Evaluation of antimicrobial and nematicidal properties of nanosized materials, and investigation of the mode of action of redox-active sulfur and selenium compounds

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Dedicated to my Loving Family

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List of Abbreviations

Abbreviation	**Definition**
AITC	Allyl isothiocyanate
A. brassisicola	*Alternaria brassicicola*
A. niger	*Aspergillus niger*
A. pullulans	*Aureobasidium pullulans*
A. thaliana	*Arabidopsis thaliana*
B. cinerea	*Botrytis cinerea*
BITC	Benzyl isothiocyanates
BM	Bead Milling
B. nitida	*Baphia nitida*
B. oryziterrae	*Bacillus oryziterrae*
B. subtilis	*Bacillus subtilis*
C. albicans	*Candida albicans*
C. coccineum	*Cynomorium coccinium*
C. elegans	*Caenorhabditis elegans*
CF	Cystic fibrosis
C. limon	*Citrus limon*
Cys	Cysteine
D. melanogaster	*Drosophila melanogaster*

List of Abbreviations

E. coli	*Escherichia coli*
EPHX2	Epoxide hydrolase 2
GI	Gastrointestinal
GPXs	Glutathione peroxidases
Grx2	Glutaredoxin 2
GSH	Glutathione
GST	Glutathione *S*-transferase
HIP	Haploinsufficiency Profiling
HOP	Homozygous Profiling
HPH	High Pressure Homogenization
HSS	High Speed Stirring
ITCs	Isothiocyanates
ID	Iodothyronine deiodinases
K. accuminata	*Kola accuminata*
K. pneumoniae	*Klebsiella pneumonia*
L. brevis	*Lactobacillus brevis*
L. esculentum	*Lycopersicun esculentum*
L. ivanovii	*Listeria ivanovii*
L. micranthus	*Loranthus micranthus*
MDR	Multidrug resistant

List of Abbreviations

M. humilis	*Mortierella humilis*
MOA	Mechanism of action
NaLyRe	Nanosizing, Lyophilization and Resuspension
OS	Oxidative Stress
OSCs	Organic Sulfur Compounds
P. aeruginosa	*Pseudomonas aeruginosa*
P. americana	*Persea americana*
P. erinaceus	*Pterocarpus erinaceus*
PITC	Phenyl isothiocyanate
P. glomerata	*Phoma glomerata*
PRDXs	Peroxiredoxins
P. thramacrophylla	*Pentacle thramacrophylla*
RNS	Reactive Nitrogen Species
RNOS	Reactive Nitrogen Oxygen Species
ROS	Reactive Oxygen Species
rpm	Revolutions per minute
RSH	Thiol
RSeS	Reactive Selenium Species
RSS	Reactive Sulfur Species
S. aureus	*Staphylococcus aureus*

List of Abbreviations

S. carnosus	*Staphylococcus carnosus*
S. cerevisiae	*Saccharomyces cerevisiae*
SCGs	spent coffee grounds
S. incanum	*Solanum incanum*
S. feltiae	*Steinernema feltiae*
SFN	Sulforaphane
SODs	Superoxide dismutases
S. incanum	*Solanum incanum*
S. typhimorium	*Salmonella typhimurium*
T. harzianum	*Trichoderma harzianum*
TrxRs	Thioredoxin reductases
TrxPs	Thioredoxin peroxidases
V. dahliae	*Verticillium dahliae*
V. longisporum	*Verticillium longisporum*
V. vinifera	*Vitis vinifera*
YKO	Yeast knock out

Summary

Complex living organisms, such as humans, animals and plants suffer from diseases which can be caused by pathogenic bacteria, fungi and nematodes. The activity of presently available antimicrobial and nematicidal agents is continuously becoming ineffective because of drug resistance arising due to excessive and inappropriate use. Therefore, an inevitable need for new sources of antimicrobials, nematicides and phytoprotectants is growing and attracting a lot of scientific interest.

The first part of the thesis encompassed the investigation of nanosized materials (plants and their waste materials, and chalcogen-containing compounds) against a broad-spectrum of microorganisms. The second part of the study comprised the mechanistic understanding of allicin and selenocyanates by exploiting a library of yeast mutants carrying deletions for specific protein functions.

Generally, the nanosized substances exhibited excellent antimicrobial and nematicidal activities. Chemogenetic screening of allicin and selenocyanates revealed hypersensitive yeast mutants with gene deletions coding for specific proteins.

In summary, the results obtained as a part of the current investigation uncover the feasibility of utilizing nanosized materials, allicin and selenocyanates against a plethora of microorganisms in Medicine and Agriculture.

Zusammenfassung

Komplexe lebende Organismen wie Menschen, Tiere und Pflanzen leiden an Krankheiten, die durch pathogene Bakterien, Pilze und Nematoden verursacht werden können. Die Aktivität der heutzutage verfügbaren antimikrobiellen und nematiziden Wirkstoffe wird aufgrund von Arzneimittelresistenzen, die durch übermäßigen und unsachgemäßen Gebrauch entstehen, kontinuierlich unwirksamer. Daher wächst der unvermeidliche Bedarf an neuen Quellen für antimikrobielle, Nematizide und Phytoprotektive Wirkstoffe, und dieszufolge auch das wissenschaftliche Interesse.

Der erste Teil der Arbeit umfasste die Untersuchung von Nanomaterialien (Pflanzen und deren Abfallstoffe sowie Chalkogen haltige Verbindungen) gegen ein breites Spektrum an Mikroorganismen. Der zweite Teil der Studie umfasste das mechanistische Verständnis von Allicin und Selenocyanaten durch die Nutzung einer Bibliothek von Hefemutanten, die Deletionen für spezifische Proteinfunktionen tragen.

Im Allgemeinen wiesen die Nanosubstanzen ausgezeichnete antimikrobielle und nematizide Aktivitäten auf. Der chemogenetische Screening von Allicin und Selenocyanaten zeigte hypersensible Hefemutanten mit Gendeletionen, die für spezifische Proteine kodieren.

Zusammenfassend lässt sich sagen, dass die Ergebnisse, die im Rahmen der aktuellen Forschungsarbeit erzielt wurden, die Anwendbarkeit von Nanomaterialien, Allicin und Selenocyanaten gegen eine Vielzahl von Mikroorganismen in Medizin und Landwirtschaft aufweisen.

Publications included in this thesis

Publication 1

Milling the Mistletoe: Nanotechnological Conversion of African Mistletoe (*Loranthus micranthus*) into Antimicrobial Materials

Muhammad Sarfraz, Sharoon Griffin, Tamara Gabour Sad, Rama Alhasan, Muhammad Jawad Nasim, Muhammad Irfan Masood, Karl Herbert Schäfer, Chukwunonso E.C.C. Ejike, Cornelia M. Keck, Claus Jacob and Azubuike P. Ebokaiwe

Antioxidants (Basel), 2018, 7(4), 60

Publication 2

No time to waste organic waste: Nanosizing converts remains of food processing into refined materials

Sharoon Griffin, **Muhammad Sarfraz**, Verda Farida, Muhammad Jawad Nasim, Azubuike P. Ebokaiwe, Cornelia M. Keck and Claus Jacob

Journal of Environmental Management, 2018, 210,114-121

Publication 3

Resuspendable Powders of Lyophilized Chalcogen Particles with Activity against Microorganisms

Sharoon Griffin, **Muhammad Sarfraz**, Steffen F. Hartmann, Shashank Reddy Pinnapireddy, Muhammad Jawad Nasim, Udo Bakowsky, Cornelia M. Keck, and Claus Jacob

Antioxidants (Basel), 2018, Volume 7(2), 23

Publication 4

Efficacy of allicin against plant pathogenic fungi and unveiling the underlying mode of action employing east based chemogenetic profiling approach

Muhammad Sarfraz, Muhammad Jawad Nasim, Claus Jacob and Martin C. H. Gruhlke

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Publication 5

Yeast Chemogenetic Screening as a Tool to Unravel the Antifungal Mode of Action of Two Selected Selenocyanates

Muhammad Sarfraz, Muhammad Jawad Nasim, Claus Jacob and Martin C. H. Gruhlke

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1. Introduction

Infectious diseases remain a main cause of morbidity and mortality in spite of substantial global efforts to fight and hinder these diseases [1]. The availability and proper use of a wide variety of antimicrobials rendered the complications linked to infectious diseases caused by opportunistic pathogens, such as *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans* and *Saccharomyces cerevisiae* significantly reduced [2]. Excessive and unintelligible administration of these agents has, nevertheless, led to a number of drug-resistant microorganisms. These microbes hamper the expected prevention and outcomes of treatment of infections leading to prolonged illness, debility, morbidity and mortality [3, 4]. Multidrug resistant (MDR) bacteria and fungi present a serious problem to human health especially in developing countries due to the over-prescription of antibiotics [5]. The issue with the extensive administration of antibiotics not only worsens the resistance in microorganisms but also escalates the cost of therapy and decreases the therapeutic options available [6].

Numerous antimicrobial and nematicidal agents are frequently employed to treat various diseases in plants, animals and humans. Nonetheless, owing to widespread resistance and environmental safety precautions, there is an urgency to re-(evaluate) natural resources as a possible source to identify alternative drugs and pesticides [7]. Therefore, new sources of antimicrobials, nematicidals and phytoprotectants in recent years to combat resistant microorganisms have been explored [8, 9]. Similarly, during the last couple of years, the approach to nanosize the entire plant or various plant parts has appeared as an alternative to conventional extraction techniques. Furthermore, deciphering the mechanisms of action of cytotoxic compounds is of utmost importance for their wider practical applications.

The present study explores the antimicrobial and nematicidal properties of nanosized materials, and uncovers the mode(s) of action of redox-active sulfur- and selenium-containing compounds, *i.e.*, allicin and selenocyanates (Figure 1).

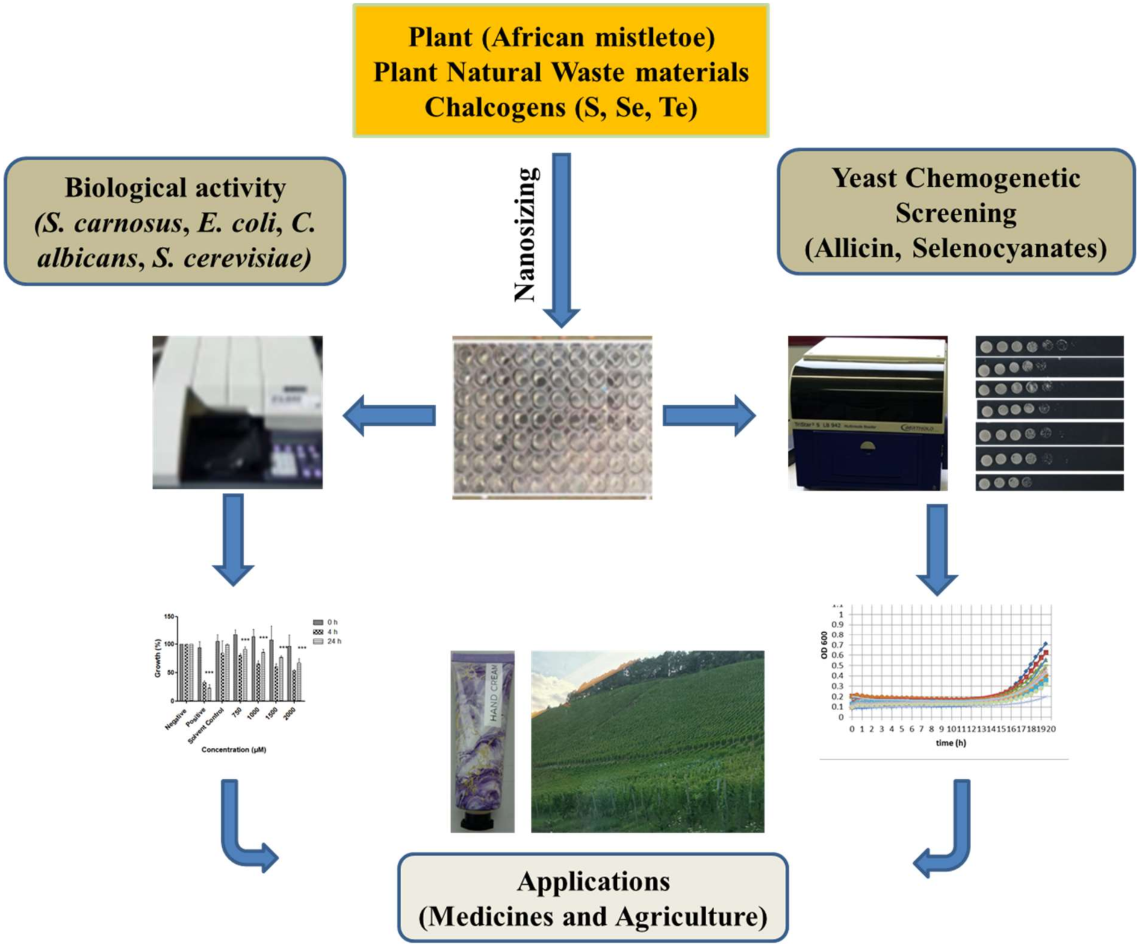

Figure 1. Scheme representing the determination of biological activity of nanosuspensions of plant, waste materials from plants and chalcogens, the mode of action of redox-active compounds (allicin and selenocyanates), and application of these materials in Medicine and Agriculture.

1.1. Pathogenic bacteria and fungi

Pathogenic microorganisms, in particular, bacteria and fungi are responsible for various health problems [1]. Among bacteria, *E. coli* are Gram-negative bacilli belonging to the family *Enterobacteriaceae*. These bacteria are nonsporulating facultative anaerobes [10]. Certain pathogenic strains of *E. coli,* for instance, diarrheagenic *E. coli* (DEC), enteroinvasive *E. coli* (EIEC) and enterotoxigenic *E. coli* (ETEC) are responsible for several diseases, *e.g.,* diarrhoea, urinary tract infections, sepsis and meningitis which may even lead to death [11, 12]. Moreover, *E. coli*, owing to its simple structure, has also gained considerable attention in both scientific research and industrial applications. The two non-pathogenic strains of *E. coli*, namely, K-12 and B strains are mostly utilized in the laboratory. Genetic and metabolic processes have been studied extensively by employing the K-12 and other related strains, whilst phage sensitivity, mutagenic assays, restriction systems and bacterial evolution have been investigated by using the B strain and other similar types [13]. In the case of Gram-positive bacteria, *Staphylococcus carnosus* belongs to the bacterial genus *Staphylococcus*. It is a coagulase-negative bacterium and comprises single and paired cocci [14].

Similarly, fungal infections cause a plethora of diseases, including superficial mycoses, sub-cutaneous mycoses, cutaneous mycoses and systemic mycoses. These fungal diseases often trigger the conditions which require extensive treatment [15]. *C. albicans*, for instance, is an opportunistic fungal pathogen which causes candidiasis [16]. These infections comprise mucosal infections (primary candidiasis) and infections of gastrointestinal epithelial cells, oropharyngeal mucosa and vaginal cells (secondary candidiasis) [17].

Baker's yeast, *S. cerevisiae* is employed widely in baking, brewing and is generally considered as safe. Nonetheless, various studies have shown that *S. cerevisiae* is responsible for numerous ailments, *i.e.,* superficial and life-threatening systemic diseases in patients with no predisposing features and in immunocompromised patients (*i.e.,* organ transplantation, broad-spectrum antibiotic treatment) or diseases (*e.g.,* AIDS, malignancy, diabetes) [18]. Resistance to antifungal drugs, as in the case of antibacterial agents, has now become a serious problem. Moreover, fungal diseases, in contrast to bacterial infections, are often overlooked. A possible reason is that fungal infections are often considered as relatively easy to treat. Furthermore, historically, bacteria and viruses are portrayed harmful, while fungi and their products can be edible. Hence, new antifungals have to be explored in order to cope with the resistance developed by pathogenic fungi against the small number of already available antifungal agents [19, 20].

There are numerous pathogenic fungi which are present in the soil and subsequently infect plants resulting in numerous plant diseases. *Verticillium* wilt, for instance, is caused by the plant pathogenic fungus, *Verticillium*, which is of increasing threat for various crops [21, 22]. The plant pathogens of genus *Verticillium* affect a wide range of economically significant crops *e.g.,* plants of family *Solanaceae* and large trees [23, 24]. Furthermore, phytopathogens of genus *Alternaria* are responsible for causing diseases in *Cruciferous* crops, such as cauliflower, broccoli, turnip and mustard [25]. *Botrytis cinerea* is a chief plant pathogenic fungus of cultivated fruits, flowers and vegetables. This fungus forms grey mould on several species of plants resulting in a major loss of economy [26].

1.2. Nematodes

Nematodes (Phylum Nematoda) pose significant problems in plants and animals producing diseases in a quarter of the global population [27]. They are one of the most ancient and diverse organisms on Earth [28]. Nematodes are also reported to be responsible for impaired physical and mental health in humans [29]. They also cause diseases in domestic and farm animals instigating enormous economic losses [30]. Synthetic pesticides or selective soil fumigants have normally been employed to control nematodes. Nevertheless, restrictions or complete prohibition of synthetic nematicides on account of safety concerns have hindered the effective eradication of nematodes [31]. Hence, there is an urgent need for the development of eco-friendly “green” nematicidal agents to tackle damage triggered by nematodes.

1.3. Oxidative stress (OS)

An imbalance between oxidants and antioxidants in favour oxidants, potentially leading to damage is termed ‘oxidative stress’ [Helmut Sies (1985) Oxidative Stress, Academic Press, New York] [32]. This imbalance mostly results in an elevation in the levels of free radicals triggering the damage of cells, tissues or whole organisms [33]. OS results in high levels of reactive oxygen species (ROS), reactive nitrogen species (RNS), reactive nitrogen oxygen species (RNOS) and reactive sulfur species (RSS) [34]. Mitochondria, transition metal toxicity and disturbed peroxisome activities represent major sources of endogenous ROS [35]. ROS are regularly produced and eliminated in all biological systems and play crucial roles in a variety of normal functions and pathological processes [36]. ROS are reactive molecules which perform vital functions in cell proliferation and differentiation in living organisms [37]. Nonetheless, OS due to excessive ROS may give rise to the functional impairment of important cellular macromolecules, such as DNA, lipids and proteins [36, 38, 39]. Moreover, OS consequences in an impaired cellular homeostasis, structure and function [40] giving rise to a number of pathological states, *i.e.,* degenerative processes, and chronic inflammatory ailments, such as lupus erythematosus, rheumatoid arthritis and psoriatic arthritis [41-44]. Therefore, it is important to maintain the normal redox homeostasis of cells for their growth and vitality. Abnormalities in cellular redox homeostasis may also trigger various cancers [45]. Since, cancer cells have elevated levels of ROS, hence, further increase in ROS and OS by exogenous agents can selectively kill cancer cells. Consequently, regulating ROS levels by targeting the redox status is a way to selectively kill cancer cells without significantly affecting the normal cells [42]. Figure 2 shows the levels of ROS in normal and cancer cells [46].

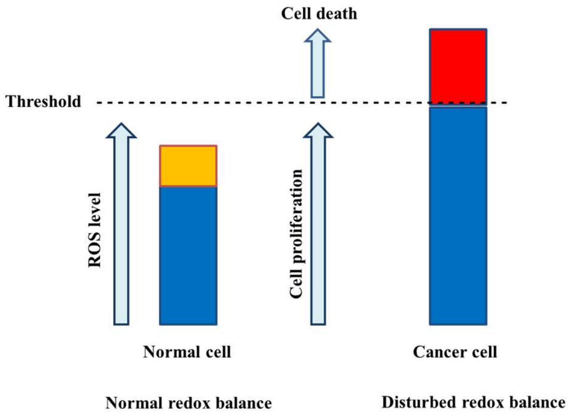

Figure 2. An overview of the redox balance in normal and cancer cells. Cancer cells have elevated levels of ROS increasing the OS which can be targeted by anticancer drugs to induce apoptosis in cancer cells. Conversely, healthy cells possess usual levels of ROS and display a normal redox balance [46].

To cope with the harmful effects of oxidants, the human body is equipped with a wide range of antioxidants. These antioxidants can be grouped into enzymatic and non-enzymatic classes [47]. Enzymatic antioxidants comprise glutathione peroxidases (GPxs), glutathione *S*-transferases (GSTs), superoxide desmutases (SODs), peroxiredoxins (Prxs), thioredoxin peroxidases (TrxPs), thioredoxin 2 (Trx2), glutaredoxin 2 (Grx2), catalases and epoxide hydrolase 2 (EPH2). Non-enzymatic antioxidants comprise molecules, such as ascorbic acid (vitamin C), tocotrienols (vitamin E) and tocopherols [48-50].

Chalcogens, such as sulfur, selenium and tellurium containing compounds have been developed which can modulate the intracellular redox status and can inhibit a broad-spectrum of pathogens [51, 52]. These compounds can be exploited in both ways; they act as antioxidants or prooxidants depending on their chemical structure and intracellular environment [53, 55]. These compounds play key roles in important biological and physiological processes, including signalling, enzymatic mechanisms and repair of biomolecules impaired by OS [56].

1.4. Plants as a source of natural compounds to combat diseases

The hunt for new lead compounds has provoked the need to re-visit the natural bioactive agents from different animals, plants, bacteria and fungi. Not surprisingly, there are a plethora of biologically active natural compounds which can be exploited efficiently to fight various diseases [57].

Plants have been a pivotal source of food and medicines for humans since centuries. The documented utilization of medicinal plants for the treatment of various illnesses dates back over five millennia to the manuscripts of remote civilizations in India and China [58]. In recent years, medicinal plants have gained ample scientific and commercial attention. Because of their greater compatibility and acceptability by the human body, natural products from plants have found a wide range of applications [59]. Nevertheless, currently pharmaceutical and medical research is being carried out for the exploration, characterization and development of natural components to cure different human diseases [60].

Medicinal plants represent a vital source of a wide range of diverse natural compounds referred to as phytochemicals. Phytochemicals are secondary metabolites of plants and include natural compounds, such as flavonoids, polyphenols, steroids, tannins, vitamins and Organic Sulfur Compounds (OSCs). Many of these phytochemicals have been employed as active ingredients of current formulations [61-63]. Natural compounds present in plants serve as an important line of defence against various pathogens. Moreover, these natural products are often less toxic to humans and environment as compared to their synthetic counterparts [64]. Plants are the prime focus of various studies because of these biologically active phytochemicals. Various parts of medicinal plants contain diverse bioactive compounds. Most of the industrial waste, *i.e.,* the cuticles, seeds, peels, pulp, leaves and stems of plants are chief sources of antioxidant, antimicrobial and antiviral compounds [65]. Plants are a renewable natural resource offering a wide range of cost effective biologically active agents as compared to synthetic compounds [66, 67].

Not surprisingly, extracts of medicinal plants are commonly utilized to treat various diseases. Nonetheless, the conversion of a whole plants or plant parts like leaves, fruit, stem or bark into a beneficial medication is a cumbersome process. For instance, isolation of active phytochemicals, generation of the extracts, isolation of fractions, and preparation of appropriate delivery systems require a variety of expensive and sophisticated technologies, suitable solvents and considerable expertise. Furthermore, these extraction techniques are time consuming, and may also result in considerable waste of the resources. Moreover, developing countries, in spite of their valuable resources of medicinal plants, are often unable to exploit them and, hence, depend on expensive imported drugs [29, 68-70].

To overcome these problems, nanotechnology has recently been explored as a new alternative to the conventional extraction approaches. This procedure exploits the milling of whole plant or plant parts to generate nanoparticles with improved absorption and bioavailability [70-72].

1.5. Nanotechnology

Solubility of a drug molecule is a crucial physicochemical characteristic for its absorption, distribution and bioavailability within the body. Drug absorption of a compound across biological membranes, which are usually lipid in nature, is related to its solubility [73]. The partition coefficient (p) is the ratio of distribution of a drug in a mixture of two immiscible solvents, for instance, water and nonpolar solvent at equilibrium. The logarithm of this ratio is thus *log P*, which is an indicator for the hydrophilicity or lipophilicity of a compound [74]. The decreased bioavailability of poorly soluble compounds is an important challenge in drug development. Nanosizing decreases the particle size and enhances the solubility, absorption and, hence, bioavailability of poorly water-soluble biologically active compounds [75, 76]. The compound must have ample lipid solubility in order to cross the biological membranes. Size reduction improves the dissolution rate and solubility of a compound to enhance its absorption and efficacy [77]. The distinctive characteristics of nanoparticles, for instance, small size, greater surface area, surface chemistry, surface charge and solubility to turn them into suitable drug carriers for the delivery of therapeutic active molecules. Nanomedicine refers to the exploitation of methods and techniques for the diagnosis, prevention and treatment of various pathological states [78]. For an effective transfer across the biological membranes, the drug molecule must be soluble in the local medium. To improve the dissolution rate, solubility and drug concentration in the body, size reduction is an important technique [77]. This concept may be elucidated by employing Noyes-Whitney equation.

$$\mathbf{dC/dt = DA\ (C_S - C)/h}$$

where, dC/dt: dissolution rate of drug particles; D: diffusion coefficient of the drug; A: total surface area of the drug particles in contact with gastrointestinal (GI) fluid; C_S: saturation solubility of the drug in solution; C: concentration of drug in GI fluid, and h: thickness of diffusion layer around each drug particle.

Size reduction is the prime target of nanosizing and can be achieved by Bead Milling (BM), High Speed Stirring (HSS) and High Pressure Homogenization (HPH) [79-81].

BM involves the interactions under high speed between the coarse material and beads in a chamber [82]. Beads comprise glass, stainless steel, zirconium oxide or ceramic materials. The size of beads and speed of milling chamber can control the size reduction [83, 84].

HSS is an industrial approach to transform bulk materials into nanosized materials. This technique involves the handling of dispersing solids in a medium. High-shear mixers or rotor-stator mixers (RSMs) are commonly employed in HSS. RSMs are usually applied for materials with higher viscosity, while high-pressure homogenizers are employed for less viscous products [85-87].

HPH is a simple, well-established and safe technique and utilized extensively in pharmaceutical industry, cosmetics and food production. Moreover, a large-scale generation of nanoparticles is feasible by relying on this technique [77].

During the last couple of years, the approach to nanosize whole plants or various plant parts has gained considerable interest and importance. Furthermore, chalcogen-containing compounds have also been utilized to yield nanoparticles [88].

The plant, waste materials obtained from plants and chalcogens, employed in the current study, are described briefly in the following sections.

1.6. *Loranthus micranthus*

The African mistletoe (*L. micranthus*) (Family *Loranthaceae)* is a semi-parasitic plant which is associated with other plants, including *Citrus limon*, *Kola accuminata*, *Pentaclethra macrophylla* and *Baphia nitida*. It is widespread in the eastern parts of Nigeria, and exhibits a wide range of biological activities [89]. This mistletoe is commonly employed in folk and traditional medicines and is described as "an all purposes herb" because of its widespread use in a diverse range of diseases. This plant is applied widely in ethno-medicine for various purposes, *i.e.,* antimicrobial, antidiabetic, anti-hypertensive, hypolipidemic, antioxidant, antispasmodic, antiepileptic and immunomodulatory properties [89-92].

The mistletoe plant also displays a diverse multitude of *in vitro* antimicrobial properties against certain antibiotic-resistant bacterial species, such as *E. coli*, *Agrobacterium tumefaciens*, *Salmonella* sp., *Proteus* sp., *Pseudomonas* sp., and fungal infections of farm animals [93]. Extensive phytochemical investigations of *L. micranthus* extracts indicate the presence of various phytochemical constituents, namely glycosides, tannins, volatile oils, steroids, alkaloids, flavonoids, terpenoids, carbohydrate and resins. The composition and, hence, the biological activity of mistletoe depend on the harvesting period and individual host tree [94]. The crude methanolic extract from the leaves of *L. micranthus*, for instance, harvested from avocado plant (*Persea americana),* was reported to possess alkaloids, terpenoids, proteins, steroids, resins, oils, tannins, flavonoids, acidic compounds, saponins, carenolides, cardiac glycosides, phlobatannins, volatile oils, carbohydrates, reducing sugar and glycosides [91, 94]. These bioactive compounds found in African mistletoe are associated with a wide array of *in vitro* antimicrobial activities against bacteria and fungi [95]. Various extracts of *L. micranthus* reveal *in vitro* antibacterial and antifungal properties against bacteria, such as *S. aureus*, *E. coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Bacillus subtilis*; and fungi particularly *C. albicans* and *Aspergillus niger* [91, 96].

1.7. Upcycling/ Turning waste into value

Modern food processing has triggered the production of various waste materials, including bio-waste, which causes hazard to the environment, animals and humans. Spent coffee grounds (SCGs), tomato stems, walnut shells and grape seeds represent good examples of such kitchen waste. This bio-waste contains a wide range of natural compounds, such as antioxidants and polyphenols. Nevertheless, this waste material has no applications and is commonly disposed of into the environment [97, 98].

1.7.1. Spent coffee grounds (SCGs)

Coffee is one of the widely consumed drinks in the world [98]. Caffeine is the most studied component of coffee, while other constituents comprise phenolic compounds, tannins, cellulose, minerals, sugars, lipids, proteins, trigonelline, xanthines, chlorogenic acid, melanoidins, vitamin precursors and diterpenes [99, 100]. Statistically, 650 kg of SCGs is produced from one ton of green coffee, while one kg of soluble coffee generates about two kg of wet SCGs [101]. SCGs contain large amounts of organic materials, in particular caffeine, polyphenols and tannins [102, 103]. SCGs find no commercial value and are currently discarded as solid waste. The disposal of SCGs needs to be properly managed since organic contents of coffee waste may exert negative impact on the environment [98]. Moreover, burning of SCGs may upsurge the release of harmful greenhouse gases into the atmosphere [104]. In a study, effects of coffee waste on human, animal and the environment have been investigated. It was explored whether coffee waste disposed of in the environment can instigate DNA damage and may produce detrimental effects on human, animal and the environment [105]. For this reason, there is an urgency for exploring ways to turn this waste into valuable products *i.e.,* upcycling.

These detrimental effects of coffee waste have stimulated the efforts to explore ways to transform coffee waste into value-added products. SCGs are conventionally employed as fertilizers in kitchen gardens. Furthermore, the activity of SCGs on *S. cerevisiae* can be exploited in specific fermentations. There are various studies focussing on the applications of coffee waste. Not surprisingly, coffee industry wastes were investigated for the feasibility of ethanol production by employing various strains of yeast. Coffee waste can also be employed to produce ethanol and, hence, biofuels [99, 100]. Similarly, coffee waste may be employed as a possible alternative to produce biofuel, biodiesel and fertilizers. Vermicomposting and coffee shampoos indicate other applications of SCGs [106-109]. Furthermore, SCGs present an easily available and inexpensive alternative to be employed as an adsorbent for the elimination of cationic dyes for the treatment of wastewater [110]. SCGs can also be utilized efficiently to remove lead ions from drinking water [111].

1.7.2. Tomato stems

Tomato (*Lycopersicun esculentum* or *Solanum lycopersicum*) is an edible red or yellow fruit of the nightshade family *Solanaceae* [112]. Tomatoes are considered as a good source of antioxidants, nutrients and phenolic compounds in the human diet [113]. Tomato fruit contains several compounds, *i.e.,* polyphenols, alkaloids, sugars, fatty acids, amino acids, organic acids, terpenes, carotenoids, hormones and various volatile substances [114]. Similarly, various studies have demonstrated that non-edible parts of tomato plant contain higher proportions of antimicrobial compounds as compared to edible parts. Tomato leaves in comparison to tomato fruits, for instance, contain higher contents of antimicrobial metabolites, *e.g.,* caffeic acid, vanillic acid, chlorogenic acid, β-phellandrene, sabinene, α-terpinene, α-tomatine and dehydro-tomatine. Extracts from tomato leaves have also shown significant *in vitro* antimicrobial activity against plant pathogenic microorganisms [115]. The extracts of whole plant, stems, leaves or roots of tomato inhibit *in vitro* the growth of pathogens, *i.e., E. coli*, *Streptococcus aureus*, *Salmonella typhimurium* and *Listeria ivanovii* [116].

1.7.3. Grape seeds

Grapes (*Vitis vinifera*) belong to the family *Vitaceae*, and contain a large number of phytochemicals. Nonetheless, only some of these agents have been reported to exhibit biological activities [117]. Grape seed extracts show *in vitro* antimicrobial activity against Gram-positive food-borne pathogens [118]. In another study, grape seed extracts inhibited the growth of *S. aureus*, and *K. pneumoniae* and *E. coli* isolated from urinary tract infection [119]. Resveratrol (3,4,5, trihydroxystilbene) is a natural polyphenol present in grapes which exhibits antioxidant and cardioprotective activities [120, 121].

Resveratrol also shows antimicrobial activities against a broad-spectrum of bacterial and fungal species [122].

1.8. Chalcogens

The elements in group 16 of the Periodic Table are referred to as chalcogens. This group contains oxygen (O), sulfur (S), selenium (Se), tellurium (Te) and the radioactive element polonium (Po).

Over the past few years, interest in the field of biologically active chalcogens has triggered the synthesis of a broad array of molecules [123]. Organic chalcogen-containing compounds have been studied for several decades because of significant biological activities against bacteria, fungi, nematodes and parasites. During the recent years, the exploitation of nanosuspensions has emerged as a possible substitute for the utilization of organic compounds as antimicrobial agents [124].

1.8.1. Sulfur

Sulfur is present in a diverse range of natural products, including some from marine and other microorganisms in the form of several OSCs [125]. Sulfur serves as an important constituent of amino acids such as methionine (Met) and cysteine (Cys). Sulfur also forms a significant part of cellular proteins and enzymes which play an essential role in several biochemical and cellular signalling pathways [126]. Various sulfur compounds present in plants, bacteria, fungi and animals exhibit distinctive biochemical properties associated with structural features, redox processes, catalytic reactions and metal binding [57]. OSCs have had an important role in pharmaceutical and agricultural avenues since centuries [127]. They are widespread in nature, *e.g.,* allicin and polysulfanes in garlic, thiocyanates and isothiocyanates in *Cruciferous* vegetables and ergothioneine in mushrooms [128, 129]. These compounds exhibit a plethora of antimicrobial and therapeutic properties [129-131]. The antimicrobial properties of thiols, thiocyanates, isothiocyanates, sulfones, sulfoxides, thiosulfinates, thiosulfonates and polysulfides place them at the centre of pharmacological interest [130]. Sulfur nanoparticles have also proved to be effective antimicrobial and nematicidal agents to combat infections caused by resistant pathogens [132, 133].

1.8.2. Selenium

Selenium was discovered in 1817 by Jöns Jacob Berzelius (1779–1848) in the reddish deposits which formed in the lead chambers at sulfuric acid plant in Gripsholm, Sweden. This element was named as 'selenium' in the honour of Greek goddess 'Selene' meaning moon [134]. Selenium was considered as an absolute poison for a long time until, in 1957, Schwarz and Foltz recognized it as a micronutrient for birds, mammals and bacteria [135].

Selenium is an important nutritional trace element associated with several physiological functions, particularly, antioxidative and antimicrobial activities [136]. Organic selenium compound, ebselen and its analogues, for instance, were synthesized and investigated for their antimicrobial and antiviral properties [137]. Organoselenium compounds exhibit *in vitro* antibacterial activity against Gram-positive (*B. subtilis* and *S. aureus*) and Gram-negative (*S. typhimurium* and *E. coli*) bacteria [138]. Various current investigations have reported selenium nanoparticles as significant antibacterial and antifungal agents [139, 140].

1.8.3. Tellurium

Tellurium was discovered by Transylvanian (today Sibiu, Romania) chemist Franz-Joseph Müller (1740– 1825) in 1782, who was investigating gold-containing ores. The name tellurium originates from the Latin word 'tellus' which means 'Earth' [141]. Tellurium is one of the important elements in the human body [142, 143]. During the recent years, tellurium nanoparticles have gained considerable interest as potential antibacterial and antifungal agents [143, 144].

Selenium- and tellurium-based nanomaterials exhibit interesting antimicrobial potential against a broad range of pathogenic strains [145, 146]. Tellurium particles have been reported to show significant *in vitro* antibacterial activities against *E. coli*, *P. aeruginosa* and *S. aureus* [147, 148]. Similarly, the antimicrobial properties of tellurium-loaded polymeric fibre meshes were explored *in vitro* against *E. coli*. These investigations revealed a dose dependent antibacterial activity of tellurium-based materials against *E. coli* [149].

1.9. Redox-active compounds employed in chemogenetic profiling

1.9.1. Allicin

Allicin is an organosufur compound produced from crushed garlic and constitutes a pivotal group of reactive sulfur species (RSS). Allicin exhibits remarkable antimicrobial activities against a broad-spectrum of bacteria and fungi [150-153]. The antimicrobial and therapeutic properties of garlic extract are predominantly derived from allicin [154]. Allicin has been reported to inhibit bacteria, *e.g., E. coli*, *Mycobacterium tuberculosis*, *Streptococcus* sp., *Staphylococcus* sp., *Bacillus* sp., *Helicobacter pylori*, *Klebsiella proteus* and methicillin-resistant, *S. aureus* (MRSA) [154, 155]. Allicin also shows significant antifungal activities both *in vitro* and *in vivo* against different phytopathogens, such as *B. cinerea*, *A. brassicicola*, *Plectospherella cucumerina* and *Magnaporthe grisea* [156-159].

1.9.2. Selenocyanates

Selenocyanates constitute an important class of reactive selenium species (RSeS). These compounds are multifunctional in nature. For instance, they are reported as effective cytotoxic agents in biology. These agents are also employed for the synthesis of different organic selenium compounds [160]. Organic selenium compounds exhibit excellent *in vitro* antibacterial and antifungal activities [161, 162]. Chemical and biological properties of selenocyanates have been studied extensively [163]. Different modes of action of selenium-containing compounds such as selenocyanates have been proposed. Isoselenocyanates, for instance, were reported to increase ROS, nitric oxide and superoxide through the redox cycle. Furthermore, these compounds showed greater cellular glutathione depletion as compared isothiocyanates (ITCs) [164].

1.10. Deciphering the mode of action of target compounds

1.10.1. Chemogenetic profiling

Understanding the mechanisms and modes of action of new compounds is of pivotal importance in chemical biology as part of drug discovery. The mechanism of a compound can be determined by gene deletions which render cells hypersensitive to that compound [165-168]. Chemogenetic screening is a technique in which a library of yeast strains is screened against the target compound(s). These yeast mutants are deleted for genes encoding particular proteins. This approach is employed to uncover the possible targets and MOA of compounds under investigation [169]. In chemogenetic profiling, the wildtype yeast and mutant strains with deleted genes are cultured in the presence of cytotoxic compounds. The yeast strains that show inhibition in growth as compared to wildtype and untreated control possibly specify the drug target [170]. This phenomenon is referred to as haploinsufficiency, and a library of approximately 1,200 mutants deleted for essential genes is available from Euroscarf (http://www. euroscarf.de/index.php?name=News) [171]. Figure 3 gives an overview of drug-induced haploinsufficiency screening in yeast.

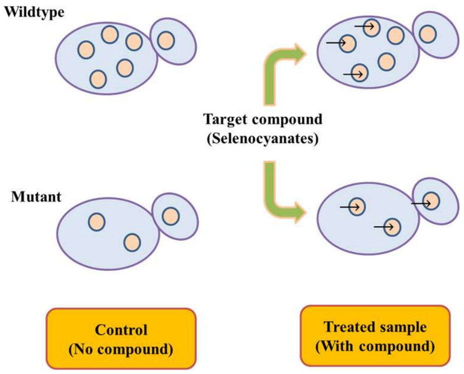

Figure 3. An overview of drug-induced haploinsufficiency in yeast. The mutant hypersensitive to test compound is deleted for genes encoding distinct protein which points towards the possible target of the compound. Wildtype exhibits less growth inhibition as compared to the mutant in response to the compound.

1.10.2. *Arabidopsis thaliana*

A. thaliana is a multicellular plant from the family *Brassicaceae*. *A. thaliana* was the first plant and the third multicellular organism to be completely sequenced after *Caenorhabditis elegans* and *Drosophila melanogaster* [172]. Because of effectiveness for genetic experiments, *A. thaliana* was originally adopted as a model organism. Small size, short generation time and prolific seed production are important features that contribute to the extensive utilization of this plant for studies in the field of plant biology [173].

2. Aims of the thesis

The prime focus of the present research is to explore new sources for compounds with potential antimicrobial and nematicidal activities for the treatment of diseases in humans, animals and plants.

The first objective of the study is to explore the antimicrobial properties of nanosuspensions produced from stem and leaves of the African plant, *L. micranthus*. This study uncovered the hidden treasure of exotic and indigenous medicinal plants to be utilized as potential drug candidates.

The second aim of the study focuses on the investigation of nanosized waste materials, comprising brewed coffee, tomato stems, walnut shells and grape seeds against the model microorganisms, and the possible applications of these agents. This study supports the notion that bio-waste may be utilized inexpensively and efficiently as a part of waste management.

The third aim of the project encompasses the feasibility of exploiting nanosized and lyophilized chalcogens with biological activity against bacteria, fungi and nematodes. These preliminary screening assays have led to the future investigations of these nanosuspensions against a plethora of microorganisms.

The fourth aim of the current study is to uncover the underlying modes of action of sulfur and selenium compounds, *i.e.*, allicin and selenocyanates. These redox-active compounds were investigated to probe potential targets and mechanisms of toxicity. The elucidation of the mechanism of these agents may contribute to their widespread applications in Medicine and Agriculture.

3. Results

3.1. Publication 1

Milling the Mistletoe: Nanotechnological Conversion of African Mistletoe (*Loranthus micranthus*) into Antimicrobial Materials

Muhammad Sarfraz, Sharoon Griffin, Tamara Gabour Sad, Rama Alhasan, Muhammad Jawad Nasim, Muhammad Irfan Masood, Karl Herbert Schäfer, Chukwunonso E.C.C. Ejike, Cornelia M. Keck, Claus Jacob and Azubuike P. Ebokaiwe

Antioxidants (Basel), 2018, 7(4), 60

3.2. Publication 2

No Time to Waste Organic Waste: Nanosizing Converts Remains of Food Processing into Refined Materials

Sharoon Griffin, **Muhammad Sarfraz**, Verda Farida, Muhammad Jawad Nasim, Azubuike P. Ebokaiwe, Cornelia M. Keck and Claus Jacob

Journal of Environmental Management, 2018, 210, 114-121

3.3. Publication 3

Resuspendable Powders of Lyophilized Chalcogen Particles with Activity against Microorganisms

Sharoon Griffin, **Muhammad Sarfraz**, Steffen F. Hartmann, Shashank Reddy Pinnapireddy, Muhammad Jawad Nasim, Udo Bakowsky, Cornelia M. Keck, and Claus Jacob

Antioxidants (Basel), 2018, 7(2), 23

3.4. Publication 4

Efficacy of Allicin against Plant Pathogenic Fungi and Unveiling the Underlying Mode of Action Employing Yeast Based Chemogenetic Profiling Approach

Muhammad Sarfraz, Muhammad Jawad Nasim, Claus Jacob and Martin C. H. Gruhlke

Journal of Applied Sciences 2020, 10(7), 256

3.5. Publication 5

Yeast Chemogenetic Screening as a Tool to Unravel the Antifungal Mode of Action of Two Selected Selenocyanates

Muhammad Sarfraz, Muhammad Jawad Nasim, Claus Jacob and Martin C. H. Gruhlke

Journal of Applied Sciences, 2019, 9(18), 3728

4. Discussion

The present work involves the exploration of antimicrobial and nematicidal activities of nanosuspensions generated from different parts of African plant, bio-waste and chalcogens, and mechanistic details of sulfur and selenium compounds *e.g.,* allicin and selenocyanates. The potential antibacterial, antifungal and nematicidal activities of the nanosized materials support the notion that nanosizing results in biologically active substances. Several synthetic antimicrobial and nematicidal compounds are available for application in the medical and agriculture arena. Nevertheless, the adverse effects of these agents to humans and the environment and widespread resistance developed by microorganisms have provoked the urgent search for 'green' plant-based alternatives [174]. During the last three decades, the developments in the nanotechnology field have revolutionized the drug discovery and development by finding avenues in the treatment of various diseases [175]. In nutshell, the investigations, performed as a part of this thesis, affirm the notion of nanosizing plant parts, bio-waste and chalocogens into biologically active substances. Furthermore, the mechanistic evaluation of redox-active compounds provides insights into their mechanisms of potential toxicity against target micro-(organisms). The highlights of findings of these studies will now be discussed in more detail.

4.1. Biological activity of nanosized African mistletoe

In the present study, different parts (leaf and stem) of African mistletoe (*L. micranthus*) were subjected to nanosizing and subsequently evaluated for their antimicrobial and nematicidal activities against Gram-negative (*E. coli*) and Gram-positive bacteria (*S. carnosus*), fungi (*C. albicans* and *S. cerevisiae*), and the model agricultural nematode, *Steinernema feltiae* [72].

The nanosuspensions of stem and leaves of *L. micranthus* exhibited a strong concentration-dependent growth inhibition of the microorganisms investigated. Intriguingly, the nanoparticles of stem demonstrated higher antimicrobial activity as compared to those of leaves. The antibacterial properties of the nanosized parts of the African plant have opened the door for its possible applications in gastrointestinal (GI) and topical infections. Hence, nanosizing techniques can be exploited efficiently to unlock the biological activities of medicinal plants. Likewise, nematicidal activity of these nanomaterials may be utilized to develop ‘green’ phytoprotectants. The impact of nanosizing on the biological properties of medicinal plants was revealed in various studies. The pre-screening toxicity studies of nanosuspensions derived from medicinal plants indicated significant antimicrobial and nematicidal activities in model microorganisms. The plants indigenous to developing countries are promising candidates as cheap alternatives as compared to expensive synthetic antibiotics. Medicinal plants with established biological activities may be exploited in such studies. The investigation of nanoparticles of medicinal plants, such as and *Pterocarpus erinaceus* and *Solanum incanum* L., uncovered remarkable antimicrobial properties possibly associated with these plants [71]. *Cynomorium coccineum* L., also referred to as desert thumb, is a parasitic plant which lacks chlorophyll but possesses unique compounds with a great diversity of biological applications. The whole plant of *C. coccineum* was nanosized and evaluated for antimicrobial activities. The preliminary screening for biological activity reported the antifungal activity of nanoparticles against the pathogenic fungus *C. albicans* [70, 176]. Nonetheless, further investigations into their modes of action and physical properties, for instance, stability, storage and potential interactions are required to allow practical applications of nanosized particles derived from plants.

4.2. Biological activity of nanosized bio-waste substances: Turning waste into value

The organic waste materials, such as spent coffee grounds (SCGs), tomato stems, walnut shells and grape seeds have been nanosized and investigated for their potential biological activities [177].

In the present study, the impact of nanosized SCGs on various model microorganisms was investigated. Intriguingly, a concentration-dependent increase in the growth of *S. cerevisiae* and *E. coli* was revealed. Increase in the concentration of SCGs stimulated the growth of the microorganisms, which points towards the growth promoting effects of coffee waste.

The nanosized tomato stems and walnut shells were also evaluated for their possible antimicrobial and nematicidal properties. Surprisingly, tomato stem nanosuspension did not show activity against any of the microorganisms investigated. This absence of antimicrobial activity is disappointing, since tomato stems are recognized to be fairly toxic. Similarly, nanosized walnut shells seem to show no activity against the model microorganisms under investigation.

Grape seed nanoparticles were also studied for their biological effects against bacteria, fungi and nematodes. These nanosuspensions did not affect the growth of bacteria at any of the concentrations employed, and inhibited the growth of yeast, *S. cerevisiae* and pathogenic fungi, *C. albicans* in a concentration-dependent manner. Nevertheless, the natural stabilizer, Plantacare®, also demonstrated antifungal activity against *C. albicans*. These particles inhibited the growth of the nematode, *S. feltiae*, in a modest yet statistically significant manner at a concentration of 1%. These investigations imply that grape seed particles are active at higher concentrations against selective microorganisms. There are several factors responsible for the specific activity of grape seed particles. Slow release of active compounds from grape seed suspension, for instance, is an important factor for the biological activity of grape seeds. Moreover, only a few compounds have been reported to exhibit biological and medicinal properties in spite of a large number of phytochemicals isolated from grapes [117]. Contrarily, the biological activities of nanosized grape seeds are attributed to the active phytochemicals and depend upon physical characteristics of nanoparticles, such as charge, shape, size and physical interactions. These findings uncover the efficient exploitation and management of bio-waste with the application of nanotechnology, hence, ‘turning waste into value’.

4.3. Activity of resuspendable lyophilized chalcogens (NaLyRe technique)

Sulfur, selenium and tellurium nanoparticles represent important and effective agents which exhibit biological activities against a plethora of microorganisms. Nanosized substances can be synthesized by exploiting a variety of avenues.

(a) Green synthesis of sulfur, selenium and tellurium nanoparticles by utilizing various natural resources such as plants and/ plant extracts [178-181].

(b) Exploitation of various bacteria, such as *S. aureus*, *S. carnosus*, *K. pneumoniae*, *Bacillus oryziterrae* and *Lactobacillus brevis*, and fungi, *e.g., Phoma glomerata*, *Aureobasidium pullulans*, *Trichoderma harzianum* and *Mortierella humilis* to generate chalcogen-containing nanosuspensions [182-186].

(c) Generation of sulfur, selenium and tellurium nanoparticles by employing various chemical methods [187-189].

The present work signifies that chalcogens (sulfur, selenium and tellurium) can be transformed into resuspendable lyophilized powders. The prime focus of the investigation was to unravel the possibility of the underlying technique of Nanosizing, Lyophilization and Resuspension (NaLyRe) which implies that combining nanosizing with lyophilization results in easy-to-handle, stable and biological active powders which can be resuspended when required [190].

Generally, a considerable activity of the resuspended chalcogen suspensions against most of the microorganisms (*S. cerevisiae*, *C. albicans*, *S. carnosus and E. coli* and *S. feltiae*) was revealed. Tellurium particles inhibited the Baker's yeast, *S. cerevisiae*, to greater extent as compared to sulfur or selenium. On the other hand, pathogenic fungus, *C. albicans*, was significantly affected by all three chalcogen particles. Nanosuspensions of chalcogens exhibited antibacterial activity against *E. coli* at the concentrations employed. Not surprisingly, the rather toxic tellurium exhibited higher activity in comparison to sulfur and selenium. In the case of *S. carnosus*, the tellurium particles reduce the growth to greater extent, while sulfur and selenium both were found to be less active.

Chalcogens were also tested in the assay of multicellular nematode, *S. feltiae*. Chalcogen particles decreased the viability of nematodes in a concentration-dependent manner. The nematicidal activity was evident even at the lowest concentration (25 µM) employed. Tellurium and selenium particles at a concentration of 25 µM showed greater nematicidal activity than the sulfur ones. It is now feasible to generate lyophilized powders of chalcogens which can be resuspended on demand with possible applications in the medical and agricultural arena.

4.4. Chemogenetic screening: From biological activity to mechanistic understanding

Understanding the mechanism(s) of cytotoxic compounds is of pivotal importance in chemistry. Chemogenetic screening is a method by which a library of selected yeast strains is screened against the compound in question. These yeast strains are deficient in genes that encode particular proteins. Thus, mutants inhibited by the compound refer to the target proteins or pathways. This technique is employed extensively to uncover the possible targets and modes action of compounds under investigation [169, 191-193].

4.4.1. Mode of action of allicin

Allicin is the major constituent of crushed garlic and responsible for a wide range of antimicrobial activities. A variety of mechanisms have been proposed to explain the antifungal activity of allicin, whilst, the modes of action of allicin associated with its pharmacological effects are not yet entirely addressed [194].

The present study indicates the possible antifungal properties of allicin against a broad-spectrum of plant pathogens, *i.e.*, *V. dahliae*, *V. longisporum, A. brassicicola* and *B. cinerea* by employing a combination of different kinds of assays, *i.e.,* zone of growth, gas phase and inhibition zone assays. Allicin exhibited remarkable antifungal activity against all the plant pathogenic fungi investigated. The potent antifungal activity of allicin *via* the gas phase indicates the potential applications of this substance as an alternative "green" fungicide (fumigant) in agriculture. Finally, a yeast chemogenetic screening was performed to uncover the mechanism of allicin. This screening resulted in greater growth inhibition of three mutants namely *Δnpa3*, *Δmcm5* and *Δhts1* as compared to the untreated controls and wildtype yeast. The hypersensitive mutants lack genes that encode specific proteins involved in DNA replication, mitochondrial translation and chromatids cohesion. These processes play an important role in the growth, cell cycle and viability of yeast cells. Hence, the inhibition of these mutants points towards the possible mechanism of antifungal action of allicin. The findings of the present study, therefore, reveal the excellent antifungal activities and mechanism(s) responsible for pronounced antimicrobial activity of allicin [195].

4.4.2. Mode of action of selenocyanates

Selenocyanates form a significant group of organoselenium compounds [196]. In the present study, selected selenocyanates were screened against a library of yeast mutants carrying gene deletions for specific redox-linked proteins. Selenocyanates inhibited the growth of five yeast mutants, *Δzwf1, Δyap1, Δtrx2, Δglr1* and *Δgnd1*, lacking genes that encode proteins involved in GSH metabolism and proteins for oxidative stress tolerance. The hypersensitive strains were also investigated in detail by plate inhibition zone assay, drop test and streak test. In another assay, the wildtype and GSH deficient mutants, *pad2* and *gr1*, of *A. thaliana*, were tested against selenocyanates. The decrease in length of root of *A. thaliana* mutants (GSH deficient) than wildtype confirmed that GSH is a key player in the protection of cells. Furthermore, GSH concentration was also measured in treated sample and untreated control. Decreased GSH level was observed in treated sample in comparison to control. The greater inhibition of *S. cerevisiae* and *A. thaliana* mutants advocates that selenium compounds affect the GSH pathway and metabolism, and subsequently promote growth inhibition of target cells [197]. Extensive studies in future by utilizing the whole library of yeast mutants are clearly required to explore the additional cellular targets of selenium-containing compounds.

5. Conclusions

In summary, the present work involves uncovering the antibacterial, antifungal and nematicidal activities of nanosized substances. The nanoparticles derived from African plant and bio-waste materials were investigated. Elemental chalcogen *i.e.,* sulfur, selenium and tellurium particles were also evaluated for their antimicrobial and nematicidal properties. The antifungal activity of allicin against plant pathogens was explored, and the mechanistic study was carried out by employing a library of yeast mutants. Finally, the mechanism of selenocyanates was deciphered by exploiting a combination of yeast chemogenetic screening and *Arabidopsis* mutants. The findings of the present study confirm the promising antimicrobial properties of nanosized materials, and decipher the mechanisms of action allicin and selenocyanates.

Nanosizing resulted in the biologically active nanomaterials employed in the current study. Various indigenous and exotic medicinal plants, comprising *P. erinaceus*, *S. incanum* L. and *C. coccinium* have been nanosized to unlock their potential antimicrobial and cytotoxic activities [69, 71]. Nanosizing of bio-waste into potential biologically active materials has made it possible to manage and upcycle this waste efficiently. Nanotechnology, in addition to vermicomposting and anaerobic digestion, can assist to process the food industry waste materials [108]. Moreover, bio-waste comprising stems, leaves, shells and seeds can be recycled cheaply yet efficiently. Thus, the approach "turning waste into value" may be helpful not only from the perspective of waste management but also upcycling. Nonetheless, the waste management/upcycling by conventional methods is expensive, tedious and requires solvents accelerating the production of residual waste. Nanosized waste materials, in contrast, present efficient examples of upcycling and management. The promising biological activities of the nanosuspensions confirm their potential applications in numerous fields, including medical and agriculture.

The antimicrobial and nematicidal activities of lyophilized nanosuspensions of chalcogens confirm the possibility of portable nanosized powders which retain the biological activity and can be resuspended when required. Thus, it is possible to achieve long term stability of chalcogens with the benefits of nanosizing and lyophilization. The NaLyRe sequence affirms the feasibility to freeze-dry chalcogens

[190] with potential applications in the fields of Medicine, Agriculture, Cosmetics and Nutrition. Chalcogen particles have been utilized efficiently for a vast array of applications including antioxidant, antimicrobial, immunomodulatory, antiviral, cytoprotective and cytotoxic activities [178-189].

Further studies are obviously required to uncover the physicochemical and nanotechnological aspects of nanomaterials. Additionally, pharmacokinetic and pharmacological properties of the nanosized substances will definitely be required to be explored for future wider applications. Ultimately, further investigations to discover the MOA of active nanosized substances are clearly required for potential applications in Medicine and Agriculture.

In the present study, reactive species such as RSS (allicin) and RSeS (selenocyanates) were subjected chemogenetic screening to decipher the mechanism of potential toxicity of these compounds against microorganisms.

The activity of allicin was determined against the plant pathogenic fungi. Allicin showed excellent antifungal activities against the plant pathogens. The mechanistic understanding of the antifungal activity of allicin was investigated by employing yeast chemogenetic screening. Allicin treatment resulted in the inhibition of yeast mutants lacking genes required for vital functions. This approach explains diverse activities of allicin against a plethora of microorganisms. Moreover, the efficacy of allicin *via* the gas phase justifies the application of allicin as bio-fumigant in the agricultural arena.

After uncovering the mechanism of antifungal activity of sulfur-containing compound (allicin), the rest part of the thesis involved the mechanistic evaluation of selenium-containing compounds (selenocyanates).

Selenocyanates demonstrated promising antimicrobial, nematicidal and cytotoxic activities particularly against resistant microorganisms. Similarly, various safety and stability assays confirm their therapeutic and agricultural applications [128]. The significant antimicrobial activities of selenium compounds have led to investigate the mechanism(s) of these highly reactive agents. Yeast chemogenetic profiling was carried out by employing yeast mutants lacking genes that encode specific proteins. The results of screening uncovered the inhibition of growth of yeast mutants deficient in GSH. The pivotal role of

GSH was also investigated in a higher multicellular organism, *A. thaliana*. Treatment of *Arabidopsis* with selenocyanate decreased the length of root of mutants as compared to wildtype *A. thaliana*. These findings affirm the protective role of GSH against OS also in plants. Likewise, these screening assays can be exploited to uncover the mechanism(s) of broad-spectrum of compounds. Future investigations are necessary to explore the additional targets of these compounds. The profiling studies in yeast are simple, inexpensive and robust as compared to tedious cell culture techniques. Numerous compounds can be screened simultaneously against the whole library of yeast mutants [198].

In conclusion, the entire plant or plant parts can be converted into nanoparticles which exhibit excellent antibacterial, antifungal and nematicidal activities. Similarly, chalcogens may be transformed into nanosized lyophilized powders which can be resuspended exhibiting improved solubility, stability and biological activity.

The mechanistic exploration of allicin and selenocyanates presents a simple, inexpensive, easy to execute and robust model for screening a plethora of compounds against a library of yeast mutants. Detailed investigations of these compounds are clearly needed for their potential applications in various fields.

6. References

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