farm composting process. Chemical and Biological Technologies in Agriculture, 3(1). https://doi.org/10.1186/s40538-016-0053-9

Spaccini, R., Cozzolino, V., Di Meo, V., Savy, D., Drosos, M., & Piccolo, A. (2019). Bioactivity of humic substances and water extracts from compost made by ligno-cellulose wastes from biorefinery. Science of the Total Environment, 646, 792–800. https://doi.org/10.1016/j.scitotenv.2018.07.334

Stupans, I., Kirlich, A., Tuck, K. L., & Hayball, P. J. (2002). Comparison of radical scavenging effect, inhibition of microsomal oxygen free radical generation, and serum lipoprotein oxidation of several natural antioxidants. *Journal of Agricultural and Food Chemistry*. https://doi.org/10.1021/jf0112320

Su, Z., Yang, Z., Xu, Y., Chen, Y., & Yu, Q. (2015). Apoptosis, autophagy, necroptosis, and cancer metastasis. Molecular Cancer. https://doi.org/10.1186/s12943-015-0321-5

Sulaiman, S. F., & Ooi, K. L. (2012). Polyphenolic and vitamin C contents and antioxidant activities of aqueous extracts from mature-green and ripe fruit fleshes of mangifera SP. Journal of Agricultural and Food Chemistry, 60(47), 11832–11838. https://doi.org/10.1021/jf303736h

Suppakul, P., Miltz, J., Sonneveld, K., & Bigger, S. W. (2003). Antimicrobial properties of basil and its possible application in food packaging. *Journal of Agricultural and Food Chemistry*. https://doi.org/10.1021/jf021038t

t'Kindt, R., Morreel, K., Deforce, D., Boerjan, W., & Van Bocxlaer, J. (2009). Joint GC-MS and LC-MS platforms for comprehensive plant metabolomics: Repeatability and sample pre-treatment. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*. https://doi.org/10.1016/j.jchromb.2009.08.041

Takahashi, S., Ohtani, T., Iida, S., Sunohara, Y., Matsushita, K., Maeda, H., … Kadowaki, K. I. (2009). Development of CoQ10-enriched rice from giant embryo lines. Breeding Science. https://doi.org/10.1270/jsbbs.59.321

Tanase, C., Boz, I., Stingu, A., Volf, I., & Popa, V. I. (2014). Physiological and biochemical responses induced by spruce bark aqueous extract and deuterium depleted water with synergistic action in sunflower (Helianthus annuus L.) plants. Industrial Crops and Products. https://doi.org/10.1016/j.indcrop.2014.05.039
Tanase, C., Cosarcă, S., & Muntean, D. L. (2019). A critical review of phenolic compounds extracted from the bark of woody vascular plants and their potential biological activity. Molecules. https://doi.org/10.3390/molecules24061182

Tarchoune, I., Incerti, A., Lachaal, M., Ouerghi, Z., Izzo, R., & NavarI-Izzo, F. (2009). Relations between antioxidant activity and salinity in basil (Ocimum basilicum Mill.). Agrochimica.

Tateo, F. (1989). The composition of various oils of Ocimum basilicum L. Journal of Essential Oil Research. https://doi.org/10.1080/10412905.1989.9697770

Theodoridis, G. A., Gika, H. G., Want, E. J., & Wilson, I. D. (2012). Liquid chromatography-mass spectrometry based global metabolite profiling: A review. Analytica Chimica Acta. https://doi.org/10.1016/j.aca.2011.09.042

Thirumurugan, D., Cholarajan, A., Raja, S. S. S., & Vijayakumar, R. (2018). An Introductory Chapter: Secondary Metabolites. In Secondary Metabolites - Sources and Applications. https://doi.org/10.5772/intechopen.79766

Ting, H. C., Yen, C. C., Chen, W. K., Chang, W. H., Chou, M. C., & Lu, F. J. (2010). Humic acid enhances the cytotoxic effects of arsenic trioxide on human cervical cancer cells. Environmental Toxicology and Pharmacology. https://doi.org/10.1016/j.etap.2009.11.009

Tiwari, A. K., & Rao, J. M. (2002). Diabetes mellitus and multiple therapeutic approaches of phytochemicals: Present status and future prospects. *Current Science*.

Tiwari, B.K., Valdramidis, V.P. and Donnel, C.P.O., Muthukumarappan, K., Bourke, P. and Cullen, P.J. (2009). Application of natural antimicrobials for food preservation. Journal of Agricultural and Food Chemistry, 57: 5987–6000

Toncer, O., Karaman, S., Diraz, E., & Tansi, S. (2017). Essential oil composition of Ocimum basilicum L. at different phenological stages in semi-arid environmental conditions. Fresenius Environmental Bulletin.

Tongnuanchan, P., & Benjakul, S. (2014). Essential oils: extraction, bioactivities, and their uses for food preservation. Journal of Food Science 79(7): 231-249.

Trevisan, S., Francioso, O., Quaggiotti, S., & Nardi, S. (2010). Humic substances biological activity at the plant-soil interface: From environmental aspects to molecular factors. Plant Signaling and Behavior. https://doi.org/10.4161/psb.5.6.11211

Trevisan, S., Botton, A., Vaccaro, S., Vezzaro, A., Quaggiotti, S., & Nardi, S. (2011). Humic substances affect Arabidopsis physiology by altering the expression of genes involved in primary metabolism, growth and development. *Environmental and Experimental Botany*. https://doi.org/10.1016/j.envexpbot.2011.04.017

Trombetta, D., Castelli, F., Sarpietro, M. G., Venuti, V., Cristani, M., Daniele, C., … Bisignano, G. (2005). Mechanisms of antibacterial action of three monoterpenes. Antimicrobial Agents and Chemotherapy. https://doi.org/10.1128/AAC.49.6.2474-2478.2005

Tronchet, M., BalaguÉ, C., Kroj, T., Jouanin, L., & Roby, D. (2010). Cinnamyl alcohol dehydrogenases-C and D, key enzymes in lignin biosynthesis, play an essential role in disease resistance in Arabidopsis. Molecular Plant Pathology. https://doi.org/10.1111/j.1364-3703.2009.00578.x

Tzin, V., & Galili, G. (2010). The Biosynthetic Pathways for Shikimate and Aromatic Amino Acids in Arabidopsis thaliana . The Arabidopsis Book. https://doi.org/10.1199/tab.0132

Uphoff, N., Kassam, A., & Stoop, W. (2008). A critical assessment of a desk study comparing crop production systems: The example of the “system of rice intensification” versus “best management practice.” Field Crops Research. https://doi.org/10.1016/j.fcr.2007.12.016

Urcoviche, R. C., Gazim, Z. C., Dragunski, D. C., Barcellos, F. G., & Alberton, O. (2015). Plant growth and essential oil content of Mentha crispa inoculated with arbuscular mycorrhizal fungi under different levels of phosphorus. Industrial Crops and Products. https://doi.org/10.1016/j.indcrop.2015.01.016

Vaccaro, S., Musculo, A., Pizzeghello, D., Spaccini, R., Piccolo, A., & Nardi, S. (2009). Effect of a compost and its water-soluble fractions on key enzymes of nitrogen metabolism in maize seedlings. Journal of Agricultural and Food Chemistry. https://doi.org/10.1021/jf901808s

Vaccaro, L., Lanari, D., Marrocchi, A., & Strappaveccia, G. (2014). Flow approaches towards sustainability. Green Chemistry. https://doi.org/10.1039/c4gc00410h

Vaccaro, S., Ertani, A., Nebbioso, A., Muscolo, A., Quaggiotti, S., Piccolo, A., & Nardi, S. (2015). Humic substances stimulate maize nitrogen assimilation and amino acid metabolism at physiological and molecular level. *Chemical and Biological Technologies in Agriculture*. https://doi.org/10.1186/s40538-015-0033-5

van Rensburg, C. E. J. (2000). An in vitro investigation of the antimicrobial activity of oxifulvic acid. *Journal of Antimicrobial Chemotherapy*, *46*(5), 853–854. https://doi.org/10.1093/jac/46.5.853

Van Rensburg, C. E. J., Dekker, J., Weis, R., Smith, T. L., Janse Van Rensburg, E., & Schneider, J. (2002). Investigation of the anti-HIV properties of oxihumate. Chemotherapy. https://doi.org/10.1159/000064919.

Van Rensburg, C. E. J. (2015). The antiinflammatory properties of humic substances: A mini review. Phytotherapy Research. https://doi.org/10.1002/ptr.5319

Varga, Z., Sabzwari, S. rafay ali, & Vargova, V. (2017). Cardiovascular Risk of Nonsteroidal Anti-Inflammatory Drugs: An Under-Recognized Public Health Issue. Cureus. https://doi.org/10.7759/cureus.1144

Veeken, A., & Hamelers, B. (2002). Sources of Cd, Cu, Pb and Zn in biowaste. *Science of the Total Environment*. https://doi.org/10.1016/S0048-9697(01)01103-2

Verpoorte, R., & Memelink, J. (2002). Engineering secondary metabolite production in plants. Current Opinion in Biotechnology. https://doi.org/10.1016/S0958-1669(02)00308-7

Vinci, G., Cozzolino, V., Mazzei, P., Monda, H., Savy, D., Drosos, M., & Piccolo, A. (2018) a. Effects of Bacillus amyloliquefaciens and different phosphorus sources on Maize plants as revealed by NMR and GC-MS based metabolomics. *Plant and Soil*. https://doi.org/10.1007/s11104-018-3701-y

Vinci, G., Cozzolino, V., Mazzei, P., Monda, H., Spaccini, R., & Piccolo, A. (2018) b . An alternative to mineral phosphorus fertilizers: The combined effects of Trichoderma harzianum and compost on Zea mays, as revealed by 1 H NMR and GC-MS metabolomics. *PLoS ONE*. https://doi.org/10.1371/journal.pone.0209664

Vines, G. (2004). Herbal harvests with a future: towards sustainable sources for medicinal plants. Plantlife International.

Vogt, T. (2010). Phenylpropanoid biosynthesis. Molecular Plant.https://doi.org/10.1093/mp/ssp106

Wang, C., Wang, Z., Peng, A., Hou, J., & Xin, W. (1996). Interaction between fulvic acids of different origins and active oxygen radicals. Science in China, Series C: Life Sciences. https://doi.org/10.1360/yc1996-39-3-267

Wannissorn, B., S. Jarikasem, T. Siriwangchai, and S. Thubthimthed. (2005). Antibacterial properties of essential oils from Thai medicinal plants. *Fitoterapia* 76:233-236

Weckwerth, W., (2003). Metabolomics in systems biology. Annu. Rev. Plant Biol. 54, 669–689.

Welti, R., Shah, J., Li, W., Li, M., Chen, J., Burke, J. J., … Wang, X. (2007). Plant lipidomics: Discerning biological function by profiling plant complex lipids using mass spectrometry. Frontiers in Bioscience. https://doi.org/10.2741/2250

Wiegand, I., Hilpert, K., & Hancock, R. E. W. (2008). Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nature Protocols, 3(2), 163–175. https://doi.org/10.1038/nprot.2007.521

William Allwood, J., Clarke, A., Goodacre, R., & Mur, L. A. J. (2010). Dual metabolomics: A novel approach to understanding plant-pathogen interactions. *Phytochemistry*. https://doi.org/10.1016/j.phytochem.2010.01.006

Wolfender, J.-L., Marti, G., & Ferreira Queiroz, E. (2010). Advances in Techniques for Profiling Crude Extracts and for the Rapid Identificationof Natural Products: Dereplication, Quality Control and Metabolomics. Current Organic Chemistry. https://doi.org/10.2174/138527210792927645

Wollina, U. (2009). Peat: A natural source for dermatocosmetics and dermatotherapeutics. Journal of Cutaneous and Aesthetic Surgery. https://doi.org/10.4103/0974-2077.53094

Wu, W. Y., & Wang, Y. P. (2012). Pharmacological actions and therapeutic applications of Salvia miltiorrhiza depside salt and its active components. Acta Pharmacologica Sinica. https://doi.org/10.1038/aps.2012.126

Xu, D., Raza, W., Yu, G., Zhao, Q., Shen, Q., & Huang, Q. (2012). Phytotoxicity analysis of extracts from compost and their ability to inhibit soil-borne pathogenic fungi and reduce root-knot nematodes. World Journal of Microbiology and Biotechnology, 28(3), 1193–1201. https://doi.org/10.1007/s11274-011-0922-0

Yang, H. L., Hseu, Y. C., Hseu, Y. T., Lu, F. J., Lin, E., & Lai, J. S. (2004). Humic acid induces apoptosis in human premyelocytic leukemia HL-60 cells. Life Sciences. https://doi.org/10.1016/j.lfs.2004.02.033

Yarkova, T. A. (2011). Chemical modification of humic acids by the introduction of indole-containing fragments. Solid Fuel Chemistry. https://doi.org/10.3103/S0361521911040136

Yen, C. C., Lu, F. J., Huang, C. F., Chen, W. K., Liu, S. H., & Lin-Shiau, S. Y. (2007). The diabetogenic effects of the combination of humic acid and arsenic: In vitro and in vivo studies. Toxicology Letters. https://doi.org/10.1016/j.toxlet.2007.05.008

Yogev, A., Raviv, M., Hadar, Y., Cohen, R., & Katan, J. (2006). Plant waste-based composts suppressive to diseases caused by pathogenic Fusarium oxysporum. European Journal of Plant Pathology. https://doi.org/10.1007/s10658-006-9058-8

Zaccardelli, M., Pane, C., Scotti, R., Palese, A.M,, Celano, G. (2012). Use of compost- as biopesticides and biostimulants in orticulture Italus Hort., 19: 17-28, 2012

Zancani M, Petrussa E, Krajňáková J, Casolo V, Spaccini R, Piccolo A, Macrì F, Vianello A (2009). Effect of humic acids on phosphate level and energetic metabolism of tobacco BY-2 suspension cell cultures. Env. Exp. Bot . 65:287–295

Zancani M, Bertolini A, Petrussa E, Krajňáková J, Piccolo A, Spaccini R, Vianello A (2011) Fulvic acid affects proliferation and maturation phases in Abies cephalonica embryogenic cells. J Plant Physiol 168:1226–1233

Zhang, A., Sun, H., Wang, P., Han, Y., & Wang, X. (2012). Modern analytical techniques in metabolomics analysis. *Analyst*. https://doi.org/10.1039/c1an15605e

Zheljazkov, V.D., Callahan, A. and Cantrell, C.L. (2008). Yield and oil composition of 38 basil (*Ocimum basilicum* L.) accessions grown in Mississippi. Journal Agricultural Food Chemistry56: 241-5.

Zheng, W., & Wang, S. Y. (2001). Antioxidant activity and phenolic compounds in selected herbs. Journal of Agricultural & Food Chemistry, 49(11), 5165–5170.

Zykova, M. V., Schepetkin, I. A., Belousov, M. V., Krivoshchekov, S. V., Logvinova, L. A., Bratishko, K. A., … Quinn, M. T. (2018). Physicochemical characterization and antioxidant activity of humic acids isolated from peat of various origins. Molecules, 23(4), 1–15. https://doi.org/10.3390/molecules23040753

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