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DIETARY SUPPLEMENTS WITH GREEN TEA MARKETED IN PORTUGAL: INFORMATION ON WEBSITES, LABELS AND ANTIOXIDANT ACTIVITY

Dissertação submetida à Escola Superior de Saúde, do Politécnico do Porto (ESS-P.Porto) para cumprimento dos requisitos necessários à obtenção do grau de Mestre Farmácia – área de especialização em Farmacoterapia e Farmacoepidemiologia, realizada sob a orientação científica da Prof. Doutora Cláudia Marta Libreiro de Pinho e co-orientação da Prof. Doutora Ana Isabel de Freitas Tavares de Oliveira.

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Abstract

The use of plants and their health benefits have been recognized over the years. Its use and consumption of herbal dietary supplements has increased in the last years in United States and Europe. Internet is one of the most required options to buy dietary supplements (DS). Several studies suggest that information on most sites is incomplete, poorly referenced, and may contain illegal claims. Thus, a conscious and informed decision is essential for consumers. *Camellia sinensis*, also known as green tea, is one of the most consumed plants worldwide and has been recognized by its antioxidant potential over the years. This activity can protect several diseases with an oxidative stress origin. For these reasons, a study was performed to evaluate the quality of information on websites selling dietary supplements with green tea. A modified DISCERN tool was used to evaluate websites from pharmacies/parapharmacies, websites from food health stores, and websites without physical space. Websites scored 1 or 2 at evaluation rating (Low – serious or extensive outcomes). These results reveal the importance of developing a checklist for the online marketing of herbal products, based on DISCERN instrument, or other. These instruments can guide health professionals, who have a very important role in directing patients to high-quality sources of information.

Legal framework for dietary supplements varies among countries, and in Portugal, DS are regulated by the *Direção Geral de Alimentação e Veterinária* (DGAV). Although there are directives published by European Commission, in Portugal, decree-law are incomplete and DS safety is placed on the substance's manufacturer or marketer. In addition, clinical trials are not required for DS in Portugal, which allows adulteration, falsification, contamination and incorrect labels. In Portugal, DS can be sold in pharmacies, parapharmacies, supermarkets, dietetic stores and online, and consumers should be able to make informed and appropriate health care choices. Also, consumers use DS labels (information provided on the packaging) and leaflets to further their understanding about some DS ingredients, relevant indication(s), directions for use, side effects, contraindications and drug-supplements interactions. For these reasons, another study was performed using an adapted form to evaluate legal and scientific information of twenty green tea DS sold in pharmacies, parapharmacies and health food stores in Portugal. From

the legal and scientific points of view none of the websites fulfilled all the analyzed criteria. Seven DS (35.0%) had indications of use similar to those mentioned in the literature. Adverse reactions and drug interactions information were rarely provided by two and three DS, respectively.

Green tea is a complex mixture of several constituents, which include phenolic compounds like catechins, flavonoids, and phenolic acids. This chemical composition is associated with antioxidant activity. Antioxidant products demand has been increasing over the last years. They are referenced as having a protective role in many diseases, like cancer, neurodegenerative and cardiovascular disorders. Considering the lack of legislation, in Portugal, that controls DS prior to its introduction in the market, the last aim of this thesis was to evaluate antioxidant activity of six green tea supplements (three isolated and three mixture of substances/plant extracts). This evaluation was performed by common assays, namely, DPPH, superoxide and Fe^{2+} chelating assays. Results showed that antioxidant activity depends on composition and concentration of each substance present in green tea's DS. In addition, green tea DS studied showed different antioxidant results at different assays performed.

These thesis results may contribute to understand the importance of quality information provided by websites selling DS, and also the importance of regulation regarding its manufacturing and selling.

Resumo

A utilização de plantas e os seus efeitos benéficos têm sido reconhecidos desde sempre. O uso de plantas e, nomeadamente, o consumo de suplementos alimentares à base de plantas tem aumentado significativamente nos últimos anos nos Estados Unidos da América, bem como na Europa. Além disso, a sua procura e aquisição através de websites tem aumentado. No entanto, vários estudos têm demonstrado que a informação presente nestes websites de venda é incompleta, raramente apresenta referências científicas e muitas vezes apresenta alegações ilegais. Posto isto, a escolha correta, consciente e informada do suplemento por parte do consumidor pode estar comprometida. A planta *Camellia sinensis*, conhecida por chá-verde, é uma das mais consumidas em todo o mundo e tem sido reconhecida ao longo dos anos pelo seu potencial antioxidante, estando descrito que possibilita a prevenção de inúmeras doenças com origem no stress oxidativo. Deste modo, foi desenvolvido um estudo no qual foi avaliada a qualidade da informação disponibilizada aos consumidores nos websites de venda de suplementos alimentares contendo chá verde. Essa avaliação foi efetuada em websites de farmácias e parafarmácias, ervanários, lojas de dietética e lojas sem espaço físico recorrendo a uma versão modificada do instrumento DISCERN. Os trinta websites avaliados obtiveram score 1 ou 2 (baixo resultado). Os resultados obtidos demonstram a necessidade de utilizar/criar uma checklist para a venda de suplementos alimentares. Esta pode ser baseada no DISCERN ou noutro instrumento semelhante. Estes instrumentos funcionarão como um guia para os profissionais de saúde, de modo a direcionar e fornecer aos consumidores informações de qualidade.

A regulamentação de suplementos alimentares varia conforme os países e cidades, e em Portugal os mesmos são regulamentados pela *Direção Geral de Alimentação e Veterinária* (DGAV). Apesar da existência de directivas europeias, os decretos-lei que regem os suplementos alimentares em Portugal são incompletos e a responsabilidade da sua qualidade é conferida ao seu fabricante ou produtor. Em adição, ensaios clínicos não são obrigatórios o que facilita adulterações, falsificações, contaminações e existência rótulos errados. Em Portugal, os suplementos são vendidos em farmácias, parafarmácias, supermercados, lojas dietéticas, ervanários e online. Deste modo, os consumidores devem

ter acesso a informações de qualidade para que a sua decisão seja consciente e segura. Além disso, os consumidores recorrem à cartonagem/rotulagem e a folhetos informativos de suplementos alimentares para obter informações acerca dos seus ingredientes, indicações de uso, efeitos adversos, contraindicações e interações. Posto isto, um estudo foi desenvolvido com base num formulário adaptado de modo a avaliar as questões legais e científicas presentes em vinte suplementos, contendo chá verde, vendidos em farmácias, parafarmácias e lojas dietéticas, em Portugal. Do ponto de vista legal e científico nenhum dos websites analisados cumpriu todos os critérios analisados. Sete suplementos alimentares (35,0%) referiram indicações de uso semelhantes às mencionadas na literatura. As informações relativas aos efeitos adversos e às interações com fármacos raramente estavam presentes, surgindo em dois e três suplementos, respetivamente.

O chá verde é uma mistura complexa, contendo compostos fenólicos como as catequinas, flavonóides e ácidos fenólicos. Esta composição está intimamente relacionada com a sua atividade antioxidante. A procura de produtos antioxidantes tem aumentado nos últimos anos. Estes apresentam propriedades preventivas em diversas doenças, como o cancro, doenças neurodegenerativas e cardiovasculares. Devido à legislação em vigor em Portugal para os suplementos alimentares, estes são colocados no mercado sem passarem por um controlo de qualidade obrigatório. Desta forma, o objetivo de outro trabalho incluído na dissertação foi a avaliação da atividade antioxidante de seis suplementos de chá verde (três suplementos com apenas chá verde como ingrediente ativo e três suplementos com mistura de outras substâncias/extratos de plantas). Esta avaliação foi realizada através de ensaios comummente utilizados, nomeadamente, o ensaio do radical DPPH, do radical superóxido e o ensaio da quelação do ferro (Fe^{2+}). Os resultados demonstraram que a atividade antioxidante depende da composição e concentração das substâncias/extratos presentes nos suplementos. Além disso, os resultados demonstraram que os suplementos apresentaram resultados distintos conforme o ensaio realizado.

Os resultados desta tese contribuem para uma melhor compreensão da importância da qualidade da informação disponibilizada em websites que vendem suplementos alimentares, e também da regulamentação de suplementos alimentares em Portugal, relativamente ao seu fabrico e comercialização.

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Abbreviations and Symbols

List of abbreviations

ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
AKT	Protein Kinase B
AMPK	Activated Kinase AMP protein
BBB	Blood Barrier Brain
CAT	Catalase
CLA	Conjugated Linoleic Acid
CYP	Cytochrome P450
DGAV	Direção Geral de Alimentação e Veterinária
DPPH	2,2-diphenyl-1-picrylhydrazyl
DRI	Daily Reference Intake
DS	Dietary Supplement
DSHEA	Dietary Supplements Health Education Act
EC	Epicatechin
ECG	(-)-epicatechin-3-gallate
EDTA	Ethylenediaminetetraacetic Acid
EFSA	European Food Safety Authority
EGC	(-)epicatechin gallate
EGCG	(-)-epigallocatechin gallate
EU	European Union
FDA	Food and Drug Administration
FRAP	Ferric Reducing Ability of Plasma
GI	Gastrointestinal
GSH	Glutathione
GSH-Px	Glutathione peroxidase
IC_{50}	Half maximal inhibitory concentration
IL	Interleukin
INFARMED	Autoridade Nacional do Medicamento e Produtos de Saúde, I.P.
LDL	Low-Density Lipoprotein

MAO	Monoamine Oxidase
NADH	Nicotinamide Adenine Dinucleotide
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NAOPH	Sodium phenoxide
NBT	Nitro-Blue Tetrazolium
NF-kB	Nuclear Factor Kappa-B
NPHs	Natural Health Products
PMS	Phenazine methosulfate
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
SMCs	Skeletal Muscle Cell
SOD	Superoxide dismutase
TGM's	Complex based on the Activation of Transglutaminase
US	United Sates

List of symbols

H_2O_2	Hydrogen Peroxide
NO•	Nitric Oxide
$^1O_2$	Singlet Oxygen
$O_2^{\bullet-}$	Superoxide Anion
O_3	Ozone
$OH^{\cdot}$	Hydroxyl Radical
$ROO^{\cdot}$	Peroxyl Radical

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CHAPTER I

General Introduction

1.1. Dietary Supplements

1.1.1. Worldwide Consumption

The use of plants and their health benefits have been recognized over the years and continues to expand rapidly across the world (Cunha et al., 2012). It is estimated that in Europe, North America and other industrialized regions, more than 50% of the population has already used medicinal plants as a complementary and/or alternative medicine at least once (Santos et al., 2008). Thus, in developed countries up to four billion people (about 80% of global population) rely on herbal medicinal products as primary source of wellness (Bodeker, 2005). As the global use of herbal medicinal products continues to grow and newer products are introduced into the market, public health issues, and concerns surrounding their safety are also increasingly recognized (Ekor, 2014).

DS are defined as concentrated sources of nutrients or other substances with a nutritional or physiological effect that increase the overall dietary intake by supplementing the normal diet (Decreto-Lei n.º136/2003). They are marketed in measured doses (e.g., as pills, tablets, capsules, liquids) (Decreto-Lei n.º136/2003).

The use of DS is widespread, and can be influenced by a number of factors such as gender, age, educational level, socio-economic status, place of residence, and ethnicity. Prevalence data on the regular use of DS by general population over the last two decades are available for several countries. The consumption of DS has increased in the last years in United States (US) and Europe (Assena, 2010; Bailey et al., 2011; De Smet, 2005). The consumption values of DS vary between 22% and 53% in the US (Bailey et al., 2011), Canada (Shakur, Tarasuk, Corey, & O'Connor, 2012), Korea (Lee & Kim, 2009), United Kingdom (Lentjes, Welch, Keogh, Luben, & Khaw, 2015), Sweden (Messerer, Johansson, & Wolk, 2001), Germany (Li, Kaaks, Linseisen, & Rohrmann, 2010) and France (Pouchieu et al., 2013). Studies in Portugal, despite scarce (Felício, 2006), follow this world tendency. For example, a study about DS consumption by Portuguese population concludes that 81% of participants consume or have already consumed DS (38% as teas/infusions/plants) (Fernandes, 2012). Another national study showed that 48% of the participants in Lisboa and Região do Vale do Tejo consumed herbal medicines or herbal supplements (Santos et

al., 2008). DS consumption represents a high globally growing market (in 2016 represented 132.8 billion USD) (Zion Market Research, 2017).

The resurgence of public interest in herbal medicines, including DS, has been attributed to several factors some of which include: i) preference to a healthy lifestyle (e.g., for the improvement of physical performance); (ii) various claims on the efficacy or effectiveness of plant medicines; (iii) the belief that these products might be effective in the prevention and treatment of certain diseases where conventional therapies have proven to be ineffective or inadequate; (iv) side effects of most modern drugs; (vi) improvements in the quality, efficacy, and safety of herbal medicines with the development of science and technology, and (viii) a movement toward self-medication (Bailey, Gahche, Miller, Thomas, & Dwyer, 2013; Dickinson & MacKay, 2014).

1.1.2. Legal Framework

The legal framework for supplements varies among countries, even when they have similar cultures, legal systems, and levels of economic development (Chow et al., 2005). For example, in Brazil, the category "dietary supplement" does not exist, and these products are placed in other food categories such as food for athletes, vitamins and/or mineral supplements, and foodstuffs with functional properties or health claims (da Justa Neves & Caldas, 2015). The distinction between foodstuffs and medicinal products is also clear in the European Union (EU) (European Parliament, 2004), although there are the so-called "borderline products", which contain substances that may have pharmacological effects at a given dose (Lachenmeier & Rehm, 2012). In the US, DS are regulated under the Dietary Supplements Health Education Act (DSHEA) of 1994 as a subset of foods and limited to those taken orally. Manufacturers must hold evidence to support their claims and they cannot make specific disease treatment claims, but only those are related to nutritional support. All products must carry a disclaimer on the label stating that claims have not been reviewed by the US Food and Drug Administration (FDA) (U.S Food & Drug Administration, 2018). In Canada, the majority of these DS are referred to as natural health products (NHPs) and are considered a subset of drugs under a specific set of regulations – the Natural Health Products Regulations. Products must undergo a premarket assessment for safety, quality and efficacy (Government of Canada, 2018).

In the EU some steps were taken, in order to harmonize the regulation of nutritional supplements, health foods, and herbal medicines. For that reason, the European Commission has published directives regulating food supplements (2002/46/EC) and herbal remedies (2004/24/EC and 2004/27/EC) (European Parliament, 2002, 2004). The Directive 2002/46/EC was implemented to safeguard human health, because in some cases excessive intake of vitamins and minerals may be harmful or cause unwanted side effects; therefore, maximum levels are necessary to ensure their safe use in food supplements (European Parliament, 2002). A very important piece of legislation for the food supplements sector is Regulation (EC) No. 1924/2006 on nutrition and health claims. According to this regulation, health claims must be based on and substantiated by generally accepted scientific evidence and must not mislead the consumer (European Parliament, 2006). They need to be authorized by the European Commission and member states under the scrutiny of the European Parliament and Council following an assessment of their scientific substantiation by the European Food Safety Authority (EFSA) (European Parliament, 2006; Quintus & Schweim, 2012).

In Portugal, DS are regulated by *Direção Geral de Alimentação e Veterinária* (DGAV) and according to the Decree-Law No. 136/2003, of 28 June, these products are defined as "concentrated sources of nutrients or other substances with a nutritional or physiological effect, marketed in dose form, with the purpose of supplementing the normal diet" (Decreto-Lei n. º 136/2003). According to Decree-Law No. 136/2003, of 28 June and Decree-Law No. 118/2015, of 23 June the labeling for DS must contain the following requirements: (i) the names of nutrients categories or substances that characterize the product or an indication of the nature of those nutrients or substances; (ii) the recommended daily dose; (iii) warning for daily intake should not be exceeded; (iv) a declaration to the effect that the supplement is not a substitute for a varied diet; (v) warning to keep out of reach of children. In addition, the DS labelling must not contain: (i) any statement attributing to the product properties of preventing, treating, or curing a human disease; and (ii) any mention stating or implying that a balanced and varied diet cannot provide appropriate quantities of nutrients in general (Decreto-Lei n. º 136/2003; Decreto-Lei n. º 118/2015).

Most of the problems associated with the use of traditional and herbal medicines arise mainly from the classification of many of these products as foods or DS in some countries. As such, evidence of quality, efficacy, and safety of these products is not required

before marketing (Ekor, 2014). Clinical trials are not required for DS in Portugal, which may promote adulteration, falsification, contamination and incorrect labels. In Portugal, these products can be sold in pharmacies, supermarkets, dietetic stores and over the internet. Therefore, the safety of DS is an important issue nowadays.

1.1.3. Online Market

Despite their popularity, the benefits of DS in general population are equivocal (Harvey, Korczak, Marron, & Newgreen, 2008; Timbo, Ross, McCarthy, & Lin, 2006). However, risks associated with dietary supplementation are also well documented, and include contamination, inadvertent outcomes and side effects. Furthermore, promotion of supplements may include misleading nutrition labels and health claims (Kwan D, 2009).

The internet is an important source of information for all themes, including health topics (Cooke & Gray, 2002). The Internet is not only used by consumers for the purchase of medicines and DS, but also for searching of health related information (60% of Europeans, 57% of the US population) (European Parliament, 2002; Wolters Kluwer Health, 2012). Consumers that use Internet to access health information and purchase DS are increasing (Thakor, Leach, Gillham, & Esterman, 2011). The difficulty for consumers and health professionals is identifying websites that provide reliable information (Saldanha et al., 2010).

According to the Wolters Kluwer's study from 2012, 65% of respondents in the US trusted the information presented on the Internet (Wolters Kluwer Health, 2012). However, more than 90% of websites contain no warning against possible supplement–drug interactions, adverse reactions, or suitability of use of the DS in children or during pregnancy. In addition, labeling, safety, and efficacy information is not required to be posted on websites selling DS, although its presence would be beneficial for the consumer to make informed purchasing decisions (Jordan & Haywood, 2007).

Several studies showed that information on most websites is incomplete, poorly referenced and many included illegal claims (Jordan & Haywood, 2007; Thakor et al., 2011). Also, many commercial websites containing food and supplement composition data link to a common source and do not contain original information themselves, so there is no guarantee that the information they provide is valid (Saldanha et al., 2010). The presentation

of misleading information represents a serious problem, because most of DS are purchased without clinician's prescription, and many patients do not report their use (Thakor et al., 2011). Of related concern, is the misperception among some consumers that herbal medicines are natural, safe and without risk. Besides that, this online selling method is fast and uncontrolled, with daily updates. So, its oversight is impossible in any country (Schmidt, 2002). Given the magnitude and amount of health information on websites and its impact on health care decisions, it is important to analyze and regulate the quality of information. What is also unclear is whether the type of website (e.g. pharmacy, parapharmacy, health food store) has any influence on the quality of information presented.

1.1.4. Green Tea [*Camellia Sinensis* (L.) O. Kuntze]

Tea is made from leaves of *Camellia Sinensis* (Theaceae family) and is one of the most consumed beverages worldwide (Cunha et al., 2012). It is estimated that about 2.5 million tons of tea are produced each year in the world, with 20% produced as green tea and mainly consumed in Asia and the Middle East (Chacko, Thambi, Kuttan, & Nishigaki, 2010).

The tea plant is believed to have originated in the Southeast Asia (Singhal, Raj, Gupta, & Singh, 2017) and is cultivated in more than 30 countries, including India, China, Sri Lanka, Kenya, Indonesia, Turkey, former Soviet Union, Japan, Iran, Bangladesh, Malawi, Vietnam, and Argentina (Cooper, Morre, & Morre, 2005).

There are at least four types of tea, based on the distinct processing techniques used for each type: white, green, oolong, and black tea (Dostal et al., 2015). Black tea is the major form of tea consumed worldwide and constitutes 78% of the world's tea production. It is produced by crushing tea leaves to allow the release polyphenol oxidase enzyme. This enzyme catalyzes the oxidation of tea catechins and as a consequence, catechins are oxidized and condensed to polymeric polyphenols (thearubigins) and oligomeric polyphenols (theaflavins), which are responsible for the color and flavor characteristics of black tea. Green tea is produced from more mature leaves which are steamed or heated immediately after harvest to minimize oxidation reactions; white tea is processed the least and uses very young leaves and leaf buds; and oolong tea is made by crushing only the rims of the tea leaves and 'fermented' under tightly controlled conditions (Botten, Fugallo, Fraternali, & Molteni, 2015; Reygaert, 2018) (Figure I).

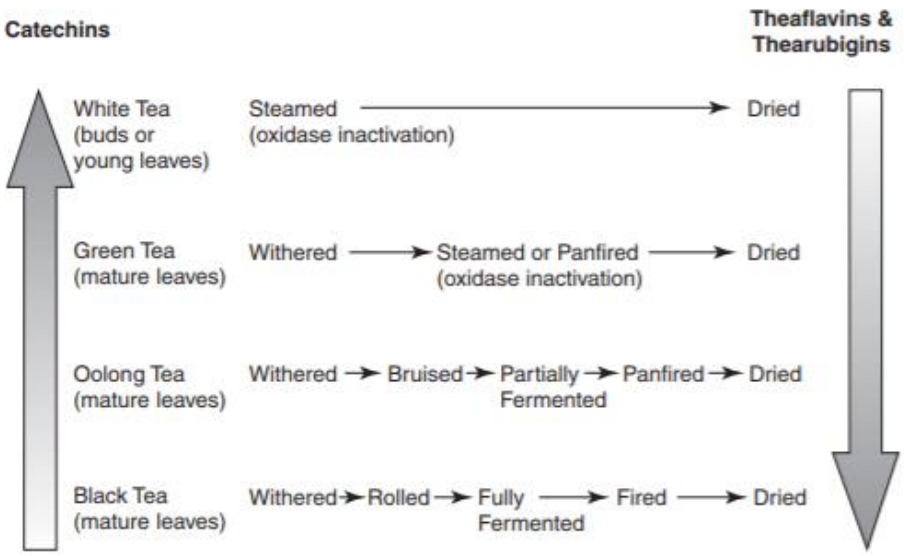

Figure I – Tea extraction processes (Cooper et al., 2005).

Among all of the different teas, however, the most significant effects on human health have been observed with green tea (Chacko et al., 2010). The plant has preventive effects on many diseases, including cardiovascular (Hodgson & Croft, 2010), neurodegenerative (Andrade & Assuncao, 2012) and cancer (Yuan, 2013). Also, antibacterial, anti-inflammatory, antidiabetic and weight loss activities have been associated to green tea (Zaveri, 2006).

1.1.4.1. Chemical Composition

Tea is a complex mixture comprised of several constituents. To produce green tea, fresh leaves of the plant are steamed and heating after harvest (Dostal et al., 2015). This process minimizes oxidation reactions, which results in high content of polyphenols (Dostal et al., 2015), including catechins, flavonoids, and phenolic acids (chlorogenic acid, caffeic acid, gallic acid) (Cunha et al., 2012). These compounds represent 25-35% of the dry weight (Wang & Lei, 2015).

Catechins (20-30% of tea dry weight) include: (-)-epicatechin (EC) (≈ 6%), (-)-epigallocatechin (EGC) (≈ 20%), (-)-epicatechin-3-gallate (ECG) (≈14%), and (-)-epigallocatechin-3-gallate (EGCG) (Wang & Lei, 2015) (Figure II).

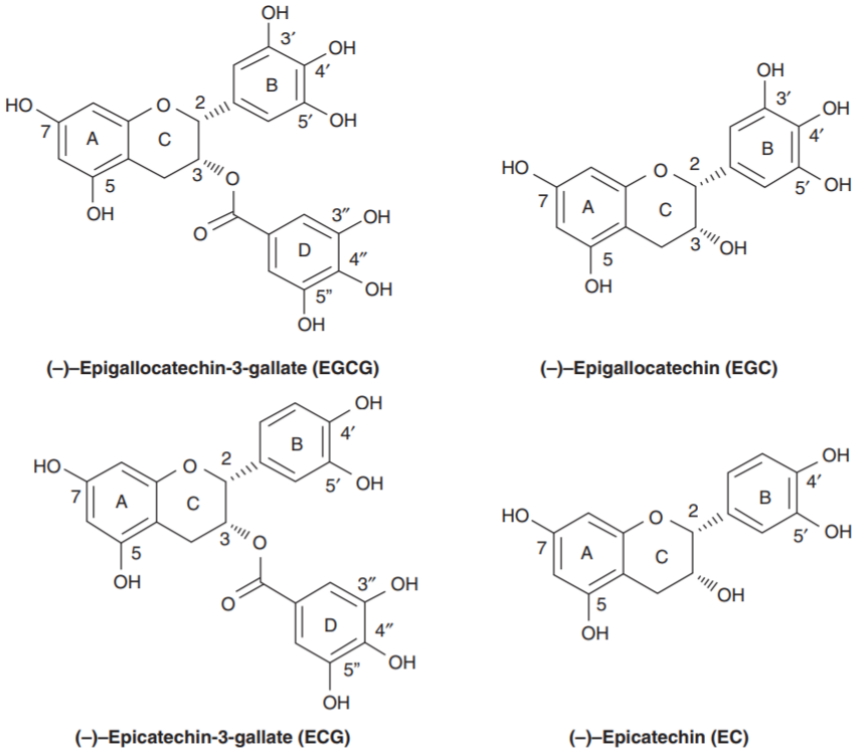

Figure II – Structures of tea catechins (Yang & Pan, 2012).

EGCG is the most abundant catechin in green tea (≈ 60%) (Bao & Peng, 2016; Reygaert, 2018). Green tea contains more catechins than the other teas, mainly because of the way it is processed after harvesting (Narotzki, Levy, Aizenbud, & Reznick, 2013). The amount of catechins in green tea can also be affected by differences in variety, origin, growing conditions, harvesting, and processing conditions (Reygaert, 2018).

Tea leaves also contain lower quantities of other polyphenols such as quercetin, kaempferol, and myricetin as well as alkaloids, such as caffeine and theobromine. One cup of green tea (with 2.5 g of tea leaves brewed for 3 min in 250 ml hot water) usually contains about 300 - 400 mg of polyphenols (130 – 180 mg of EGCG) and between 50 and 100 mg of caffeine (Singhal et al., 2017). In addition, green tea has proteins (mainly enzymes) (15%), carbohydrates (cellulose, pectins, glucose, fructose, sucrose) (5%), lipids (linoleic and linolenic acids), vitamins (B, C and E), pigments (chlorophyll and carotenoids), mineral and trace elements (4-9%) and volatile compounds (0.1%) (Cunha et al., 2012).

1.1.4.2. Absorption and Metabolism of Catechins

In order to be effective in the body, catechins present in green tea need to be bioavailable after consumption. The bioavailability of tea polyphenols is dependent on the molecular size and number of phenolic groups. Only a small fraction of tea catechins present in the intestinal tract after drinking tea can be absorbed, and therefore considered to be bioavailable (Cai et al., 2018). Once in the body, catechins undergo metabolic processing in the liver and small intestine and colon (Reygaert, 2018). After tea consumption, they undergo Phase II and III metabolism in enterocytes or hepatocytes and exerted from bile, feces and urine. Catechins and their conjugated metabolites, catabolized small molecular phenolic acids can be distributed in various organs and tissues, where they perform various biological actions (Warden, Smith, Beecher, Balentine, & Clevidence, 2001) (Figure III).

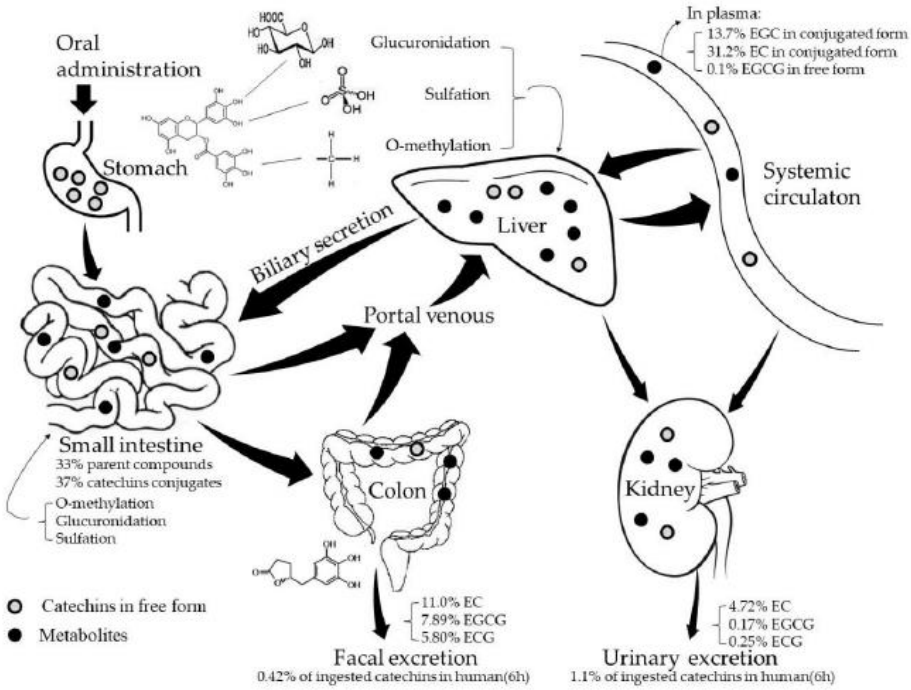

Figure III – Metabolism of green tea catechins (Cai et al., 2018).

The levels of catechins peak concentrations are affected by an individual's metabolism and of course by the amount of catechins in the ingested type of green tea (Reygaert, 2018). The peak concentrations of catechins and their metabolites occur in blood plasma between 1.5 and 2 hours after ingestion and in urine between 4 and 6 hours after ingestion (Reygaert, 2018). Some studies showed that approximately 1.68% of catechins

were present in human's plasma (0.16%), urine (1.1%) and feces (0.42%) after tea ingestion over 6 hours (Cai et al., 2018). Generally, the levels found in the body are directly proportional to the amount of catechins consumed (Clifford, van der Hooft, & Crozier, 2013).

1.1.4.3. Pharmacological Actions of Green Tea

Nowadays green tea has been studied for its potential beneficial health effects (Yang, Wang, & Sheridan, 2018). The plant has been shown to have anticarcinogenic, anti-inflammatory, antimicrobial, and antioxidant properties (Reygaert, 2018).

1.1.4.3.1. Anticarcinogenic Effects

Polyphenols present in green tea promote anticarcinogenic effect. Many studies report that tea polyphenols inhibited the formation and development of tumors at different organ sites, in animal models (Yang, Wang, Lu, & Picinich, 2009). Various molecular targets of EGCG in cancer therapy are the molecules and pathways involved in cell survival and growth (Gaur & Agnihotri, 2014). In fact, catechins control cellular proliferation factors; apoptosis (by augmenting the expression of apoptotic protein expression, namely p53 and p21) and angiogenesis in tumor cells; oxidative stress; NF-κB signaling; pro-inflammatory cytokines; cell cycle; metastasis related enzymes; and suppress the expression of the prostate specific antigen and androgen receptor transcriptional activity (Pandey, Shukla, & Gupta, 2010; Shirakami, Sakai, Kochi, Seishima, & Shimizu, 2016).

1.1.4.3.2. Anti-inflammatory Effects

Tissue injury or exposure to bacterial endotoxins, pro-inflamamtory cytokines, mitogens and viral proteins triggers immune-inflammatory reactions, such as the arachidonic acid pathway. Furthermore, there is an activation of nuclear factor kappa-B (NF-κB), which regulates chronic inflammatory reactions and pro-inflammatory cytokine production (Gaur & Agnihotri, 2014; Maroon, Bost, & Maroon, 2010). Inflammation is a component of many conditions and diseases including aging, arthritis, cancer, cardiovascular diseases, diabetes, and obesity. Green tea polyphenols (especially EGCG) reduce nitric oxide (NO) production, an important mediator of inflammation; scavenge reactive oxygen species (ROS) formed during the inflammatory process from the damaged

cells, thereby downregulating t NF-κB; inhibits pro-inflammatory cytokines, such as interleukin (IL)-1β (Gaur & Agnihotri, 2014).

1.1.4.3.3. Antimicrobial Effects

Green's tea antimicrobial effects are related to catechins (Reygaert, 2018). It blinds bacterial cells membrane, which interferes with various bacterial processes and can damage cell strains (Steinmann, Buer, Pietschmann, & Steinmann, 2013). Green tea has the capacity to diminish bacterial's ability to bind to host cells (Reygaert, 2018) and inhibits the ability of the bacteria to bind to each other to form biofilms, which are significant in pathogenesis (Blanco, Sudano-Roccaro, Spoto, Nostro, & Rusciano, 2005). In addition, damage to the cell membrane also results in loss of function to transmembrane transporter proteins, which are responsible for secretion of toxins and efflux of substance such as antimicrobial agents (Nakayama et al., 2015). Green tea extracts possess antimicrobial activity against the methicillin-resistant *Staphylococcus aureus* (MRSA), *Clostridium botulinum, Bacillus cereus, Escherichia coli, Klebsiella pneumonia and Salmonella* (Saeed et al., 2017; Yoda, Hu, Zhao, & Shimamura, 2004). Also, against *Helicobacter pylori*, viruses (e.g. hepatitis, HIV, rota-, entero- and influenza virus), yeasts, filamentous fungi, *Chlamydia*, mycoplasmas and parasites (Taylor, Hamilton-Miller, & Stapleton, 2005).

1.1.4.3.4. Antioxidant Effects

Green tea has the ability to directly scavenge Reactive oxygen species (ROS) (including superoxide, hydroxyl radical and hydrogen peroxide), it inhibits the redox sensitive transition metal ions and transcription factors, pro-oxidant enzymes, and induce antioxidant enzymes (Xiang et al., 2016). Green tea is rich in antioxidants and its activity are well documented (Saeed et al., 2017). The imbalance between ROS and antioxidants results in oxidative stress, responsible for cellular damage (Saeed et al., 2017). Catechins in green tea increase serum superoxide dismutase activity and aorta catalase enzyme and have a protective role against oxidative stress (Chacko et al., 2010). *In vitro* antioxidant activity assessment methods are often used to screen and confer antioxidant potential to plants or their phytochemicals and sometimes to understand the probable mechanism of action of plant antioxidants (Foyer & Noctor, 2005). Green tea antioxidant activity can be also

revealed by ferric reducing ability of plasma (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assays (Langley-Evans, 2000; Nanjo et al., 1996). Green tea, especially EGCG, may exert its beneficial effects by modulation of mitochondrial functions, alteration of the cell cycle, and mitochondria-related apoptosis (Oliveira et al., 2016).

1.1.4.4. Therapeutic Uses of Green Tea

Research into the effects of green tea on human health has shown that it can be an important dietary factor in the prevention and treatment of various diseases such as arthritis, cancer, cardiovascular diseases, diabetes and obesity, infections, and in neurologic and oral health (Reygaert, 2014).

1.1.4.4.1. Overweight/Obesity and Diabetes

Overweight, obesity and diabetes are emerging as major global health issues, and the closely related metabolic syndrome also predisposes individuals to cardiovascular diseases (Yang, Zhang, Zhang, Huang, & Wang, 2016). Increasing evidence shows that obesity is associated with insulin resistance, chronic low-grade inflammatory responses, and oxidative stress (Suliburska et al., 2012). Obesity is a result of an increase in fat mass which is caused by increase in the size of fat cells (Reygaert, 2018). Green tea has been shown results in these cases by decreasing absorption of lipids and proteins, thus reducing calorie intake. The plant also interferes with activated kinase AMP protein (AMPK) by tea polyphenols that are bioavailable in the liver, skeletal muscle, and adipose tissues; decreases gluconeogenesis and fatty acid synthesis; and increases catabolism (Yang et al., 2016). These mechanisms also decreased total plasma and LDL (low-density lipoprotein) cholesterol, triglycerides, and blood pressure (Suzuki, Pervin, Goto, Isemura, & Nakamura, 2016). All these actions have an important role in obese and/or diabetic people (Grandl & Wolfrum, 2018; Iqbal, Al Qarni, Hawwari, Alghanem, & Ahmed, 2018). Clinical physiology studies show that diminished insulin-stimulated vasodilation is consistently in subject with obesity and/or diabetes (Munir, Chandrasekaran, Gao, & Quon, 2013). This insulin resistance is associated to type 2 diabetes and in some cases the insulin production is also affected (Munir et al., 2013). In this metabolic disorder green tea has been shown to increase insulin receptor sensitivity and stimulate glucose-induced insulin secretion (Fu et al., 2017).

Thereafter the positive effects of green tea supplementation have been observed particularly in the prevention and control of type 2 diabetes (Harrison et al., 2011).

1.1.4.4.2. Cardiovascular Diseases

Several studies suggest that catechins prevent the incidence of detrimental cardiovascular events, and also lower the cardiovascular mortality rate (Bhardwaj & Khanna, 2013). Studies have shown that green tea polyphenols have favorable effects on systemic risk factors and direct effects on the vasculature and platelets that might account for reduced cardiovascular risk (Deka & Vita, 2011; Kushiyama, Shimazaki, Murakami, & Yamashita, 2009; Nakachi, Matsuyama, Miyake, Suganuma, & Imai, 2000). Green tea has the ability to prevent atherosclerosis, hypertension, endothelial dysfunction, ischemic heart diseases, cardiomyopathy, cardiac hypertrophy and congestive heart failure by decreasing oxidative stress, preventing inflammatory events, and reducing platelet aggregation (Bhardwaj & Khanna, 2013). Additionally, they interfere with vascular growth factors, which inhibit vascular smooth muscle cell proliferation and thrombogenesis by suppressing platelet adhesion; could protect vascular endothelial cells and enhance vascular integrity and regulate blood pressure (Bhardwaj & Khanna, 2013). All these documented mechanisms indicate the positive effects of green tea in the prevention and treatment of cardiovascular diseases (Kuriyama et al., 2006).

1.1.4.4.3. Neurodegenerative Diseases

Green tea has been found useful against neurodegenerative diseases such as Alzheimer's disease, and Parkinson's disease (Saeed et al., 2017). EGCG inhibits apoptosis in some neurons and prevents neurotoxicity caused by β-amyloid in neuronal hippocampus cells (Mancini et al., 2017; Saeed et al., 2017). In addition, EGCG also prevented the lesions caused by Parkinson's disease (Levites, Amit, Youdim, & Mandel, 2002). The anti-inflammatory and antioxidant properties of green tea also protect neurons, and green tea metabolites have been shown to cross the BBB (blood brain barrier) (Faria et al., 2011). When flavonoids cross the BBB, they can control molecular traffic and buffer against changes in the systemic circulation (Faria et al., 2011).

1.1.4.4.4. Oral health

Oral health represents another green tea prevention area. Numerous studies have suggested beneficial effects of green tea on oral conditions such as dental caries, periodontal diseases and halitosis (Khurshid, Zafar, Zohaib, Najeeb, & Naseem, 2016). It has been shown that catechins increase the activity of oral peroxidases, preventing the development and progression of periodontitis, and reducing dentin erosion and tooth loss, and it has also a role in improving bad breath (Kushiyama et al., 2009).

The beneficial role of fluoride, present in green tea, is well known as it inhibits bacterial growth and helps remineralization of dental tissues (Zafar, 2015). Also, green tea can abolish bad breath by suppressing anaerobic bacteria and eliminating the production of volatile sulfur compounds, responsible for halitosis (Rassameemasmaung, Phusudsawang, & Sangalungkarn, 2013). Periodontal health is inversely related to green tea consumption (Koyama et al., 2010). Green tea plays a supportive role in the maintenance of periodontal health, where EGCG can restrict the development and colonization of harmful bacteria such as *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Prevotella nigrescens* (Makimura et al., 1993).

1.1.4.5. Toxicity

Bioavailability is a factor positively associated with the severity of green tea's catechins toxicity. For example, fasting increases EGCG bioavailability (Chow et al., 2005) leading to increased severity of adverse effects when consumed on an empty stomach. Also, the risk of adverse effects is likely increased by factors that increase the bioavailability of these flavanols such as genetic polymorphisms in metabolizing enzymes (Miller et al., 2011) and herb-drug interactions.

A number of plant extracts are recognized as posing a drug interaction liability when combined with conventional therapeutics (Albassam & Markowitz, 2017). Green tea has various bioactive compounds that are absorbed, metabolized and eliminated similar to many drugs. Therefore, possible interactions between tea constituents and drugs as competitive substrates or inhibitors are expected. Tea catechins may directly bind to drugs and decrease their absorption, bioavailability and their biological activities. Tea catechins may also increase or decrease the expression (or activities) of drug-metabolizing enzymes and drug transporters (Yang & Pan, 2012).

At modest consumption levels, green tea generally appears unlikely to result in clinically significant effects on the disposition of drugs metabolized by cytochrome P450 (CYP) enzymes (Albassam & Markowitz, 2017). In fact, it can inhibit or promote drug absorption or elimination by interacting with intestinal and hepatic organic anion transport peptides responsible for drug absorption from the intestinal lumen and bloodstream removal (Kim et al., 2017).

Green tea has antioxidant properties, and when some compounds with antioxidant properties are combined, different interactions may occur showing various effects that may be synergistic, antagonistic or additive (Ranjbar Nedamani, Sadeghi Mahoonak, Ghorbani, & Kashaninejad, 2015). Several studies have reported adverse effects related to green tea, and most of 50% were considered sufficiently documented for causality assessment (Pittler, Schmidt, & Ernst, 2005; Shaw, Leon, Kolev, & Murray, 1997; Valli & Giardina, 2002). In general, side-effects were associated with green tea leaves derivates and involved mainly with acute hepatotoxicity (Di Lorenzo et al., 2015). The components most frequently indicated as responsible for hepatotoxicity are catechins and its gallic esters (Galati, Lin, Sultan, & O'Brien, 2006). The hepatotoxicity can be imputed to the capability of EGCG or its metabolites to induce oxidative stress in the liver (Mazzanti et al., 2009).

Caffeine is a methylated xanthine, alkaloid, and an important compound of green tea (Colon & Nerin, 2014). In humans, caffeine acts as a central nervous system stimulant. However, the excessive can produce negative effects in the organism such as anxiety disorders (Colon & Nerin, 2014). In fact, the concomitant use of green tea and caffeine needs an informed supervision. Some drug interactions and/or related problems to caffeine intake are: monoamine oxidase (MAO) inhibitors, including furazolidone, procarbazine and selegiline. In this case, large amounts of caffeine may produce dangerous cardiac arrhythmias or serve hypertension because of caffeine's sympathomimetic side effects of caffeine (Colon & Nerin, 2014). These interactions can be controlled by the amount of caffeine intake. The amount of caffeine in tea beverage is determined by the brewing conditions of time, temperature, leaf size, and amount of tea (Wiseman, Balentine, & Frei, 1997).

Many studies related interactions of green tea with cardiovascular drugs (Werba et al., 2018), potentially leading to reduced drug efficacy or increased risk of drug toxicity (Catapano et al., 2017). EGCG, ECG, EGC, EC are responsible for these interactions (Werba et

al., 2018). These drug interactions reported in humans increased in the last years and now include simvastatin, rosuvastatin, nadolol, sildenafil, tacrolimus and warfarin (Catapano et al., 2017). Green tea contains vitamin K, which probably promotes the antagonism of warfarin by green tea (Catapano et al., 2017). Vitamin K concentration in green tea products depends on the dilution and amount of tea leaves used to brew the tea (Catapano et al., 2017; Mills et al., 2011). Also, the amount of tea consumed per day will play a major role in determining the actual amount of exogenous vitamin K consumption (Catapano et al., 2017; Mills et al., 2011).

Safety and tolerability of green tea's long-term use has not been well defined (Bun, Bun, Guedon, Rosier, & Ollivier, 2006; Teschke, Zhang, Melzer, Schulze, & Eickhoff, 2014). In conclusion, the risk of interactions might be higher in patients who consume high volumes of green tea and infusions with high catechin content (Werba et al., 2018). The risks associated with high dose of green tea are: drinking a large amount may cause neural tube birth defect in infants due to folic acid antagonism due to caffeine, catechins and tannic acids in green tea; discolors the dental plaque but not the teeth itself; increased bleeding time and risk of bladder cancer; stain esthetic restorative material in oral cavity; insomnia, anxiety, irritability, nausea and headaches; stomach upset and diuresis; heart irregularities, tremor and restlessness (Singhal et al., 2017) .

There are a few contraindications known associated with green tea consumption. Individuals with weakened cardiovascular systems, renal diseases, thyroid hyperfunction, elevated susceptibility to spasm, and certain psychic disorders (e.g., panicky states of anxiety) should use green tea with caution. Also, green tea's intake during pregnancy is contraindicated. Lactating women should also limit caffeine intake to avoid sleep disorders in infants as well as children (Wollschlaeger et al., 2003).

1.1.4.6. Posology

Clinical studies reveal that a regular long-term daily ingestion of tea is safe and contributes significantly to the prevention of some diseases; however, the dosage intake is depending on clinical situation and desired therapeutic effect (Wollschlaeger et al., 2003).

A green tea infusion contains about 300 - 400 mg of polyphenols (Singhal et al., 2017), depending on the type of tea, harvesting variables, and brewing methods (Yamimoto, 1997). EMA recommends 1.8–2.2 g of whole or comminuted herbal substance in 100–150

mL of boiling water as an herbal infusion, 3-5 times daily (Committee on Herbal Medicinal Products, 2013). In case of powered herbal substance, EMA recommends 390 mg 3 times daily up to 5 time (Committee on Herbal Medicinal Products, 2013). The use in children and adolescents under 18 years of age is not recommended time (Committee on Herbal Medicinal Products, 2013).

1.2. Oxidative Stress and Antioxidant Defenses

Radical species or free radicals have one or more unpaired electrons in their valence shell. They are produced at low levels during normal physiological conditions and are scavenged by endogenous antioxidant systems (Filaire et al., 2013). The harmful effect of free radicals causing potential biological damage is termed oxidative stress (Donzelli et al., 2006; Valko et al., 2007). In fact, oxidative stress results from the metabolic reactions that use oxygen and represents a disturbance in the equilibrium status of prooxidant/ antioxidant reactions in living organisms (Valko et al., 2007).

ROS are products of normal cellular metabolism and represent the most important class of radical species generated in living organisms (Valko et al., 2007). Chloroplasts and mitochondria are the two main powerhouses and sites of ROS generation within plant cell (Kasote, Katyare, Hegde, & Bae, 2015). Biologically, significant ROS elements include hydroxyl radical ($OH^{\cdot}$), superoxide anion ($O_2^{\cdot-}$), nitric oxide ($NO^{\cdot}$), hydrogen peroxide (H_2O_2), ozone (O_3), singlet oxygen ($^1O_2$), and peroxyl radical ($ROO^{\cdot}$) (Aceti, Beghetti, Martini, Faldella, & Corvaglia, 2018).

Oxidative stress results from the overproduction of ROS in the organism that exceeds the endogenous antioxidant capacity for them to be eliminated (Sifuentes-Franco, Pacheco-Moises, Rodriguez-Carrizalez, & Miranda-Diaz, 2017). ROS imbalance can damage cellular lipids, proteins or inhibit DNA normal function (Valko et al., 2007). Depending on the type of antioxidants, intensity, and time of redox imbalance as well on the type of cells, oxidative stress can influence synthesis of antioxidant enzymes, repair processes, promote inflammatory responses and/or affecting immune system, apoptosis, cell proliferation and damaging key biomolecules (DNA, membrane lipids, enzymes (D'Arena et al., 2018; Kousteni, 2011). For these reasons, oxidative stress has been implicated in several human diseases (Valko et al., 2007). Oxidative stress is involved in several age-

related conditions (cardiovascular diseases, chronic obstructive pulmonary disease, chronic kidney disease, neurodegenerative diseases, and cancer), including sarcopenia and frailty (Liguori et al., 2018).

1.2.1. Endogenous and exogenous antioxidant defenses

Exposure to free radicals has led organisms to develop an extensive range of antioxidant defenses, both endogenous and exogenous (Filaire et al., 2013). These defenses can be modified by exercise, training, nutrition and aging (Dekkers, van Doornen, & Kemper, 1996) and can be divided into three main groups: antioxidant enzymes, chain breaking antioxidants, and transition metal binding proteins (Figure IV).

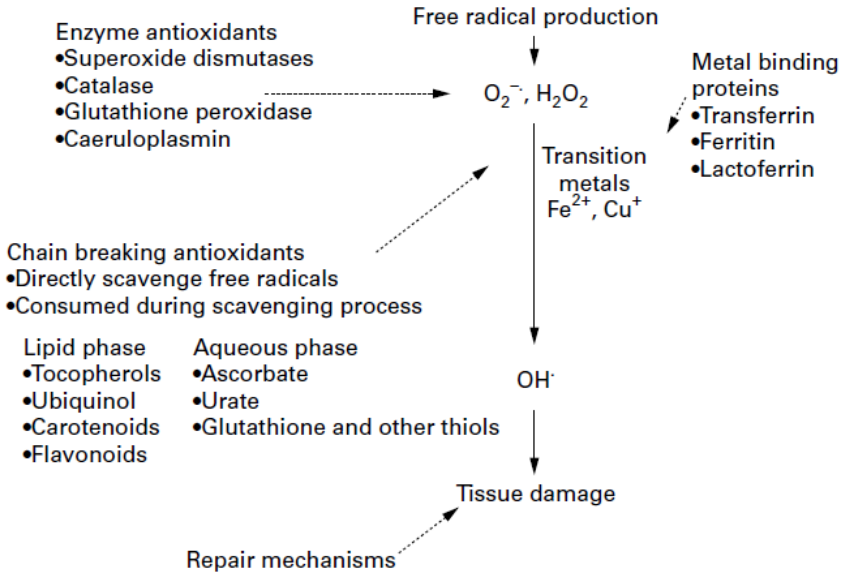

Figure IV – Antioxidant defenses against free radical attack (Halliwell & Gutteridge, 1990)

Antioxidant enzymes catalyze the breakdown of free radical species, usually in intracellular environment. Transition metal binding proteins prevent the interaction of transition metals (e.g. iron and copper) with hydrogen peroxide and superoxide producing highly reactive hydroxyl radicals. Chain breaking antioxidants are electron donors and react preferentially with free radicals before important target molecules are damaged.

Antioxidants can be synthesized *in vivo* or taken as dietary antioxidants (Kasote et al., 2015). Plants have long been a source of exogenous antioxidants (Kasote et al., 2015).

They may act as radical scavengers, peroxide decomposers, hydrogen donors, electron donors, singlet oxygen quenchers, enzyme inhibitors, or metal-chelating agents (Frei, Stocker, & Ames, 1988). Their effect depends on concentration, polarity, medium, and presence of other antioxidants (Sifuentes-Franco et al., 2017).

Antioxidant defense protects biological systems from free radical toxicity and includes both endogenous and exogenous molecules (Liguori et al., 2018). Endogenous antioxidants include enzymatic and non-enzymatic pathways (Liguori et al., 2018). Antioxidant enzymes are superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione-S-transferase and glucose-6-phosphate dehydrogenase (Birben, Sahiner, Sackesen, Erzurum, & Kalayci, 2012). The non-enzymatic antioxidants are molecules that interact with nitrogen species (RNS), and terminate the free radical chain reactions (Liguori et al., 2018). The non-enzymatic antioxidants are bilirubin, α-tocopherol (vitamin E), β-carotene, albumin and uric acid (antioxidant capacity in plasma) (Wu, Kosten, & Zhang, 2013). Exogenous antioxidants consist of low molecular weight antioxidants like ascorbic acid (vitamin C), α-tocopherol, phenolic antioxidants, oil lecithins, selenium, zinc, and drugs such as acetylcysteine (Pisoschi & Pop, 2015).

Plants have an efficient complex enzymatic and non-enzymatic antioxidant defense systems to avoid the toxic effects of free radicals. Plants synthesize antioxidant enzymes (glutathione and ascorbate) within the chloroplast stroma and cytosol using nicotinamide adenine dinucleotide phosphate (NADPH) as the ultimate electron donor. In addition, they generate ascorbic acid during aerobic metabolism (Kasote et al., 2015). In chloroplasts and protoplastids, plants also synthetize vitamin E. Plant antioxidants such as ascorbic acid and flavonoids have been shown to be the best exogenous antioxidants (Foyer & Noctor, 2005). In addition, several studies have showed that polyphenols are the most common natural products to improve the function of stressed mitochondria (Brand et al., 2018).

1.2.2. Antioxidant Activity and Green tea

Fruits, vegetables, and some other natural products which are rich in antioxidants can reduce oxidative stress *in vivo*, and might be an effective approach for preventing diseases (Liu et al., 2018). Most of the observed therapeutic effects of plants have been linked to their potent antioxidant activity (Foyer & Noctor, 2005).

It is estimated that more than 4000 flavonoids have been identified in plants, and the list is constantly growing (Harborne & Williams, 2000). In addition, tea, coffee, red wine, cereals and chocolate contribute for daily total polyphenol intake (Scalbert, Johnson, & Saltmarsh, 2005). Consumption of tea intensifies the body's antioxidant activity, and increase activity of basic antioxidant enzymes such as: glutathione reductase, GSH-Px, CAT, glutathione S-transferase and quinone reductase (Winiarska-Mieczan, 2018). The antioxidant properties of green tea result from their chemical composition (view 1.4.1.1.). It was demonstrated that the antioxidant potential in blood plasma after drinking green tea is increased by 34%, while after drinking black tea the increase is 29% (Serafini, Ghiselli, & Ferro-Luzzi, 1996). The same study also demonstrated that drinking 300 ml of green tea increases the antioxidant capacity of blood plasma by 40% in 30 minutes; however, even 80 minutes after consumption, this value reaches the baseline (Serafini et al., 1996).

Over the last years, several studies have been performed in order to investigate the antioxidant activity of green tea. Table I summarizes the studies carried out in the last ten years (from 2008 to 2018), regarding the evaluation of *in vitro* antioxidant activity of green tea.

Some authors evaluated this antioxidant activity isolated or compared green tea to black tea and other plants (Anesini, Ferraro, & Filip, 2008; Jung, Song, & Choe, 2016; Kerio, Wachira, Wanyoko, & Rotich, 2013; Tsai, Tsai, Chien, Lee, & Tsai, 2008). Also, some studies were developed to understand the correlation between green tea antioxidant proprieties and prevention of several diseases or human conditions (Annunziata et al., 2018; Posadino et al., 2017; Qin, Guo, Li, Wang, & Kim, 2013; Rani, Arora, Kaur, & Manhas, 2018; Wang et al., 2014; Zhong et al., 2011). Results presented in table I highlight the importance of green tea in human health and its capacity as an effective antioxidant.

Table I – Studies regarding the evaluation of in vitro antioxidant activity of green tea, in the last 10 years.

Reference	Aim of the study	Methods	Results/Conclusion
Anesini et al., 2008	Determine the total polyphenol content and *in vitro* antioxidant capacity of green and black tea cultivated and industrialized in Argentina.	– Twelve samples of eight brands. – Assays: Ferric thiocyanate method and DPPH free-radical scavenging assay.	– Green tea showed a higher polyphenol content than black tea. – Antioxidant activities were well correlated with the total polyphenol content.
Tsai et al., 2008	Evaluate the antimicrobial activity against bacteria, total antioxidant capacity and phenolic constituents of methanolic extracts from different herbs compared with those of green tea	– Methanolic extracts. – Assays: DPPH radical scavenging activity, trolox equivalent antioxidant capacity, oxygen radical absorbance capacity.	– Green tea exhibited the highest antioxidant capacity. – Green tea may be an effective potential source of natural antioxidants.
Zhong et al., 2011	Determine the effects of tea catechins incubation on cell proliferation, cell membrane integrity, antioxidant enzyme activities, and antioxidant enzyme mRNA and protein expression in H_2O_2 stress –induced skeletal muscle cells (SMCs) of goats.	– Tea catechins were isolated from green tea leaves. – Assays: CAT activity, CuZn-superoxide dismutase activity, and GPx activity	– CAT and CuZn-SOD mRNA expression levels were increased by different concentrations of tea catechins incubation. – Tea catechins affected antioxidant status in SMCs by modulating antioxidant enzyme activities at mRNA and protein expression levels.
Kerio et al., 2013	Tea products from different cultivars were analyzed for total polyphenols, catechin profiles and *in vitro* antioxidant activities.	– Polyphenols and catechins extracts were prepared from tea leaves. – Assays: DPPH radical scavenging activity.	– Antioxidant potency of teas is dependent on the predominant flavonoid compound, the type of tea cultivar and the processing method. – Cyanidin-3-O-glucoside was the anthocyanin most highly correlated with antioxidant activity.

Table I – Studies regarding the evaluation of in vitro antioxidant activity of green tea, in the last 10 years (Continuation).

References	Aim of the study	Methods	Results/Conclusion
Quin et al., 2013	Evaluate the *in vitro* wound healing potential of chitosan green tea polyphenols complex based on the activation of transglutaminase (TGM) genes in epidermal morphogenesis	– Methanolic extraction (leaves) with different concentrations (30%, 40%, 50%, 60%, 70%), time (2 to 6 h), and temperature (20°C to 80°C). – Assays: reducing power; DPPH method; chelating ability.	– Green tea polyphenols showed antioxidant properties. – The efficacy of chitosan green tea polyphenols in wound healing based on these results may be ascribed to its antioxidant properties and activation of the expression of TGMs.
Wang et al., 2014	The conditions for extracting polysaccharides from tea (*Camellia sinensis* L.) fruit peel were studied (temperature, time, and liquid/solid ratio)	– Polysaccharides from fruit peel of tea. – Assays: FRAP, α-glucosidase inhibitory activity, 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS).	– Remarkable scavenging activity on ABTS, reducing activity, and inhibitory potential against α-glucosidase *in vitro*. – Tea fruit peel is a potentially valuable renewable bioresource for the development of polysaccharide antioxidants.
Jung et al., 2016	Investigate the effects of the addition of gardenia seed, green tea, and cactus pear to rice batter on the chemical quality of lotus root bugak, including lipid oxidation, contents of antioxidants and pigments, and *in vitro* antioxidant activity	– Assays: reducing power and DPPH radical scavenging activity.	– *In vitro* antioxidant activity of lotus root bugak increased with the addition of gardenia seed, green tea, or cactus pear. – Green tea and gardenia seed could improve the health and food functionality of antioxidation for lotus root bugak, respectively.
Posadino et al., 2017	Investigate the impact of Polyphenol E on prostate cancer cells, analyze the potential signals involved and elucidate whether anti- or pro-oxidant effects may be implicated.	– Polyphenon E (standardized green tea extract).	At the tested concentrations, Polyphenol E did not exert any antioxidant activity, eliciting instead a pro-oxidant effect at concentrations 30 and 100 μg/ml (consistent with the observed Polyphenol E cytotoxicity).

Table I – Studies regarding the evaluation of in vitro antioxidant activity of green tea, in the last 10 years (Continuation).

References	Aim of the study	Methods	Results/Conclusion
Rani et al., 2018	Evaluate phenolic compounds as antioxidants and chemopreventive drugs from *Streptomyces cellulosae* strain TES17 isolated from rhizosphere of *Camellia sinensis*	– Strain TES17 isolated from soil sample collected from rhizosphere of tea plant – Assays: DPPH radical scavenging assay; ABTS radical scavenging activity; Superoxide anion scavenging assay; Phosphomolybdenum assay; Reducing power assay; Lipid peroxidation assay.	– The extract of *Streptomyces cellulosae* strain TES17 demonstrated significant antioxidant activity with percentage inhibition of 78.47, 91.08 and 82.08 for DPPH, ABTS and superoxide radical assays at 5 mg/mL, respectively. – The antioxidant capacity of extract was well correlated with its total phenolic content and total flavonoid content. – *S. cellulosae* strain TES17 isolated from the rhizosphere of *Camellia sinensis* plant produces potent compounds with antioxidant activity, further might be developed into therapeutic drugs to combat oxidative stress.
Annunziata et al., 2018	Evaluation of colon bio accessibility and antioxidant activity of tea polyphenolic extract.	– 80% methanolic extract (*v/v*) of tea polyphenols was obtained from green, white and black tea. – Assays: DPPH and ABTS.	– After *in vitro* GI digestion, tea polyphenol bioaccessibility and antioxidant activity are higher in the colon than in the duodenum. – Polyphenols are poorly absorbed in the duodenum. However, they can exert their antioxidant and anti-inflammatory activities in the lower gut, resulting in a novel strategy for the management of gut-related inflammatory diseases.

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CHAPTER II

Quality of information on websites selling dietary supplements with green tea

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Abstract

Introduction: The use of plants and its health benefits have been recognized over the years. Nowadays, internet is one of the most required options to buy dietary supplements. However, several studies suggest that information on most sites is incomplete, poorly referenced, and may contain illegal claims. This study aims, therefore, to evaluate the information on Portuguese websites where supplements with green tea can be purchased.

Methods: Cross-sectional survey of 30 websites, including pharmacies/parapharmacies, health food stores, and online shopping websites. They were identified through Google® in December 2017, and a modified version of DISCERN instrument was used to determine information quality. Rating scores were analyzed and quality was assessed according to the scores and websites content.

Results/Discussion: Overall, all websites scored 1 (20%) or 2 (80%) and no site received a score of 3, 4 or 5. The online shopping websites had the best results. However, in pharmacies and parapharmacies the results were most consistent. It was also observed that in all websites, questions one and four showed the lowest score: "Is it clear what sources of information were used to compile the information on the e-commerce website (other than the author or producer?"; "Does the webpage provide details of additional sources of support and information?". These results can be explained by the legislation related to dietary supplements. In Portugal, as well as in other European countries, many products containing medicinal plants are sold as dietary supplements and are not covered by applicable legislation for herbal medicines. Products with green tea have contraindications, adverse effects and precautions associated with its use. Nevertheless, the majority of websites scored in this study provided insufficient or poor-quality information about these topics.

Conclusion: This study's results strongly support the need for improved website information regarding its selling products. Also, consumer education about the benefits and risks of buying dietary supplements online is needed for, as informed and safe choice.

Keywords: Green tea, Dietary Supplements, DISCERN, Information, Quality, Websites

Introduction

Plants have been used for centuries for food and medicine, and in many developing countries, populations still rely on traditional medicine for primary health care (Prinsloo et al., 2018). The use of herbal remedies, has also been widely accepted in developed countries, including United Kingdom, France and Germany (Braun et al., 2010; Calapai, 2008; Ekor, 2014). Phytotherapy has gained popularity in developed and developing countries because herbal medicines are seen as a safe, nonaggressive and holistic approach to healing (Kristanc & Kreft, 2016).

Nowadays, Internet has become an essential health information searching tool and an easy method to buy dietary supplements (DS). Health consumers are increasingly using the Internet to access health information and purchase health products (Thakor et al., 2011). The advantages of the Internet as a source of health information include easy access to a massive volume of information and ease of updating information. Online health information can make consumers better informed, maximizing, therefore, health outcomes (Jallon, 1997). However, several studies suggest that information on most sites is incomplete, poorly referenced, and may contain illegal claims (Walji et al., 2004), as it is not always written by health professionals. Moreover, most information search engines do not use any kind of filter to select trustworthy information (Batchelor & Ohya, 2009). One concern and public health issue is internet's health information quality. Due to its deregulated nature, it is impossible to control the information that in uploaded online. So, health information on the web varies substantially in quality, accuracy, and readability (Moody et al., 2007). Therefore, the quality of information available online needs to be ensured, and attempts have been made to estimate the individual risk of finding an inadequate information regarding various health conditions (Maloney, Ilic, & Green, 2005).

Camellia Sinensis (L.) O. Kuntze, known as green tea, is one of the most consumed plants in the world, and an important plant used as an antioxidant with abundant health benefits (Hodgson & Croft, 2010). It is also an example of a widely used herbal medicine marketed extensively online. Besides direct green tea consumption, various processed goods (including cosmetics, medicines, and food additives) containing green tea have been developed over the years. Green tea leaves contain antioxidative catechins, what justifies

its many health benefits such as antibacterial, anti-inflammatory, antidiabetic and weight loss activities (Zaveri, 2006).

Given the rapid grown of green tea market (Park, 2008), its easy online purchase, and the adverse reactions, contraindications and interactions associated with its use, it is important that health websites contain high quality information in order to adequately inform consumers. What is also unclear is whether the type of website (e.g. pharmacy, parapharmacy, health food store) has any influence on the quality of information presented.

Similar studies performed with other plants suggest that the information presented on websites is poor (Bessell, Anderson, Sansom, & Hiller, 2003; Thakor et al., 2011). Several solutions have been proposed to address the information quality and reliability issues found on websites. The DISCERN quality index tool is one way to evaluate the reliability and quality of online health information and treatment choices (Khazaal et al., 2009).

For all the above mentioned, this study aims to evaluate the overall website quality selling green tea supplements.

Methods

Search strategy

To select the different websites evaluated in the present work, a Google® search was performed on December 2017, in order to identify websites that sold green tea supplements in Portugal, using the following search terms: [Buy] AND [Green Tea OR *Camellia sinensis*] AND [Capsules OR Tablets] AND [Pharmacy OR Parapharmacy OR Food Health Store].

Inclusion/exclusion criteria

To stratify the results, the following inclusion criteria were applied: a) supplements containing green tea isolated or in combination with other plants and/or substances; b) green tea supplements in capsules or tablets; c) portuguese websites; d) three categories of websites, namely, online pharmacies/parapharmacies, online food health stores, and online websites without a physical space. As for exclusion criteria: websites that only refer green tea information or their biological activities; duplicate websites and supplements. Through

this method, the first ten websites, retrieved by Google®, of each category (thirty in total) were selected.

Data collection procedures

DISCERN instrument is a validated 16-item questionnaire on a continuous rating scale of 1 (definite NO) to 5 (definite YES), used to evaluate quality of written information on health-related websites. Any rating between 1-5 indicates that some of the elements assessed by the items are presented to a certain extent (Chanock et al., 1999).

The 16 questions are categorized into three sections Section 1 (questions 1 to 8) assesses reliability, dependability and trustworthiness of a website (with a maximum score of 40 points) (Chanock et al., 1999). Treatment choices quality information is measured on Section 2 (questions 9 to 15) with a maximum score of 35 points; and Section 3 (question 16), evaluates overall publication quality rating. So, the score that a website can reach would be between 0-80 points and each website was classified as "excellent" (68–80), "good" (55–67), "fair" (42–54), "poor" (29–41), or "very poor" (16–28) (Charnock et al., 1999).

In this work a modified version of DISCERN was used to evaluate the quality of the 30 websites selling dietary supplements containing green tea (Thakor, Leach, Gillham, & Esterman, 2011). Briefly, the modifications consisted of: (i) removing the first three questions of section one due to the lack of relevance to e-commerce websites, (ii) adding specific and objective descriptors for responses to questions 9 through 15 (section two), (iii) changing question 10 from treatment benefit to specific indications for green tea, (iv) dividing question 11 into three separate questions, related on adverse reactions, drug interactions and contraindications, and (v) adding a question on ease of access to information. The final section (question 16) assessed the overall quality rating of online e-commerce websites (Thakor et al., 2011).

The modified DISCERN instrument was tested for face validity by three researchers, on a small sample of six e-commerce websites, consisting of two sites from each category. Data were analyzed using Microsoft Excel® version 2016.

Results

Of the 30 websites scored, modified DISCERN tool indicated that all websites fell below the maximum score (80 points). Applying the modified DISCERN tool, the score average for the questions regarding reliability was 27.1 (in a maximum of 80 possible points). The websites without a physical space scored 27.5, the websites from health food stores scored 27 and finally the websites from pharmacies/parapharmacies scored 26.9.

Regarding overall quality, the maximum score achieved by one of the websites according to DISCERN tool was 37/80 (related to one online food health store), and the lowest one was 19/80 (related to one website without a physical space). The overall average score was 34.8/80 and classified as "poor". Analyzing by categories, websites without a physical space have an average score of 40.3 points, pharmacies/ parapharmacies have an average score of 32.7 points and online health food stores have an average score of 31.3 points. Also, DISCERN instrument rated 9 (30.0%) websites as "very poor" (3 websites from pharmacies/parapharmacies, 5 websites from food health stores, and 1 website without physical space).

Based on the item 16 of the modified DISCERN tool (section 4 – Overall Rating of the Website) all websites scored 1 or 2 (Low – serious or extensive outcomes). Six websites scored 1 (20%) and 24 scored 2 (80%) (Figure I).

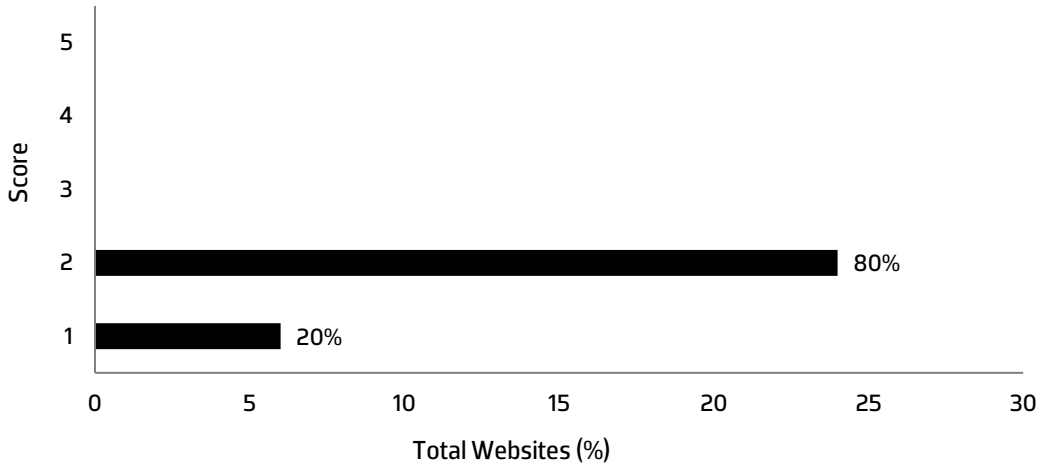

Figure I – Overall quality rating of websites selling dietary supplements (DISCERN instrument question 16)

Analyzing the results per category, 90% of the online pharmacies/parapharmacies, 80% of the online food health stores, and 70% of the websites without a physical space score 2 (Table I).

Table I – Overall quality rating of websites selling dietary supplements per category (DISCERN instrument question 16)

Quality rating	Category of Website						Total	
	Pharmacy/ Parapharmacy		Health Food Store		Websites without physical space			
	N	%	N	%	N	%	N	%
1	1	10	3	30	2	20	6	20
2	9	90	7	70	8	80	24	80
3	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0
Total	10	100	10	100	10	100	30	100

For all the categories, question 15 (Is the information related to green tea most visible and easy to access?) had the best score, with an average of 3.9; 4.0 and 4.1 for websites without a physical space, online pharmacies/parapharmacies, and online health stores, respectively. The questions 6 (Does the webpage describe how green tea works?) and 7 (Does the webpage describe the indications of green tea?) also presented good scores, in comparison to the rest. The questions with lower average (score 1) were 1, 4, 11, 12 and 13 for online health stores; questions 1, 4, 8, 10 and 13 for online pharmacies/parapharmacies; and questions 1, 4, 8, 10 and 11 (Figure II).

The questions 1 [Is it clear what sources of information were used to compile the information on the e-commerce website (other than the author or producer)?] and 4 (Does the webpage provide details of additional sources of support and information?) were common, regarding the lowest score, in all websites categories (Figure II).

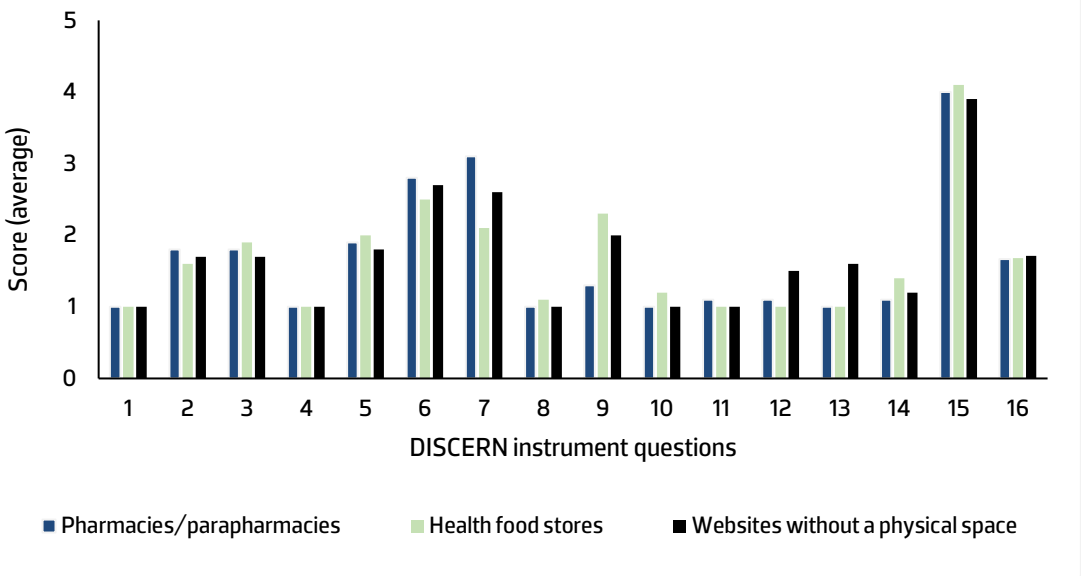

Figure II- Quality ratings for the 30 websites per DISCERN instrument question (average).

Discussion

Online health information can harm as well as heal and many quality criteria have been suggested to help consumers identify misleading, inaccurate, or harmful information (Walji et al., 2004). As more people are searching the web for health-related information, there is a need to provide credible and useful data.

Although there are more than 90 described tools for evaluating online health information, in this study the modified DISCERN tool was chosen because it represents a structured approach to assess information quality published online, on websites selling DS (Thakor et al., 2011).

This study has shown that the information quality on most websites is generally poor. The fact that this study identified almost all websites scoring "poor" is a negative aspect of the present quality of online health information. This finding is consistent with other studies (Martin-Facklam, Kostrzewa, Schubert, Gasse, & Haefeli, 2002; Walji et al., 2004). For example, in their study, Martin-Faclam et al., (2002) revealed that content quality for websites about St. John's wort, as reflected by stating clinically relevant drug-drug interactions and the correct indication for its use, is low. Also, Walji et al., (2004) analyzed 150 websites retrieved from a search for the three most popular herbs: ginseng,

ginkgo and St. John's wort and their purported uses. They concluded that 38 websites (25%) contained statements that could lead to direct physical harm if acted upon, and 145 websites (97%) omitted information. Finally, Thakor et al., 2011 showed that the majority of websites analyzed in their study were poorly rated with a concerning lack of information about the interaction between *Hypericum perforatum* and some drugs. Most sites also failed to provide sufficient information about contraindications and adverse effects of hypericum treatment.

The results observed in this study can be explained by the legislation related to DS. In Portugal, as well as in other European countries, many products containing medicinal plants are sold as DS and are not covered by applicable legislation for herbal medicines (Decreto-Lei n. º 136/2003). In addition, labeling, safety, and efficacy information is not required to be posted on websites selling DS, although its presence would be beneficial for the consumer to make informed purchasing decisions (Jordan & Haywood, 2007).

Thus, manufacturers may claim "indications" for their products without support from appropriate studies (Martin-Facklam et al., 2002).

Also, in many DS information was presented in a sensational, emotive or alarmist way, for example: "This product offers a possibility of a slender body, even for the lazy ones"; "powerful antioxidant"; "the purest green tea extract on the market".

DS with green tea have indications of use, however there are also adverse reactions, contraindications and interactions associated with its use. Nevertheless, the majority of websites analyzed provided insufficient or poor-quality information about these topics. Almost all websites omitted drug interactions and adverse reactions. This is concerning because many consumers perceive "natural" products as safe. Further, many plants that may be safe when used alone interact with conventional medications (Walji et al., 2004). Green tea's EGCG, ECG, EGC, EC are responsible for these interactions (Werba et al., 2018). Many studies related interactions with cardiovascular drugs (Werba et al., 2018), potentially leading to reduced drug efficacy or increased risk of drug toxicity (Catapano et al., 2017). However, the risk of interactions might be higher in patients who consume high volumes of green tea and infusions with high catechin content (Werba et al., 2018). Because pharmacokinetic interactions were mentioned in only one website (from an online health store), patients might combine green tea with other drug therapy, unaware of adverse drug interactions that might reduce the efficacy of their treatment.

Also, none website referred sources of information used to compile the information on e-commerce website (other than the author or producer). Citation of scientific sources may serve as indicators of reliable drug information (Allam, Schulz, & Krauthammer, 2017).

This study also showed best overall results for websites without a physical space. This can be explained because in pharmacies, parapharmacies and health food stores, consumers can always resort to the professional for counselling (Kwan et al., 2008). However, because this counseling cannot happen in websites without a physical space, they need to have more information available.

Conclusion

Both consumers and professionals should be made aware that websites providing information currently have widespread variability in quality. The development of a checklist for the online marketing of herbal products, based on DISCERN instrument, or other similar instrument, may help prevent important concerns from being overlooked, such as drug interactions and contraindications. These instruments can guide health professionals, who have a role in directing patients to high-quality sources of information. It is important to notice that online information is essential for an adequate purchase. Also, consumer education about the benefits and risks of buying dietary supplements online is needed for, as an informed and safe choice. The results of this study strongly support the need for improved online information regarding its selling products.

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Annex I

MODIFIED DISCERN TOOL

SECTION 1: *Is the publication reliable?*

1. Is it clear what sources of information were used to compile the information on the e-commerce website (other than the author or produced)?

Rating this question:

No		Partially		Yes
1	2	3	4	5

HINT:

- Check whether the main claims or statements made about treatment choices are accompanied by a reference to the sources used as evidence, e.g. research study or expert opinion.
- Look for a means of checking the sources used such as a bibliography/reference list or the addresses of the experts or organizations quoted, or external links to the online sources.

Rating note: In order to score a full '5' the publication should fulfill both hints. Lists of additional sources of support and information (Question 7) are not necessarily sources of evidence for the current publication.

2. Is it clear when the information used or detailed on the e-commerce website was produced?

Rating this question:

No		Partially		Yes
1	2	3	4	5

HINT look for:

- dates of the main sources of information used to compile the publication
- date of any revisions of the publication (but not dates of reprinting in the case of print publications)
- date of publication (copyright date).

Rating note: The hints are placed in order of importance – in order to score a full '5' the dates relating to the first hint should be found.

3. Is the information balanced and unbiased?

Rating this question:

No		Partially		Yes
1	2	3	4	5

HINT look for:

- a clear indication of whether the publication is written from a personal or objective point of view
- evidence that a range of sources of information was used to compile the publication, e.g. more than one
- research study or expert evidence of an external assessment of the publication.

Be wary if:

- the publication focuses on the advantages or disadvantages of one particular treatment choice without reference to other possible choices
- the publication relies primarily on evidence from single cases (which may not be typical of people with this condition or of responses to a particular treatment)
- the information is presented in a sensational, emotive or alarmist way.

4. Does the webpage provide details of additional sources of support and information?

Rating this question:

No		Partially		Yes
1	2	3	4	5

HINT look for:

- suggestions for further reading or for details of other organizations providing advice and information about the condition and treatment choices.

5. Does the webpage refer to areas of uncertainty?

Rating this question:

No		Partially		Yes
1	2	3	4	5

HINT:

- Look for discussion of the gaps in knowledge or differences in expert opinion concerning treatment choices.

Be wary if:

- the publication implies that a treatment choice affects everyone in the same way, e.g. 100% success rate with a particular treatment.

SECTION 2: *How good is the quality of information on treatment choices?*

These questions apply to the treatment (or treatments) described in the publication. Self-care is considered a form of treatment throughout this section.

6. Does the webpage describe how Green Tea plant works?

Rating this question:

No		Partially		Yes
1	2	3	4	5

HINT:

- Look for a description of how Green tea acts on the body to achieve its effect.

7. Does the webpage describe the indications of Green Tea?

Rating this question:

No		Partially		Yes
1	2	3	4	5

HINT:

- Benefits can include controlling or getting rid of symptoms, preventing recurrence of the condition and eliminating the condition, both short-term and long-term.

Judging criteria:
1) No
2) Yes, indications not recommended by German Commission E monograph or EMA
3) Yes, a mixture of indications not recommended and recommended by German Commission E monograph and EMA
4) Yes, only one indication recommended by German Commission E monograph or EMA
5) Yes, more than one indication recommended by German Commission E monograph or EMA

8. Are drug interactions mentioned?

Rating this question:

No		Partially		Yes
1	2	3	4	5

Judging criteria:
1) Not mentioned OR wrong drugs mentioned OR includes the statement "no interactions present"
2) At least one correct drug class is mentioned
3) Yes, 2-4 correct drugs are explicitly mentioned
4) Yes, more than 5 drugs are mentioned
5) More than five drug interactions are mentioned AND the consumer is advised to consult a health professional if they have any of the contraindications

8.1. List of drugs interactions mentioned (circle all that apply):

Iron	
Bronchodilators:	E.g. Theophylline
Coronary Vasodilators	
Tamoxifen	
Warfarin	
Stimulants (Central Nervous System)	

9. Are contra-indications of Green Tea treatment mentioned?

Rating this question:

No		Partially		Yes
1	2	3	4	5

Judging criteria:
1) Not mentioned OR wrong contra-indications mentioned OR includes the statement "no contraindications present"
2) At least one correct contraindication is mentioned
3) Yes, 2-4 correct contraindications are explicitly mentioned
4) 5-7 contraindications are mentioned
5) More than seven contraindications are mentioned AND the consumer is advised to consult a health professional if they have any of the contraindications

9.1. List of contraindications mentioned (circle all that apply):

Known hypersensitive to green tea
Breastfeeding
Pregnancy
Hyperthyroidism
Gastric and duodenal ulcers
Cardiovascular problems (hypertension, arrhythmia)
Gastritis
Psychological disorders (anxiety)
Irritable bowel syndrome

10. Are potential adverse effects of Green Tea treatment mentioned?

Rating this question:

No		Partially		Yes
1	2	3	4	5

Judging criteria:
1) Not mentioned OR wrong adverse effects mentioned OR includes the statement "no adverse effect present"
2) At least one correct adverse effect is mentioned
3) Yes, 2-4 correct adverse effects are explicitly mentioned

4) 5-7 adverse effects are mentioned
5) More than seven adverse effects are mentioned AND consumer is advised to consult a health professional if they experience an adverse effect with Green Tea

10.1. List of adverse effect mentioned (circle all that apply):

Irritability
Insomnia
Nervousness
Tachycardia
Anxiety
Gastrointestinal mucosa irritability
Hypertension
Headache
Tremor

11. Does the website describe what would happen if no treatment is used?

Rating this question:

No		Partially		Yes
1	2	3	4	5

HINT: Look for

- a description of the risks and benefits of postponing treatment, of watchful waiting (i.e. monitoring how the condition progresses without treatment) or of permanently forgoing treatment.

12. Does the website describe how the treatment choices affect overall quality of life?

Rating this question:

No		Partially		Yes
1	2	3	4	5

HINT: Look for

- description of the effects of the treatment choices on day-to-day activity
- description of the effects of the treatment choices on relationships with family, friends and careers.

13. Is it clear from the website that there may be more than one possible treatment choice?

Rating this question:

No		Partially		Yes
1	2	3	4	5

HINT: Look for

- a description of who is most likely to benefit from each treatment choice mentioned, and under what circumstances
- suggestions of alternatives to consider or investigate further (including choices not fully described in the publication) before deciding whether to select or reject a particular treatment choice.

14. Does the website provide support for shared decision-making?

Rating this question:

No		Partially		Yes
1	2	3	4	5

HINT:

- Look for suggestions of things to discuss with family, friends, doctors or other health professionals concerning treatment choices.

SECTION 3: *Ease of access to information*

15. Is the information related to Green Tea most visible and easy to access?

Rating this question:

No		Partially		Yes
1	2	3	4	5

Judging criteria:
1) No information is cited on first click of the URL
2) Only the name of the product is cited on first click of the URL; a link to another website is provided
3) Only the name of the product is cited on first click of the URL; a link to another page is provided
4) The product name, price and indications are cited on first click
5) Information relating to indications, drug interactions, contraindications and adverse effects, is cited on first click

SECTION 4: *Overall Rating of the Website*

16. Based on the answers to all of the above questions, rate the overall quality of the website as a source of information about treatment choices

Rating this question:

No (*Serious or extensive shortcomings*)		**Partially** (*Potentially important but not serious shortcomings*)		**Yes** (*Minimal shortcomings*)
1	2	3	4	5

The original version of the DISCERN instrument is freely available from www.discern.org.uk

CHAPTER III

Dietary supplements with green tea marketed in Portugal and labelling information assessment

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Abstract

Introduction: Consumption of dietary supplements (DS) has increased in the last years in United States (US) and Europe. The legal framework for supplements varies among countries, and in Portugal, DS are regulated by *Direção Geral de Alimentação e Veterinária* (DGAV) according to Decree-Law No. 136/2003, 28 June. For that reason, clinical trials are not required for DS in Portugal, facilitating adulteration, falsification, contamination and incorrect labels. Therefore, DS safety is an important issue nowadays. For that reason, this study aims to evaluate overall information quality present in labels/leaflets of DS with green tea, marketed in Portugal.

Methods: A cross-sectional survey was performed from December 2017 to February 2018. Information present in labels/leaflets of green tea DS marketed in Portugal were collected from stores. Products were evaluated according to the information required by law as well as overall composition, indications of use, plant part used, posology, adverse effects, supplement-drug interactions, and contraindications. Descriptive statistics were analysed using Microsoft Excel®.

Results/Discussion: A total of twenty DS were analyzed. In case of DS with multiple active ingredients most common substances, besides green tea, were conjugated linoleic acid (CLA), chromium, L-carnitine and vitamin E. Regarding the evaluation criteria according to legal view, one supplement presented maximum score (7 points) and ten DS had the lowest score (6 points). In case of evaluation criteria according to scientific view, only one supplement presented 8 points (in a maximum of 9). Seven DS (35.0%) had indications of use similar to those mentioned in the literature. Adverse reactions and drug interactions information were rarely provided by two and three DS, respectively.

Conclusion: Portugal compared to other countries in Europe or US has a weak and incomplete legal framework for DS. Manufactures still do not include much information in labels, because DS are still considered as "natural" and safe. New Regulation procedures should be created and a higher control of existing ones should be established in Portugal. Consumers can make informed health care choices, only when fully informed about the safety, efficacy and quality of products consumed for health promotion and disease prevention.

Keywords: *Camellia sinensis*, Dietary Supplements, Green tea, Information, Labels, Quality

Introduction

DS are defined as concentrated sources of nutrients or other substances with a nutritional or physiological effect that increases the overall dietary intake by supplementing the normal diet. They are marketed in measured doses (e.g., as pills, tablets, capsules, liquids) (Decreto-Lei n. º 136/2003). DS consumption has increased in the last years in US and Europe (Assena, 2010; Bailey et al., 2011; De Smet, 2005) as a strategy for disease prevention, for the correction of inadequate lifestyle habits, and for the improvement of physical performance (Bailey et al., 2011; Bailey et al., 2013; Dickinson & MacKay, 2014). In Portugal, DS consumption follows this world tendency (Fernandes, 2012). In 2017, one million and 983 thousand individuals (representing 23.2% of the portuguese residents with 15 years or more) reported the intake of vitamins and/or other supplements over the previous 12 months (Grupo Marktest, 2017).

Supplements legal framework varies among countries, even when they have similar cultures, legal systems, and levels of economic development (Chow et al., 2005). In the European Union (EU) some steps were taken, in order to harmonize the regulation of nutritional supplements, health foods, and herbal medicines. For that reason, European Commission has published directives regulating food supplements (2002/46/EC) and herbal remedies (2004/24/EC and 2004/27/EC) (European Parliament, 2002, 2004). In Portugal, DS are regulated by the *Direção Geral de Alimentação e Veterinária* (DGAV). However, the responsibility for supplement's safety is placed on the manufacturer or marketer of the substance, and not on DGAV.

According to Decree-Law No. 136/2003, 28 June and Decree-Law No. 118/2015, 23 June the DS labelling must contain the following requirements: (i) names of nutrients or substances categories, that characterize the product or an indication of those nutrients or substances nature; (ii) reference daily intake; (iii) warning for daily intake should not be exceeded; (iv) a declaration to the effect that the supplement is not a substitute for a varied diet; (v) warning to keep out of reach of children. In addition, the DS labelling must not contain: (i) any statement attributing to the product properties of preventing, treating, or curing a human disease; and (ii) any mention stating or implying that a balanced and varied diet cannot provide appropriate quantities of nutrients in general (Decreto-Lei n. º 136/2003).

The requirements for placing on the market DS do not comprise a safety assessment, and in accordance with Regulation (EC) No.178/2002, a food is by nature safe. For that reason, clinical trials are not required for DS in Portugal, facilitating, therefore, adulteration, falsification, contamination and incorrect labels. Other problems related to DS consumption include adverse reactions, contraindications and drug-supplements interactions. Therefore, DS safety is an important issue nowadays (DGAV, 2018).

In Portugal, DS can be sold in pharmacies, parapharmacies, supermarkets, dietetic stores and over the internet, and consumers should be able to make informed and appropriate health care choices. Also, consumers use DS labels (information provided on the packaging) and leaflets to increase their understanding about some DS ingredients, relevant indication(s), directions for use, side effects, contraindications and drug-supplements interactions. Consequently, DS labels and leaflets must deliver useful, accurate information and in an understandable manner (Nabors, Lehmkuhl, Parkins, & Drury, 2004).

For all the above mentioned, the present study aimed to evaluate the overall information quality present in labels and leaflets of DS with green tea, marketed in Portugal.

Methods

Search strategy

In order to select the green tea supplements to be used, a Google® search was first performed on December 2017, in order to identify online pharmacies/parapharmacies and online food health stores that marketed these products in Portugal. The following search items were used: [Buy] AND [Green Tea OR *Camellia sinensis*] AND [Capsules OR Tablets] AND [Pharmacy OR Parapharmacy OR Food Health Store]. First DS appearing on websites were selected and information present in labels and leaflets was collected in stores. For data collection an adapted form was used (Jordan & Haywood, 2007) with eighteen questions divided in two parts: (1) Information related to DS legislation; and (2) Information related to scientific topics of the plant. Additionally, information regarding plant´s scientific name; plant part used; presence of standardized extract, presence of leaflet, and presence of vitamins and minerals was also recorded (Annex I).

Inclusion/exclusion criteria

For DS selection, the following inclusion criteria were applied: a) supplements containing green tea isolated or in combination with other plants and/or substances; b) green tea supplements in capsules or tablets; c) portuguese websites; d) two categories of websites, namely, online pharmacies/parapharmacies and online food health stores. As for exclusion criteria: websites that only refer green tea information or their biological activities; duplicate websites and DS. Through this method, the first ten DS, retrieved by Google®, of each category (twenty in total) were selected.

Evaluation criteria and quality score

Each DS was evaluated according to the required information by portuguese law (legal view) and to relevant information for both professional and general public (scientific view). Each item analysed was marked as being 'present' (1 point), 'absent' (0 points) or 'incomplete' (0.5 points).

From a legal view, a point has been assigned when information was in agreement with Decree-Law No. 136/2003, of 28 June and Decree-Law No. 118/2015, of 23 June (maximum of 7 points). From a scientific view information related to composition, indication of use, adverse effects, interactions, contraindications, posology, common and scientific name, and plant part used were analysed based on literature (maximum of 9 points). Descriptive statistics were analyzed using Microsoft Excel®.

Results

Green tea DS selected from pharmacies/parapharmacies and health food stores websites were analyzed, after collecting its labels and leaflets in stores. Regarding the results, products were delivered as tablets (5; 25.0%) and capsules (15; 75.0%); the majority were multi-ingredient (17; 85.0%) versus single (3; 15.0%). Composition of the twenty DS analyzed are presented in Table I. In case of DS with multiple ingredients most common substances were CLA, chromium, L-carnitine and vitamin E (Table I).

Table I – Composition of DS analyzed.

Number of DS	Ingredients of DS
1	*Camellia sinensis*, CLA
2	*Camellia sinensis*, CLA, L-Carnitine
3	*Camellia sinensis*, Chitosan, Vitamin C
4	*Camellia sinensis*, *Taraxacum officinale, Vitis vinífera*
5	*Camellia sinensis*, *Fructus rubis, Coffea arabica, Garcinia cambogia, Euterpe oleacea, Citrus grandis, Fucus vesiculosus,* Chromium
6	*Camellia sinensis*
7	*Camellia sinensis, Centella asiatica, Taraxacum officinale, Phaseolus vulgaris*
8	*Camellia Sinensis*, CLA
9	*Camellia sinensis,* Chitosan, *Garcinia Cambodia*, *Peumus Boldus, Rhamnus sagrada, Passiflora incarnata*
10	*Camellia sinensis*, CLA, *Ilex paraguariensis,* Vitamin E
11	*Camellia sinensis*
12	*Camellia sinensis*, CLA, *Ilex paraguariensis,* Vitamin E
13	*Camellia sinensis,* β-carotene, Vitamin C
14	*Camellia sinensis ,Rhodiola rosea, Coleus forskohlii, Centella asiatica, Equisetum arvense, Ruscus aculeatus, Capsicum frutescens*, Chromium
15	*Camellia sinensis, Rhodiola Rosae L., Coleus forskohlii,* Caffeine, Acetil-L-Tyrosine, Tyrosine, Phenylalaine, β-Sitosterol, Chromium, Selenium, Iodine
16	*Camellia sinensis*
17	*Camellia sinensis*, CLA, Vitamin E
18	*Camellia sinensis*, CLA
19	*Camellia sinensis*, CLA, L-Carnitine
20	*Camellia sinensis*, L-Carnitine, *Paullinia cupana, Citrus aurantium, Theobroma cacau*, Chromium

Evaluation criteria according to legal view

Table II shows, for a legal view, the number of DS that referred each of the seven possible topics. All DS have one point in topic 1 (statement of dietary supplement), 2 (nutrients or substances categories), 4 (reference to daily intake of the DS can´t be exceeded), 5 (DS must not be used as a substitute for varied food regime), 6 (DS should be kept out of the children's reach) and 7 (absence mention that attributes prophylactic, treatment or curative properties in human diseases) (Table II).

In topic 3 (reference daily intake for DS) 0.5 point were attributed for an incomplete answer. For this topic, only DS number 3 obtained a score of one. DS number 4, 10, 12, 14, 15, 17 and 18 have recommended daily dose for other substances present in the products, namely vitamin E, chromium, selenium and iodine (Table II).

Finally, for this part of the form, DS number 3 has the best score (7 points) and 12 supplements had the lowest score (6 points) (Table II).

Evaluation criteria according to scientific view

Table III shows, as for scientific aspects, the number of DS that referred each of the nine possible topics. All DS indicated the green tea's common name, information on active substances and its exact quantity (quantitative composition) and posology (in labels and/or leaflets) (Table III). In case of posology for DS with only green tea as an active ingredient (three DS), all referred the recommended daily intake dose extract.

Regarding green tea's scientific name, 90.0% of DS indicated scientific name (*Camellia sinensis*). However, in two DS it wasn´t correctly written. Analyzing the plant part used in green tea, 50.0% of DS indicated this information in the product (leaves). Seven (35.0%) mentioned indications of use similar to those mentioned in the literature. Warnings about potential adverse effects, contraindications/precautions of use and interactions appears in two (10.0%), sixteen (80.0%) and three (15.0%) DS, respectively. DS number 20 had the highest score (8 points) and numbers 1 and 3 had the lowest score (4 points) (Table III).

Table II – Topics for evaluation criteria according to legal view for the twenty DS

Topics for evaluation criteria according to legal view	Dietary Supplements																				Total	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	N	%
1. A statement that product is a dietary supplement	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	**20**	**100**
2. Description of the nutrients or substances categories which characterize the supplement	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	**20**	**100**
3. Reference daily intake of the DS	0	0	1	0.5	0	0	0	0	0	0.5	0	0.5	0	0.5	0.5	0	0.5	0	0	0.5	**4.5**	**22.5**
4. A disclaimer that the reference daily intake of the DS can´t be exceeded.	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	**20**	**100**
5. A disclaimer that DS must not be used as a substitute for a varied food regime.	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	**20**	**100**
6. A disclaimer that DS should be kept out of the reach of children.	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	**20**	**100**
7. Absence mention that attributes prophylactic, treatment or curative properties in human diseases	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	**20**	**100**
TOTAL (7 possible points)	**6**	**6**	**7**	**6.5**	**6**	**6**	**6**	**6**	**6**	**6.5**	**6**	**6.5**	**6**	**6.5**	**6.5**	**6**	**6.5**	**6**	**6**	**6.5**		

Table III – Topics for evaluation criteria according to scientific view for the twenty DS

Topics for evaluation criteria according to scientific view	Dietary Supplements																				Total	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	N	%
1. Common name	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	**20**	**100**
2. Scientific name	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	**18**	**90**
3. Information on active substances and their exact quantity (quantitative composition)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	**20**	**100**
4. Plant part	0	0	0	1	0	1	1	0	1	0	1	0	1	1	1	1	0	0	0	1	**10**	**50.0**
5. Posology	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	**20**	**100**
6. Indication of use	1	1	0	1	0	0	0	1	0	0	1	0	0	0	1	0	0	0	0	1	**7**	**35**
7. Adverse effects	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	**2**	**10**
8. Contraindications/ Precautions of use	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	**16**	**80**
9. Interactions	0	0	0	0	0	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	**3**	**15**
TOTAL (9 possible points)	**4**	**5**	**4**	**6**	**5**	**6**	**7**	**6**	**6**	**6**	**7**	**6**	**6**	**6**	**7**	**6**	**5**	**5**	**5**	**8**		

Table IV describes adverse effects, contraindications/precautions of use and interactions of green tea in DS with single-ingredient *versus* DS with multi-ingredients. Analyzing the results, only DS with more than one active ingredient referred adverse effects, with the most common being gastrointestinal disorders (DS number 8 and 20). Other adverse reactions listed included irritability and insomnia (DS number 8), and electrolyte imbalances (DS number 20).

Regarding contraindications/precautions of use, sixteen DS related some of this information in the products. The most common was hypersensitivity or allergy to any of the components (mentioned in sixteen DS). DS number 18 also refers hypersensitivity or allergy to milk or fruits of the Rosaceae family. In case of DS with one single active ingredient (green tea) only one DS (DS number 11) didn´t recommend their use in pregnancy or breastfeeding; for DS with multi-ingredients, twelve products mentioned these contraindications. Other contraindications/precautions of use included contraindication to children below 12 years old, patients with cardiac diseases (hypertension), hyperthyroidism, patients with colon inflammatory diseases and abdominal pain, prolonged use, or intake with meals in order to minimize adverse effects (Table IV).

Regarding interactions with drug, DS number 10, 12 and 17 referred substances that interact with green tea metabolization. All these three DS mentioned oral anticoagulants and/or antiplatelet agents as harmful substances (Table IV).

Regarding DS´s uses, seven (35%) products indicated the situations where DS containing green tea can be used. The most common uses related to DS containing green tea were weight control. Some claims present in products included "burn fat", "adipose tissue metabolism", "thermogenic", "appetite control" and "cellulitis".

In this study, DS information regarding the presence of a leaflet and vitamins/minerals was collected. Only five (25.0%) DS had leaflet. Some DS (9; 45.0%) presented vitamins and minerals in its composition, being chromium and vitamin E the most popular (present in four and three DS, respectively). Selenium, iodine and vitamin C were also present in DS (Table I).

Table IV – Adverse effects, contraindications/precautions of use and interactions present in single– ingredient vs multi–ingredient twenty green tea DS.

	DS (single–ingredient)	DS (multi–ingredient)
Adverse effects		**(DS 8)** Gastrointestinal disorders: diarrhea, flatulence, abdominal pain, nausea; Irritability and insomnia **(DS 20)** Electrolyte imbalances (mostly cardiac patients). Abdominal pain and cramps
Contraindications/ Precautions of use	**(DS 6,11,16)** Hypersensitivity or allergy to any of the components **(DS 11)** Not recommended for pregnant women, children and breasting women	**(DS 5, 7, 8, 9, 10, 12, 14, 15, 17, 18, 19, 20)** Hypersensitivity or allergy to any of the components. Not recommended for pregnant and breasting women **(DS 8, 20)** Not recommended for patients with cardiac diseases (hypertension) and hyperthyroidism. Not recommended for children under 12 years old **(DS 5)** In case of thyroid disease alerted medical monitoring **(DS 8)** Recommended DS intake with meals to minimize adverse effects **(DS 14)** DS has chromium: diabetics patients have to monitoring their values **(DS 15)** Not recommended use in athletes undergoing doping. Daily intake at night **(DS 18)** Hypersensitivity or allergy to milk or fruits of the Rosaceae family (e.g. peach, strawberry, apricot, pear fruit) **(DS 20)** Not recommend in patients with colon inflammatory diseases (Crohn's disease) and abdominal pain. Not recommended prolonged use.
Interactions		**(DS 10, 12, 17)** Oral anticoagulants and/or antiplatelet agents as harmful substances

Discussion

DS are classified as food and their purpose is to supplement the normal diet. This definition requires legal provisions of the food law (Noble, 2017). Its consumption requires some control, because these products can have adverse effects and contraindications, possibly endangering consumer's health. Several studies related poor quality control, safety, misbranding, and adulteration to supplementation in many countries (Pawar & Grundel, 2017).

This study provides an information overview of green tea DS marketed in Portugal, and focuses on the legal information required in DS labelling and also on scientific information important for the effectiveness, quality and safety profile of these products. In order to have access to this information, labels and leaflets present in supplements were collected from pharmacies, parapharmacies and food health stores.

Supplements legal framework has been regularly updated in many countries, however, US (FDA) and Canada (Natural Health Products Regulations) have the most controlled guidelines for safety, quality and efficacy at DS selling (Government of Canada, 2018; U.S Food & Drug Administration, 2018). Europe has been following these guidelines and European Commission has published directives regulating food supplements (2002/46/EC) and herbal remedies (2004/24/EC and 2004/27/EC) (European Parliament, 2002, 2004). Despite laws and directives in other countries and Europe, DS in Portugal still have a poor quality and safety control.

According to the portuguese law, DS labelling need to have the following disclaimers: "dietary supplement", "recommended daily dose of the DS can´t be exceeded", "DS must not be used as a substitute for a varied food regime", "DS should be kept out of the children's reach". In addition, DS have to mention "recommended daily dose" and the warning that "DS are not intended to diagnose, treat, cure, or prevent any disease". DS also need to describe the categories of nutrients or substances that characterize the supplement which characterize the supplement. These disclaimers are required in Portugal for selling a DS, and this study showed that information on many DS can be incomplete, poorly referenced, and may contain illegal claims. For example, many DS doesn't reference daily intake of the products. This topic is very important for consumers, because failure recommended daily dose (excessive intake) may cause overdose.

Almost all supplements (87.5%) included the scientific name in the label/leaflet and all the twenty DS analyzed mentioned plant´s common name. However, and based on the literature, 80.0% referred scientific name correctly – *Camellia sinensis* (L.) Kuntze (Committee on Herbal Medicinal Products, 2013; Thorne Research Inc. 2002; Wollschlaeger et al., 2003). None DS analyzed provided scientific evidence in support of the products presented. Scientific information is important to give credibility to these products, but may also serve to lull a consumer into a false sense of safety and effectiveness. Manufacturers and vendors of DS know when and how to use scientific terminology as marketing tools, but when combined with the omission of key safety information, this only increases the likelihood of consumer use and potential harm (Owens, Baergen, & Puckett, 2014).

Plants used for production of herbal medicines are commonly presented as powder obtained from dry plant, dry extract or standardized dry extract (Feltrin & Chorilli, 2010). However, efficacy of dry plant or dry extract cannot be compared to standardized dry extract, simply because there is no standard for the concentration of those substances considered as crucial for the pharmacological action (Feltrin & Chorilli, 2010). It is important to use products with the same extract used in clinical studies (standardized), to ensure the same percentage of content, especially regarding those substances responsible for plant therapeutically effect (Feltrin & Chorilli, 2010). In this study, seven (35.0%) DS mentioned green tea extract in their label. However, only one supplement (number 13) referred the use of green tea standardized dry extract (Standardized Green Tea Extract (leaf) – polyphenols 200 mg [50%]). Posology mentioned in DS with green tea as the only active ingredient (three DS) were comparable to the daily intake dose recommended in literature (daily intake dose extract for DS number 6, 11 and 16 was 1500, 1200 and 1500 mg, respectively). According to Gruenwald, (2004) a daily dose of 300 to 400 mg of polyphenols is typical. For adjuvant treatment of control weight diets and functional asthenia, recommended dose is 1.170 to 1.950 mg of powdered herbal substance daily (corresponding to approximately 35 to 80 mg of caffeine) (Committee on Herbal Medicinal Products, 2013)

Only ten (50%) DS mentioned the leaves as the plant part used in green tea, which is in agreement with literature (Committee on Herbal Medicinal Products, 2013; Gruenwald, 2004; Wollschlaeger et al.,2003).

According to Portuguese law, DS are not intended to diagnose, treat, cure, or prevent any disease, so disease claims are prohibited in these products. However, indications of use

may be a request in products to promote their selling. Only 35% of DS indicate its purpose of use and many of them were not in agreement with German Commission E monograph (treatment of diarrhea and asthenia) or by European Medicines Agency (EMA) (relief of fatigue and sensation of weakness). In this study, all DS with indication of use referred overweight as its main objective. Other recommendations of use/effects like "diuretic effect", "strengthens the immune system" "lowers cholesterol levels" were also referred. Many studies have shown that green tea has a positive effect on the metabolism of lipid by different mechanisms (Huang et al., 2014; Sae-tan et al., 2011; Suzuki-Sugihara et al., 2016). However, controversies regarding the effects of green tea for overweight or obesity still remain (Diepvens et al., 2006; Mielgo-Ayuso et al., 2014).

Green tea is one of the most popular beverages worldwide, and many preventive or therapeutic effects on common diseases are being attributed to its consumption and strongly publicized. This may encourage patients to consume these products as "natural". However, green tea products may interfere by various mechanisms with the absorption, oral bioavailability, or activity of different drugs, potentially leading to reduced drug efficacy or increased risk of drug toxicity (Werba et al., 2015). Therefore, it is necessary to gather information on the ingredients in DS to facilitate medication safety efforts (Pawar & Grundel, 2017). In this study, sixteen (80%) supplements mentioned contraindications/ precautions of use. However, adverse reactions and drug interaction information were rarely provided by two and three DS, respectively. This might happen because it is not mandatory for manufacturers to provide this kind of information on DS label.

Regarding contraindications, hypersensitivity or allergy to any of the components and use in pregnancy or breastfeeding, these warnings were present in almost supplements. This kind of information is important, because a relatively large number of women use DS during pregnancy and believe that they are safe (Baudischova, Straznicka, Pokladnikova, & Jahodar, 2018).

In this study, the most cited drug interactions included the concomitant use of DS containing green tea with oral anticoagulants and/or antiplatelet agents. Most data in the literature embrace the view that green tea may antagonize the effect of warfarin (Cheng, 2007; Izzo, 2012; Izzo, Di Carlo, Borrelli, & Ernst, 2005; Wittkowsky, 2008). However, there is also a general agreement in assigning to the green tea-warfarin interaction a low level of evidence (Izzo, 2012) and a low probability of occurrence (Holbrook et al., 2005). Another

supplement-drug interaction referred in the literature is related to the use of green tea with simvastatin. Green tea can increase the exposure to some cardiovascular drugs (Werba et al., 2018). These drug-interaction increase simvastatin levels (Werba et al., 2018). Consumers need to be aware that there is a wide interindividual variability of the green tea's effect on drug kinetics and, in some subjects, it might be clinically relevant, especially in people who assume large volumes of green tea and/or catechin-enriched products (Werba et al., 2015).

Green tea is generally considered a safe and non-toxic beverage. However, there is a lack of tolerance and safety data on supplements containing green tea. The average cup of green tea, however, contains from 10-50 mg of caffeine and overconsumption may cause irritability, insomnia, nervousness, and tachycardia (Cunha et al., 2012; Mason, 2001). Adverse effects mentioned in DS analyzed included gastrointestinal disorders (diarrhea, flatulence, abdominal pain, nausea), irritability and insomnia, electrolyte imbalances (mostly cardiac patients), and abdominal pain and cramps.

Finally, some DS analyzed in this study have vitamins and minerals (chromium, vitamin E, selenium, iodine, and vitamin C). Annexes I and II of Regulation (EC) No. 1170/2009, defines which vitamins, minerals and units are allowed in DS. The results of the present study showed that all vitamins and minerals are in agreement with literature. DS number 5, 14, 15 and 20 reported having 40 μg of chromium. Vitamin E was reported as present in DS 10, 12 and 17 at 20 mg. DS 3 reported having 80 mg of vitamin C and DS 15 has selenium (200 μg) and iodine (150 μg). Doses and units for all DS are in agreement to Annexes I and II of Regulation (EC) No. 1170/2009 (Parlamento Europeu, 2009).

Conclusion

Portugal compared to other countries in Europe or US has a weak and incomplete legal framework for DS. Only seven disclaimers are required by Portuguese law, and manufactures still do not include all of them in label and/or leaflet of DS. Scientific information is not required in DS marketed in Portugal. This information's omission can promote several drug–DS interactions. These interactions can originate adverse effects in consumers, which could endanger them and consequently overload health care facilities them in danger and overload hospitals and health centers. Stimulating Innovation Management of Polypharmacy and Adherence in The Elderly (SIMPATHY) project estimates that 8.6 million hospital admissions in Europe every year are caused by adverse drug event (SIMPATHY Consortium, 2017). For these reasons, new rules should be created and a higher control of existing ones should be established in Portugal and Europe for safety of their population. This study suggests that existing DS labels may not be satisfactory and require improvements to better support safe and appropriate use.

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Annex I

Data collection form of information in DS containing green tea [adapted from Jordan & Haywood (2007)]

Pharmaceutical form _____

Type of Dietary Supplement (single- or multi-ingredient) _____

Part I – Information about food supplements legislation

A statement that product is a dietary supplement
Presence of supplements' nutritional categories or substances that characterize the product or a specific reference to its nature.
A disclaimer that DS are not intended to diagnose, treat, cure, or prevent any disease.
Reference daily intake of the DS
A disclaimer that the recommended daily dose of the DS can´t be exceeded.
A disclaimer that DS must not be used as a substitute for a varied food regime.
A disclaimer that DS should be kept out of the reach of children

Part II – Scientific plant monograph information

Vulgar name
Scientific name
Information on active substances and their exact quantity (quantitative composition)
Plant part
Posology
Indication of use
Adverse effects
Contraindications/precautions of use
Interactions

Part III – Supplementary questions

Patient information leaflet
Vitamins and minerals present (units)

CHAPTER IV

Antioxidant activity of dietary supplements with green tea marketed in Portugal

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Abstract

Introduction: Green tea is recognized by its antioxidant potential. Nowadays, the marketing of DS claiming protection against free radicals has increased. However, most labels include no data about its antioxidant properties. So, this study aims to evaluate antioxidant activity of six green tea DS sold in pharmacies and health foods stores in Portugal.

Methods: Six commercial DS containing green tea were evaluated for *in vitro* antioxidant capacity by different methodologies: antiradical activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH), superoxide and metal chelating activity. EDTA, ascorbic acid and quercetin were used as standards.

Results/Discussion: Great variability in antioxidant activity was seen among all DS, as well as different patterns of antioxidant capacity depending on the method used. Scavenging activities of the Fe^{2+} chelating, DPPH and superoxide radical for all DS studied were observed in concentration-dependent patterns. For Fe^{2+} chelating activity assay, only sample B showed antioxidant results (IC_{50} = 2681.6 ± 92.0 μg/mL). Sample E showed the best IC_{50} value for DPPH assay (31.6 ± 2.3 μg/mL). In superoxide assay IC_{50} values range from 44.7 to 265.3 μg/mL. In this study DS with only green tea as active ingredient showed the best results, with an IC_{50} value of 31.6 ± 2.3 μg/mL. In all samples (except for sample C), and for superoxide radical scavenging activity, IC_{50} values were lower than the positive control, ascorbic acid (IC_{50} = 77.5 ± 10.9 μg/mL), a well-known antioxidant. The variety of results obtained may be related to the composition and concentration of samples studied and the antioxidant mechanisms underlying.

Conclusion: *In vitro* antioxidant activity of commercial dietary antioxidant supplements varies considerably among products, which is expected given the different botanical origins, different preparation processes, formulations, and concentrations. DS containing green tea with other substances didn´t show better results than DS with only green tea extract. These results show the need to standardize DS in terms of its antioxidant capacity to match required doses to the oxidative status of consumers.

Keywords: Antioxidant Activity, *Camellia Sinensis*, Dietary Supplements, DPPH, Green Tea, Metal Chelating Activity, Superoxide

Introduction

Tea is made from leaves of *Camellia sinensis* and is one of the most consumed beverages worldwide (Cunha et al., 2012). Among all of the different teas, the most significant effects on human health have been observed with green tea (Chacko et al., 2010). This plant has preventive effects on many diseases, including cardiovascular (Hodgson & Croft, 2010), neurodegenerative (Andrade & Assuncao, 2012) and cancer (Yuan, 2013), and biological activities like antibacterial, anti-inflammatory, and antidiabetic have been associated to green tea (Zaveri, 2006). Green tea is also one of the most common natural ingredients included in over-the-counter weight loss products available in many countries, because its extracts contain caffeine and catechin polyphenols (Janssens et al., 2016).

Green tea is a complex mixture of several constituents, which include phenolic compounds (Dostal et al., 2015) like catechins, flavonoids, and phenolic acids (chlorogenic acid, caffeic acid, gallic acid) (Cunha et al., 2012). This chemical composition is associated with antioxidant activity, by the ability to directly scavenge reactive oxygen species (ROS) (Xiang et al., 2016).

ROS are products of normal cellular metabolism and represent the most important class of radical species generated in living organisms (Valko et al., 2007). The imbalance between ROS and antioxidants results in oxidative stress, responsible for cellular damage (Saeed et al., 2017). Oxidative stress is involved in several age-related conditions (cardiovascular diseases, chronic obstructive pulmonary disease, chronic kidney disease, neurodegenerative diseases, and cancer) (Valko et al., 2007).

Over the last years, several studies have been performed in order to investigate the antioxidant activity of green tea using either *in vitro* or *in vivo* approaches (Anesini et al., 2008; Frei & Higdon, 2003; Jung et al., 2016; Kerio et al., 2013). Tea polyphenols act as antioxidants *in vitro* by scavenging ROS and reactive nitrogen species (RNS) and chelating redox-active transition metal ions. They may also function indirectly as antioxidants through inhibition of the redox-sensitive transcription factors; inhibition of pro-oxidant enzymes; and induction of phase II and antioxidant enzymes (Frei & Higdon, 2003).

In vitro antioxidant activity assessment methods are often used to screen and confer antioxidant potential to plants or their phytochemicals and sometimes to understand the possible mechanism of action of plant antioxidants (Foyer & Noctor, 2005). Green tea

antioxidant activity can be revealed by different antioxidant assays like 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging and metal chelating activity (Langley-Evans, 2000; Nanjo et al., 1996; Valentao et al., 2001).

According to Yang et al. (2011) DS provide 25% of dietary total antioxidant capacity in US adults. In the last years, it has become easier to find in the market several new products claiming antioxidant properties. Moreover, these are becoming an important parameter to assess products' quality and in the future antioxidant properties can be part of DS labelling (Almeida et al., 2011; Costa et al., 2012). Polyphenolic phytochemicals are the most frequently occurring phytochemicals in DS with *in vitro* antioxidant properties (Proteggente et al., 2002). As mentioned above, antioxidant activity of plant extracts or purified bioactive compounds has been intensely researched. However, data regarding antioxidant activity of formulations already marketed is scarce (Almeida et al., 2011; Costa et al., 2012). Data, in literature, regarding antioxidant properties in DS containing green tea is also spare. So, this study aims to evaluate antioxidant activity of six green tea DS marketed in Portugal, using different antioxidant assays.

Methods

Chemicals

Nitroblue tetrazolium (NBT), 2,2-diphenyl-1-picrylhydrazyl (DPPH), reduced form of nicotinamide adenine dinucleotide (NADH), N-phenylmethazonium methosulfate (PMS), quercetin, and ferrozine were from Sigma-Aldrich (St. Louis, MO, USA). Ascorbic acid and pyruvate were purchased from Panreac (Barcelona, Spain). Ethylenediaminetetraacetic acid (EDTA) was purchased from VWR (Portugal). All other chemicals were of analytical reagent-grade.

Samples and Samples Preparation

Samples were DS containing green tea commercially available in Portugal and labeled with antioxidant activity. Six samples (three DS with only green tea as an active ingredient and three DS with multi-ingredients) were selected from pharmacies, parapharmacies and health food stores. In order to compare its antioxidant activity, samples were prepared using

the formulation available: pill and capsule. Samples A, C and D are mixtures, whereas samples B, E and F represent green tea only (Table I).

Table I – Composition of study samples

Sample	Composition	Formulation	Label's recommended daily dose
A	Green Tea and Grape Seeds Lyophilized Coextract *(gallic acid 20% procyanidins)*, 300 mg; Dandelion (root), 60 mg; *(caffeic acid derivatives 0.18%)*; Green tea leaf (powder), 548 mg	Capsule	Three capsules/day
B	Green tea (*Camellia sinensis* (L.) Kuntze) leaf *(minimum of 2% caffeine)*, 300 mg	Capsule	Two capsules before breakfast + two capsules before lunch
C	Green tea *(minimum of 6% caffeine)*, 300 mg; Guarana (*minimum of 22% caffeine)*, 300 mg; Bitter orange, 300 mg; L-carnitine, 200 mg; Cocoa, 100 mg; Choline, 82.5 mg; Chromium, 40 μg	Pill	Two tablets/day (after meals)
D	Selenium, 200 μg; Chromium, 160 μg; Iodine, 160 μg; Green tea extract, 520 mg; Acetyl-L-tyrosine, 400 mg; Caffeine, 400 mg; *Rhodiola rosea*, 320 mg; *Coleus forskohlii*, 320 mg; Tyrosine, 200 mg; β-Sitosterol, 60 mg; Phenylalanine, 80 mg	Capsule	In the first week start with one capsule 30min before breakfast and before lunch. In the second week take two capsules each time.
E	Green tea leaf, 500 mg	Capsule	Two capsules after breakfast + one capsule before lunch
F	Standardized Green Tea Extract (leaf) *polyphenols 200 mg (50%)*, 400 mg; Green tea leaf (powder), 100 mg	Capsule	one to two capsules/day at meals

Each formulation was weighted and recommended daily dose of each DS was dissolved in 150 mL of distilled water in order to obtain the concentration of the stock-solution. Pills and the inner part of the capsules were dissolved in distilled water. Several dilutions of each DS were prepared to perform the antioxidant activity assays.

Antioxidant Activity

Antioxidant activity of all samples was evaluated by DPPH radical scavenging activity, superoxide radical scavenging activity, and Fe^{2+} chelating activity. The sample concentration providing 50% inhibition (IC_{50}) was obtained by plotting the inhibition percentage against sample concentrations. EDTA, ascorbic acid and quercetin were used as standards.

DPPH radical scavenging activity

DPPH scavenging procedure was performed in accordance with Lima et al., (2007) with minor modifications. After addition of 19.4 μL of each sample to DPPH (175 μL), the reduction of DPPH absorption was measured at 515 nm using a plate reader spectrophotometer. Radical scavenging ability was calculated using the following formula:

$$DPPH \text{ inhibition } (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$ A = Absorbance

Superoxide radical scavenging activity

This procedure was performed using the PMS-NADH nonenzymatic assay as previously described by Valentao et al., (2001). The reaction mixture used was: 26.1 μL of each sample + 75 μL NADH (166 μM) + 150 μL NBT (43 μM). After incubation at 30ºC for 3 min, 10 μL of PMS (70.5 μM) was added. The absorbance was determined over time at 560 nm. Superoxide radical scavenging ability was calculated using the following formula:

$$\text{Superoxide Radical Scavenging ability } (\%) = 100 \text{ x } [(\text{AC-AS})/\text{AC}],$$

AC = absorbance of the control

AS = absorbance of the sample (DS or standard)

Fe^{2+} chelating activity

This procedure was performed in accordance with Russo et al., (2005) with some modifications. Briefly, 50 μL of each sample at different dilutions were added to 50 μL of a ferrous sulphate solution ($FeSO_4$) (0.12 mM of) and 50 μL of ferrozine (0.6 mM). The mixture

was then shaken vigorously and left at room temperature for 10 min. After incubation, absorbance was measured spectrophotometrically at 562 nm. The results were expressed as percentage of inhibition of the ferrozine – Fe^{2+} complex formation (Russo et al., 2005).

Statistical Analysis

GraphPad Prism® 7.0 (GraphPad Software, Inc; San Diego, USA) was used for statistical analysis. Data were presented as mean ± SD of at least three independent experiments.

Results

In this study, antioxidant activities of DS containing green tea were evaluated by applying three common methods, iron chelating assay and DPPH and superoxide radical scavenging activity. All the DS proved to have free radical scavenging activity but to different extent (Table II). Scavenging activities of the Fe^{2+} chelating, DPPH and superoxide radical for all DS studied were observed in concentration-dependent patterns (data not shown).

Table II - Antioxidant activities of studied DS samples.

Sample	IC_{50} (μg/mL)		
	Antioxidant Assay		
	DPPH free radical Scavenging	Superoxide radical scavenging Activity	Fe^{2+} chelating ability
A	39.3 ± 16.7	63.6 ± 7.6	n.d.
B	34.0 ± 17.4	62.8 ± 4.5	2681.6 ± 92.0
C	45.5 ± 1.0	265.3 ± 5.4	n.d.
D	n.d.	57.7 ± 0.8	n.d.
E	31.6 ± 2.3	65.6 ± 0.3	n.d.
F	n.d.	44.7 ± 3.2	n.d.

n.d.: not determined

In the measurement of DS scavenger activity against the DPPH radical, sample E showed the best IC_{50} value (IC_{50} = 31.6 ± 2.3 µg/mL) when compared to other samples. Antioxidant activity of tested samples, based on IC_{50} values, followed the order: sample E > sample B > sample A > sample C. In samples D and F, the determination was not possible, in the range of tested concentrations. According to the results, none DS is as good as the standard quercetin (IC_{50} = 5.2 ± 0.1 µg/mL (Table III).

Analysing the results for superoxide radical scavenging activity, best IC_{50} value was shown by sample F (44.7 ± 3.2 µg/mL), followed by samples D, B, A, E and C. In all samples (except for sample C), IC_{50} values were lower than the positive control, ascorbic acid (IC_{50} = 77.5 ± 10.9 µg/mL, a well-known antioxidant (Table III).

Finally, in case of iron chelating activity, only sample F presented an IC_{50} value (2681.6 ± 92.0 µg/mL). Again, and according to the results, all DS are not as good as the standard EDTA (5.4 ± 0.2 µg/mL). (Table III).

Table III – IC_{50} values for standards.

Antioxidant Assay	Positive control	IC_{50} value (µg/mL)
DPPH	Quercetin	5.2 ± 0.1
Superoxide	Ascorbic acid	77.5 ± 10.9
Fe^{2+} chelating activity	EDTA	5.4 ± 0.2

Discussion

Plants are a rich source of natural bioactive compounds and many of them can act as antioxidants (Noreen, Semmar, Farman, & McCullagh, 2017). Polyphenols are the most interesting group of green tea leaf components, and therefore green tea can be considered an important dietary source of polyphenols, particularly flavonoids (Cabrera, Artacho, & Gimenez, 2006). The role of flavonoids and related compounds, as components responsible, in part, for the protective effects of a fruit- and vegetable-rich diet has become an increasingly important area of human nutrition research (Del Rio et al., 2013).

Dietary sources and intake of polyphenols contribute for health promotion and reduce the risk of chronic diseases (Bolling, McKay, & Blumberg, 2010). *In vitro*, animal

studies, and clinical trials employing biomarkers of oxidative stress status, provide strong evidence that green tea polyphenols may play a role in the risk and pathogenesis of several chronic diseases, especially cardiovascular disease and cancer. In addition, several studies have showed antidiabetic, antibacterial, anti-inflammatory, and hypocholesterolemic properties of green tea (Feng et al., 2001; Fernandez, Pablos, Martin, & Gonzalez, 2002; Pan, Jankovic, & Le, 2003).

DS with antioxidant properties are marketed as single or multi-ingredient products, from natural or synthetic origin, and are presented in a variety of forms including tablets, pills, capsules, powders, drinks or supplements bars (Almeida, Barreira, Oliveira, & Ferreira, 2011). In this study six DS were analysed. Its selection was based in the different components included in the available formulations, either as single active components or in different combinations (Table I). The antioxidant components comprise hydrophilic (e.g. polyphenols) molecules and natural extracts (e.g. *Rhodiola rosea*, *Paullinia cupana* and *Coleus forskohlii*) (Arantes et al., 2018; Sista et al., 2018; Takshak & Agrawal, 2015).

Tea polyphenols act as antioxidants *in vitro* by scavenging ROS and RNS and chelating redox-active transition metal ions (Lima et al., 2007). Therefore, in order to extensively characterize the antioxidant potential of DS with green tea extracts there is a need for combining several different methods. In this study, antioxidant activities of different DS samples were assessed, by applying different methods, based on metal ion chelation and free radical scavenging. As expected, results regarding antioxidant activity of the different commercial dietary antioxidant supplements show great variability, reflecting their diverse composition and concentrations (Table II).

DPPH radical scavenging assay is a commonly method used due to excellent reproducibility, stability, commercial availability and by the fact that it is an easy measurement method (Silva et al., 2004). DPPH is a stable nitrogen-centered free radical, the color of which changes from purple to yellow upon reduction by either the process of hydrogen- or electron- donation (Nabavi, Ebrahimzadeh, Nabavi, Eslami, & Dehpour, 2011). As a result of a color change from purple to yellow the absorbance decreased when the DPPH radical was scavenged by an antioxidant through donation of hydrogen to form a stable DPPH-H molecule (Matthaus, 2002). Several studies have shown green tea antioxidant activity by DPPH scavenging assay (Frei & Higdon, 2003; McKay & Blumberg, 2002; Satoh, Tohyama, & Nishimura, 2005). Dietary supplements A, B, C and E were able to

reduce DPPH in a dose dependent manner with the IC_{50} values ranging from 31.6 to 45.5 µg/mL. In this study sample E has the lowest IC_{50} (31.6 ± 2.3 µg/mL, followed by sample B (34.0 ± 17.4 µg/mL). These two samples represent DS with only green tea has a bioactive ingredient. A lower IC_{50} value represents a stronger DPPH scavenging capacity, and a higher antioxidant potential (Lima et al., 2007).

The antiradical activity against superoxide radical can be measured using a nonenzymatic assay using a PMS-NADH system (Valentao et al., 2001). Superoxide radical is formed in almost all aerobic cells as a result of one oxygen electron donation, being relevant in the oxygen toxicity mechanism (Magalhaes et al., 2008). Superoxide is a week radical but it may cause severe damage to the cell by generating hydroxyl radical and singlet oxygen (Ahmad et al., 2013). In this assay, sample F presented best value of IC_{50} (44.7 ± 3.2 µg/mL). This sample can deliver a high recommend daily dose of green tea to consumer (1500 mg) which may explain the observed results.

Transition metal ions, such as Cu^{2+} and Fe^{2+}, can catalyze the generation of reactive oxygen species and result in lipid peroxidation and DNA damage (Stohs & Bagchi, 1995). Therefore, the ability of tea polyphenols to chelate metal ions, such as iron and copper, may contribute to their antioxidant activity by preventing redox-active transition metals from catalyzing free radical formation (Rice-Evans et al., 1997). However, it is not clear whether metal chelation is a physiologically relevant antioxidant activity, because most transition metal ions are bound to proteins *in vivo* where they cannot participate in metal-catalyzed free radical formation (Frei & Higdon, 2003). In the Fe^{2+} chelating ability, ferrozine reacts with ferrous ions to form a magenta complex that absorbs at 562 nm (Lopez et al., 2016). The absorbance is directly related to iron in the fly (Lopez et al., 2016). Green tea catechins possess well-established metal-chelating properties. Structurally important features defining their chelating potential are the 3´,4´-dihydroxyl group in the B ring (Hider et al., 2001), as well as the gallate group (Guo et al., 1996; Kumamoto et al., 2001). However, in this study only one sample showed an IC_{50} value (2681.6 ± 92.0 µg/mL). Results observed in this assay can be explained by differences in green tea mechanism, dilutions used, or sample extraction methods (Chen, 2013).

In all the assays performed, best results were given by a DS with only green tea as a bioactive compound. These results showed that DS containing green tea as single ingredient may be more effective as antioxidant compared DS with multi-ingredients, which

are not in agreement with the study performed by Almeida et al., (2011). Almeida et al., (2011) showed that, considering DPPH scavenging activity, a DS containing vitamins A, C and E, L-cysteine chloridrate, powdered extracts of green tea, red wine and pycnogenol, zinc glycinate, taurine, L-glutathione, manganese glycinate, Spirulina, *G. biloba*, *S. marianum* and *Gotu kola* extracts, selenomethionine, copper lysinate and riboflavin-5-phosphate; and a DS containing disodium selenium, vitamins A, C and E were the most powerful supplements (with a IC_{50} value of 0.052 ± 0.001 μg/mL and 0.12 ± 0.02 μg/mL, respectively).

Several studies have been evaluating this antioxidant potential of single green tea products or extracts. Lowe, Gana & Raham (2015) indicated that a green tea extract, taken as a DS, for 14 days can increase the leukocyte activity and the total plasma antioxidant status (Lowe, Gana & Raham, 2015). Another study performed by Yu et al., (2017), which was a randomized, double-blinded, placebo-controlled phase II clinical trial, demonstrated that high-dose of green tea extract intake, for approximately 12 months, were associated with liver enzyme elevation in a small proportion of healthy postmenopausal women (Yu et al., 2017).

In contrast, several authors showed better antioxidant activity for green tea combined with other substances. For example, El-Begati (2016) found that a mixture of grape seed and green tea extract was more effective as antioxidant than each extract separately. Another study performed by Taghizadeh et al., (2017) indicated that taking green tea, capsaicin and ginger for 8 weeks among overweight women had beneficial effects on weight, BMI, markers of insulin metabolism and plasma GSH levels. Finally, Liu Z., Lou, Jia, Wang & Li, (2016) concluded that there is a synergistic interaction with *Potentilla Fruticosa L.* leaves extract plus green tea extract regarding antioxidant activities.

However, this synergic effect depends on the type of substances present in mixture and its concentration. For example, sample C has guarana (*Paullinia cupana*), bitter orange (*Citrus aurantium*) and cocoa (*Theobroma cacao*) extract, which were used principally for their content in caffeine and thermogenic effect. *Citrus aurantium* extract and its primary protoalkaloidal constituent *p*-synephrine are extensively used in weight management products and as thermogenic agents (Stohs, Preuss, & Shara, 2012). Sample D, besides green tea, has *Rhodiola rosea* and *Coleus forskohlii* extract. *Rhodiola rosea* has biologically active substances including flavonoids and phenolic glycosides (salidrosides and rosavin) (Kelly, 2001). *In vitro* studies have been shown its antioxidant potential (Battistelli et al.,

2005; De Sanctis et al., 2004). However, in a randomized double-blind trial with *R. rosea* supplementation (600 mg/day), no effects were observed in exercise-induced muscle damage and inflammatory markers (Jowko et al., 2018). In addition, *R. rosea* supplementation showed no influence on oxidative stress parameters (Jowko et al., 2018).

Conclusion

The antioxidant activity of fruits and vegetables has been thoroughly investigated but less is known about this activity in DS. DS are formulated from different plant species, have different compositions, and concentrations of active ingredients, so it´s expected that they will vary in its antioxidant ability. In case of green tea, this plant is a higher phenolic source, however, antioxidant activity can vary based on botanical origin, preparation process, concentrations and forms. DS studied showed different antioxidant results at different assays performed. Also, DS containing green tea with other substances didn´t show better results than DS with only green tea extract. These results show the need to standardize DS in terms of its antioxidant capacity to match required doses to the oxidative status of consumers.

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CHAPTER V

General Conclusion

The prevalence of DS use has increased dramatically over the last years, and DS have become a matter of consumer interest. For that reason, concerns about ingredient misidentification, safety, and quality assurance continue to be important for the industry and the public.

Regulators, health professionals and manufacturers often disagree on how much quality testing is necessary for supplements. However, DS have precautions of use, adverse effects and contraindications. For this reason, DS shouldn't be regulated the same way as foods. In 2007, FDA created new good manufacturing practices rules. These guidelines should be extended and/or adapted to Europe, Portugal included, where DS regulation seems to be incomplete and poor. In Portugal, many herbal products are produced in the country or imported, and sold in places where, in a general way, dietetic products are dispensed. So, these products are not subject to intervention from Autoridade Nacional do Medicamento e Produtos de Saúde, I.P. (INFARMED), and therefore their quality, safety and efficacy is not guaranteed for the consumer. According to Portuguese law, DS are not intended to diagnose, treat, cure, or prevent any disease, so disease claims are prohibited in these products. However, indications of use may be a request in products to promote their selling. Demonstrating efficacy requires clinical studies with well-defined products and rigorous experimental designs, and the studies must be replicable.

Green tea is one of the world's most popular beverages and has been associated with a number of health benefits. However, the plant also has drug interactions, adverse effects, contraindications and precautions of use. Given the widespread use of green tea supplements and data suggesting benefits with respect to disease prevention, the work herein presented intended to study the information on websites, labels and antioxidant activity of DS with green tea marketed in Portugal.

Consumers are increasingly using the Internet as a source of health information or to buy health products, like DS. However, the content of health information on websites is unregulated, so consumers are exposed to information that may be inaccurate and misleading. This thesis showed that the use of validated instruments is necessary to reduce the risks from patients accessing misinformation. Also, Portuguese regulation does not obligate manufactures to describe scientific information at DS labels, just because DS are regulated as foods and consequently are considered safe products. However, this kind of information is essential for consumers safe and can interfere with conventional treatments.

This thesis also showed that the majority of websites analyzed provide insufficient or poor quality information about the benefits and risks of product use. It was also observed some differences in the quantity and type of information between different types of websites (pharmacies, parapharmacies, health food stores and websites without physical space). If consumers not have a follow-up by a health professional, they may not know the real dangerous of green tea DS intake. Based on the results found in this thesis, we recommend more rigorous regulation for the online marketing of DS.

Nowadays, it is possible to find in the market several products claiming their antioxidant activities, and this kind of information is becoming an important parameter to assess the quality of a DS. However, data regarding antioxidant activity of products already marketed is scarce, because labels often lack information regarding effective antioxidant capacity values.

In this thesis, DS analyzed showed different antioxidant results with different assays performed. Also, DS containing green tea with other substances didn´t show better results than DS with only green tea extract. In case of green tea, this plant is a higher phenolic source, however, antioxidant activity can vary based on botanical origin, preparation process, concentrations and forms. Clinical studies are not required, and pharmacological actions are not guarantee. Manufacturing practice rules should be necessary for standardize DS formulation and ensure its quality.

Dietary supplements can offer significant health benefits, but may also cause an increased health care cost and adverse effects. Appropriate clinical trials that evaluate the use and efficacy of different DS may be critical for our health care system. Challenges in supplement science and its regulation provide new opportunities for researchers and regulators to work together both nationally and internationally, and this cooperation will have an impact on health care. Some steps can be made in order to have a better DS regulation. These included reclassification of the available products with a review of their advertised benefits and potential risks; manufacturers of DS should follow to the same standards as drug manufacturers; and regulatory agencies should encourage the implementation of an adequate post marketing surveillance program.

Limitations

This study is limited to searching websites with DS in the form of tablets and capsules; to searching websites based on a specific plant extract (green tea); also to a relatively low number of websites and products. All of the search was performed within a three month time period, so changes may have occurred during this time, and affect data. We choose only Portuguese websites, and could be interesting to compare different realitys.

Future Perspectives

As future perspectives it could be interesting to analyze other information present on websites, namely the scientific references supporting the DS offered, testimonials from consumers; prices and extra information provided by websites. It could be also important to extend the selected websites to other categories (like supermarkets) or websites from other countries. Antioxidant studies and legal requirements analyzed should be amplified to other DS categories with high consumption by population (e.g. multivitamins, sports nutrition). In case of antioxidant activity, could be interesting to perform other assays [e.g. thiobarbituric acid assay (TBA); 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay; and ferric reducing/antioxidant power (FRAP) assay}. We could also determine the total phenolic and flavonoid contents of the DS in order to compare these results to antioxidant activity.