Trop J Nat Prod Res, June 2024; 8(6):7355-7367 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Role of New Nanosized Feed Additives as Inhibitors of Certain Gram-Positive and Gram-Negative Bacteria and as Anti-Mycotoxigenic Agents

Nesrine. H. Youssef[1], Alnaji A. Almansori[2], Amany H. Shams[3] and Mayada A. Sabra[4*] and Pousy A. Salaheldin[5]

[1]*Regional Centre for Food and Feed (RCFF), Agricultural Research Center, Microbiology and Mycotoxins labs, Dekhila Port, Alexandria, Egypt.*
[2]*Botany Department, Faculty of Science, Derna University. Derna 417230. Libya.*
[3] *Bacterial Plant Diseases and Molecular Bacteriology Laboratory, Plant Pathology Department, Faculty of Agriculture El Shatby. Alexandria University. 21545.*
[4*]*Agricultural Department of Botany (Agricultural Microbiology), Faculty of Agriculture (Saba Basha), Alexandria University, Alexandria, Egypt,*
[5]*Agricultural Research Center, Horticultural ResearchInstitute, Forestry Department, Alexandria, Egypt.*

ARTICLE INFO

Article history:
Received 13 March 2023
Revised 14 May 2024
Accepted 29 May 2024
Published online 01 July 2024

ABSTRACT

Recently, because of the world's critical conditions, the development of new feed additives and the creation of non-traditional double-function feed additives are essential and for improving feeds quality by reducing the content of certain bio-contaminants. This study aimed to reduce environmental pollutants and create effective and economical inhibitors of bio-contaminants by evaluating the aptitude of these feed additives such as particles Miswak (*Salvadora persica*), black mulberry leaves, and seaweeds (*Sargassum linifolium* and *Posidonia oceanica*) algae with different concentrations (500 and 1000 µl) and (50 and 100 µl) before and after nanosizing process, respectively, to reduce the growth of certain gram-positive and gram-negative bacteria, such as *Enterobacter cloacae* and *Staphylococcus aureus*, , as well as the production of the fungus *Aspergillus flavus* aflatoxin (B1). Our data illustrated that all the tested particles of Miswak, black mulberry leaves, and *Sargassum linifolium* and *Posidonia oceanica* algae possessed antibacterial activity against the tested bacteria, which increased after the nanosizing process, but *S. aureus* was more susceptible to the tested nanosized treatments than *E. cloacae*. Treatments with nanosized Miswak had the greatest inhibitory effect on both tested bacteria, with 96.84% regarding *S. aureus* and 95.185% in the case of *E. cloacae* at a concentration of 50 µl and dilution10^{-6}. The aflatoxin detoxification capabilities of these treatments were tested. *Posidonia oceanica* (P.O.) particles were the best detoxifier agent before and after the nanosizing process, followed by blue mulberry leaves and *S. linifolium* particles. Nanosized treatments, especially *Posidonia oceanica*, an antibacterial and aflatoxin detoxifier agent, are recommended.

***Keywords*:** Antimicrobial activities; plant parts and algae nanoparticles; *Enterobacter cloacae staphylococcus aureus Aspergillus flavus;* AflatoxinB_1.

Introduction

Enterobacter cloacae is a gram-negative, optionally anaerobic, rod-shaped bacterium that belongs to the ubiquitous Enterobacteriaceae family. Additionally, *E. cloacae* causes many human diseases, such as many nosocomial infections with high resistance to fluoroquinolone .[1] Urinary tract infections (U.T.I.s), respiratory infections, soft tissue infections, osteomyelitis, and endocarditis. *Enterobacter* species can be present in water, potatoes, certain foods, soil, human skin surfaces, and sewage.[2], and they have become increasingly important as phytopathogens. For example, *E. cloacae* has been associated with the internal decomposition of onion.[3]

*****Corresponding author. E mail: Mayada555@alexu.edu.eg
Tel:+02001001538498

Citation: Youssef, NH, Almansori, AA, Shams, AH, Sabra MA, Salaheldin, PA. Role of New Nanosized Feed Additives as Inhibitors of Certain Gram-Positive and Gram-Negative Bacteria and as Anti-Mycotoxigenic Agents. Trop J Nat Prod Res. 2024; 8(6): 7355-7367. https://doi.org/10.26538/tjnpr/v8i6.4

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

In addition, other species of this complex of *E. cloacae* have been reported to cause mulberry, alfalfa seeds, and cassava.[4,5,6] as well as, potato soft rot in Egypt.[7] *Staphyllococcus aureus* is a major commensally opportunistic mammalian pathogen and one of the most dangerous human pathogenic staphylococcal species.[8] It invades animal and human skin and mucosal membranes and causes fatal infectious diseases in a plethora of animal hosts. This is harmful to animals' health and can serve as a reservoir for the human *staphylococcus* bacteria[9].

Fungal secondary metabolites called aflatoxins, one of certain *Aspergilli* that commonly occur in food and feed and pose a health risk to consumers.[10, 11] Food and feed processing and suitable storage conditions can further reduce mycotoxin levels by physical removal and decontamination by chemical or enzymatic transformation of mycotoxins into less toxic products.[12] Plants are natural sources of antibacterial and antifungal agents, and the carbohydrate composition, total phenolic content and in vitro antioxidant activities of fruits and leaves of many mulberry species have been studied.[13] The total phenolic content was reported to be the highest in black and red mulberry leaves. It may exhibit good nutritive and antioxidant activity in all varieties .[14] Miswak (*Salvadora persica*) is frequently taken from the Arak tree's roots also, and some sticks collected from El-Minia governorate, Egypt, are made extracts from its twigs and stem.[15] The beneficial effects of Miswak on oral hygiene and dental health are partially due to its mechanical action and mainly due to its pharmacologic action and chemical constituents.[16] The biological effects (antibacterial and antifungal) of *Salvadora persica* are due to

its high total phenolic and flavonoid contents and its effective antioxidant activity.[17,15]

Conversely, marine algae contain a large range of secondary bioactive metabolites, such as alkaloids, polyketides, cyclic peptides, polysaccharides, phloro-tannins, diterpenoids, sterols, quinones, and glycerol-lipids, as antimicrobial agents; moreover, marine algae are considered a new source of bioactive compounds for drug production.[18,19] Many bioactive components in algae inhibit the growth of some gram-positive and gram-negative pathogenic bacteria.[20] The brown algae *Sargassum linifolium* is a marine macroalgae (Phaeophyta) considered the second most common group of seaweed.[21] This alga produces diverse polysaccharides with valuable biological activities.[22] The extract of *Sargassum* spp. was reported to minimize spectrum toxicity in rats .[23] *Posidonia Oceanica* is a green algae ball that pollutes Egyptian beaches and is of great interest for polluting the Mediterranean Sea.[24] *Posidonia oceanica* (Po) extract has a high total phenolic content. *P. Oceanica* has antifungal and antibacterial activities.[25]Copper (Cu) is a trace element necessary for biological utility and essential to the functional immune system while supplemental copper added to pig diets can enhance daily growth and feed conversion ratio in addition to reducing microcytic hypochromic anaemia .[26] Table (1) summarizes the importance of copper as a feed supplement in several animal species.

Table 1: Total copper requirements per ppm dry matter of complete feed for ruminants, broiler chickens and rabbits according to E.U. Feed Industry recommendations.

Species	Categories	Total Cu requirements in	References
Ruminants:	Calve	15	N.R.C. (National Research Council), (2007a)
Bovine	Dairy Cows	35	
	Cows for reproduction	35	
	Cattle	30-35	
Caprine	Dairy goats	10-25	
	Goats for reproduction	10-25	
Ovine	Dairy sheep	15	Ojo *et al*., (2009)
Broiler chickens	Chickens from 1 to14 days age	126	Tumová *et al*., (2002)
	Chickens from 15 to 41day	35	
Rabbits	Rabbits	30	Li *et al.*, (2021)

Over the past two decades, nanoscience has advanced dramatically and found utility in various industries, including biomedical and agricultural .[27] Inorganic nanoparticles have shown great structural flexibility and functionality and have been applied in pharmaceutical applications for the treatment of diseases and for targeted delivery.[28,29] Among most inorganic nanoparticles, copper nanoparticle (CuNP) antiseptics show a wide spectrum of activity, and their microbial resistance is much less than antibiotics.[30] Chemical precipitation processes usually produce these sustainable (CuNPs) nanoparticles.[31] The antibacterial properties of silver have been known for more than 100 years, and it is used in treatments.[32] Copper nanoparticles (CuNPs) can interact with the organelles of microorganisms and with thiol groups in enzymes and proteins, [29,33] which leads to the accumulation of copper in vacuoles and cell walls as granules and inhibits microorganism growth.[34] CuNPs have greater bactericidal activity due to their larger surface area, which results in synergistic effects that exert antibacterial activity.[35,36] These nanoparticles disrupt bacterial membranes, increase cell permeability, and prevent cell division, resulting in cell death. Moreover, it interacts with bacterial membrane proteins, phospholipids, lipoproteins and lipoteichoic acids. It reduces their colonization and surface adhesion .[34] Cu ions and Cu nanoparticles are considered high antibacterial agents against a wide range of bacteria, including *Escherichia coli*, *Salmonella enterica*, *Staphylococcus aureus* and *Listeria monocytogenes*, and it is considered an essential nontoxic metal for human health.[37] The goal of this study was to determine the antibacterial activity of certain plant parts and alga extracts in reducing the growth of *E. cloacae* and *S. aureus*, and to study the effects of these extracts as antifungal and anti-mycotoxigenic agents on reducing the growth and the amount of aflatoxin produced by *Aspergillus flavus*. This research also sought to ascertain the function of the nanosizing of certain plant parts and algae using copper sulphate, which is usually added to rumen feed,[38] to inhibit these tested bacteria and fungi and to compare the efficacies of these processes. On the other hand, our study focused on determining the phytochemical properties and proximate analysis of these plant parts and minimizing seacoast waste during mining by recycling and reusing this *P. oceanica* biomass waste as a feed additive.

Materials and Methods

Bacterial strains used

The *Enterobacter cloacae* strain *Enk*1 (accession: LT592256) and *Staphyllococus aureus* strain ATCC 29213 were obtained from Department of Microbiology, Faculty of Agriculture, Alexandria University, Egypt . The *Aspergillus flavus* strain NRRL3357 obtained from the National Research Centre, Dokki, Cairo (CAICC), Egypt.

Testing the ability of the isolate to produce aflatoxin (confirmatory test)

To ensure the accuracy of our findings, we meticulously confirmed the ability of the *A. flavus* isolate to produce mycotoxins. The fungal strain was carefully grown on 'Yes' medium and tested using the precise agar plug method. Subsequently, an HPLC apparatus was used, following the established protocols of [39,40]. As we have observed, this rigorous process ensures that aflatoxins can be produced in isolation.

Preparation of plant parts and other materials

Miswak *(Salvadora persica)* stems samples were collected from fences located 35 kilometers from the city of Sharm El Sheikh, which represents a natural fence around an area to stabilize sand dunes, on May 2021 Figure (1, A).Seagrass balls of *Posidonia Oceanica* were collected from agglomerated balls on the polluted western coast of Rocky Bay of Abu Qir, Alexandria, Egypt (longitudes 30◦05′- 30◦22′ E and latitudes 31◦16′- 31◦21′ N), during spring 2021 Figure (1, B). Alexandria black mulberry fresh leaves were collected from Antoniadis Station, Alexandria , Egypt (Latitude: 31° 11' 1.20" N Longitude: 29° 56' 33.59" E). Liophylized brown algae *(Sargassum linifolium*) were purchased from the National Institute of Oceanography and Fisheries, NIOF, Egypt, Faculty of Sciences, Alexandria University Figure (1, C).All plant parts were gently washed with sterilized water, surface sprayed with 20 g each of 70% alcohol, and oven-dried for 72 hrs. at 40 °C to prevent degradation of all their active ingredients.[41,42,43]The sterilized pieces were finely ground after sterilization and kept at 4 °C for further analysis. 20 mL of ethanol was added for each 6 g plant leaves and kept in the dark for 16 h, then blended in a sterilized blender. Afterward, the mixture was filtered and, kept in sterilized bottles at -4°C until their use for further analysis.

Preparation of plant parts and other materials
Leaf samples, *P. oceanic* balls (P.O.) and Miswak sticks (M) were surface sterilized using 70% alcohol spray, cut into small pieces and oven-dried at 40 °C to avoid any degradation of active components.[44] After drying, the samples were ground into fine powder using a sterilized electric grinder.

Proximate analysis
The proximate nutritional composition of the plant samples used, including moisture, crude protein content, ash, crude fat, and crude fibre contents, was determined using NIRS according to.[45,46] The proximate composition of the tested samples was determined at Protein Lab RCFF (Abees Branch) using a Foss NIRs TW DA1650 instrument (Serial No 91758059 Denmark). The total carbohydrate content was estimated using NIRS according to.[47]

Determination of the total phenolic content, total flavonoids, and antioxidant activity of the tested plant parts
The plant samples' total phenolic and flavonoid contents were spectrophotometrically assayed at the EL-Shatby Agriculture Faculty Central Lab according to.[48,49] The spectrophotometric method is less expensive and faster than other analytical chromatographic techniques, as measured spectrophotometrically by the Folin–Ciocalteu colorimetric method, using gallic acid as an equivalent (G.A.E.) per gram of sample. The absorbance of phenolic compounds was also tested and analyzed as whole spectra because it is more accurate than estimation based on absorption at single wavelengths, samples were analyzed in three replications.[50] The total phenolic content was determined with the help of a standard cure prepared from a pure phenolic standard (gallic acid).

Determination of total flavonoid content (TFC)
The determination of flavonoids in the tested plant extracts was carried out after complexation with aluminium chloride according to.[51,52] Briefly, 0.5 ml of each plant extract and standard solution (0.01-1.0 mg/ml) were mixed with 2 ml of distilled water, followed by the addition of 0.15 ml of sodium nitrite (5% $NaNO_2$ w/v) solution. After 6 minutes, 0.15 ml of 10% $AlCl_3$(w/v) solution was added. The reaction time and $AlCl_3$ concentration were 2.5–7.5%. Six minutes later, 2 ml of sodium hydroxide (4% NaOH, w/v) were added. The final volume was adjusted to 5 ml with the immediate addition of distilled water, and the samples were homogenized thoroughly via direct dilution. The absorbance of each mixture was determined as mg equivalent per gram of sample at 510 nm. All analyses of all samples were performed in triplicate.

Determination of the antioxidant activity
The antioxidant activity was estimated according to .[53,54] using DPPH radical scavenging ability. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical is a stable purple free radical that reacts with a hydrogen donor obtained from Sigma–Aldrich (Egyptian Bureau). DPPH was used to test the antioxidant activity, and the capacities of the extracts to scavenge free radicals were determined as described by.[55,56]
The calculation equation was:$(DPPH)\ \% = [(Ab - Abs)/Ab]\ x\ 100$
where Ab is the blank absorbance value, and Abs is the sample absorbance value.
Gas Chromatography–Mass spectrometry analysis using a G.C. (Agilant Technology 7890A) coupled with a mass selective detector (M.S.D., Agilent 7000TripleQuad) equipped with an Agilent HP-5ms capillary column T.G.–5MS (30 m × 0.25 mm × 0.25 µm film thickness) was performed as previously described by .[54,57]

Figure 1. (A) Miswak (*Salvadora persica) (*B) Sea grass ball of *Posidonia Oceanica* (C) Brown algae *(Sargassum linifolium)*

Green Biosynthesis of CuNPs
Preparation of chemicals and reagents
Copper sulphate pentahydrate ($CuSO_4\ 5H_2O$) was purchased from Sigma–Aldrich, Egypt Bureau. Double deionized and distilled water were obtained from the Central Laboratory of Agriculture Faculty, El Shatby, Alexandria University.

Preparation of plant parts and plant byproducts
Small pieces of plant samples were washed with distilled water, oven-dried at 40 °C, ground in a sterilized electric grinder and dropped into 100 ml of double deionized water in a 250 ml glass beaker at 80 °C for 10 min. An ultrasound sonicator was used to disturb and destroy plant cell walls according to,[58] which improved and augmented the ability of the water to penetrate the cells and obtained a high-quality compound extraction yield. Afterwards, the extract was filtered through Whatman No. 41 filter paper to obtain aqueous plant extracts, which were frozen.

Biosynthesis of CuNPs
Plant sample aqueous extracts (100 ml) were gently mixed with 4 g of $CuSO_4.\ 5H_2O$ using magnetic stirring at room temperature (25 °C) for 4 h. [59] The conversion of the blue color, which is characteristic of copper sulphate pentahydrate, changed to a brown color within 10-15 minutes, indicating the formation of CuNPs due to the reduction of copper ions from Cu (II) ions to Cu. [35] The clear supernatant of the samples was obtained by centrifugation at 3000 rpm for 10 min at room temperature. The obtained copper nanoparticles were dried in an oven at 80-90 °C for 4 h for further analysis.
Characterization of the CuNPs
Determination of the structure of the synthesized CuNPs and the distribution of these nanoparticles was confirmed by using ultrasonic liquid processing (U.L.P.) (BANDELIN, German, 20 kHz), which involved direct immersion in the reaction solution for 5 min at room temperature. The surface of the prepared CuNPs was studied via a Fourier transform spectrophotometer, and the morphology and size distribution of the CuNPs were investigated via transmission electron microscopy.[60]

Fourier transform infrared (FTIR) Spectroscopy
The chemical constituents responsible for the reduction of the synthesized CuNPs were studied by using FT-IR spectroscopy. FTIR analysis was performed using Bruker Tensor 37 (Germany). Fourier transform infrared (FT-IR) spectroscopy was performed in the range of 3500–500 cm^{-1}, and all the measurements were recorded in transmittance (T%) mode at room temperature.[61]
Transmission electron microscopy (T.E.M.)
The surface morphology and size of the synthesized CuNPs were analyzed via transmission electron microscopy (JEM-1400Plus-Japan).[62] The test was carried out by dispersing the samples in an ethanol solution through sonication for 15 min and centrifuging for 10 min at 10000 rpm. Drops of the green synthesized CuNPs were poured onto carbon-coated gold grids and then left to dry naturally. A size distribution histogram was obtained by using Nano Measurer Software.[27]

Bacterial Culture Activation (Bacterial Experiment)
Each bacterial strain was activated in brain-heart broth for 24 h and incubated at 25 °C. After incubation, the bacterial cultures were harvested individually by centrifugation at 600 rpm for 10 min and then washed twice with phosphate buffer. The turbidity was adjusted (with a spectrophotometer) to an optical density (O.D.) of 0.85 at 600 nm. A total of 200 µl of the cell suspension of each bacterial strain was inoculated into nutrient agar and incubated at 25 °C for 48 h with *Staphylococcus aureus* according to.[42] and with the *Enterobacter clocae* standard method using serial dilutions according to.[63,64] The plate counts were recorded as serial dilutions. A bacterial treatment experiment was performed before and after the nanosizing process with the following treatment concentrations: 500 and 1000 µl before the nanosizing process and 50 and 100 µl after the nanosizing process.

Preparation of fungal broth (Aspergillus flavus experiment)
Thirty-nine 250 ml conical flasks were each filled with 100 ml (yeast extract and sucrose) broth medium and then inoculated with 4 mm of *A. flavus* inoculum. Treatment concentrations were 500 and 1000 microlitres for non-nanosized plant extracts and 50 and 100 microlitres for nanosized plant extracts. Each treatment was repeated, and the plants in the control group were kept in an incubator at 30 °C for fifteen days. The fungal mass was gently removed, and the sample was oven-dried for 72 h at 70 °C and then weighed. Aflatoxin levels were estimated in all nontreated and in both treated non-nanosized and nanosized fungal treatments.

Statistical analysis
The statistical analyses were carried out using the CoStat program, version 6.303, according to.[65] and the analysis of variance technique (CoHort software, Monterey, C.A., U.S.A.). The phytochemical property and proximate analysis data are presented as the mean ± standard deviation ($L.S.D_{0.05}$), and the values were considered statistically significant when $p \leq 0.05$.

Results and Discussion

Phytochemical analysis of the tested treatments
The total phenolic and flavonoid contents in the plant samples were spectrophotometrically measured at the El-Shatby Agriculture Faculty Central Lab according to.[48,51]
Table (2) shows that the brown algae *S. linifolium* was the richest plant material tested in this study, with a flavonoid content of 211g/ml and TPC of 329.82 mg GAEs/g DW and an antioxidant activity of 83.6%, followed by blueberry leaves and then Miswak, with a flavonoid content of 55.66 and 48.27 g/ml, TPC of 70.94 and 66.13 mg GAEs/g DW. and antioxidant activity of 47.87% and 39.2%, respectively. However, *P. oceanica* was the poorest plant material used in this study. Our findings concur with those, [66] who reported that *Saragassum sp*. is considered a rich source of carotenoids and many bioactive compounds, such as terpenoids, sterols, sulphated polysaccharides, polyphenols, sargaquinoic acids, sargachromenol, and pheophytin. Our results concerning the flavonoid content of Miswak were consistent with those of ,[67] reported that Miswak root contains a small number of flavonoids.[67] Though, the phytochemical properties of our studied *P.* oceanica were poorer than those of ,[68] which indicated that the origin and year of the collected alga additionally have significant roles in the phytochemical properties of algae and plants.[69] Our findings are closely in agreement with those of,[46] who found strong variability between different collections. Moreover, the flavonoids, total phenol content, and antioxidant activity of Miswak (*Salvadoa persica* root stick) and black mulberry (*Morus nigra*) leaves did not match those of ,[70,14] nor did they match those of, [12], which is simply because the phytochemical properties of plants differ according to the collection time in the same seasons, the cultivation season, and the origin of the cultivated plant. Our data are relatively consistent with those of,[25] who reported that *Posidonia oceanica* (P.O.) extract has a high total phenolic content.

Proximate analysis of the tested treatments
The proximate analysis of the tested plant parts and algae was carried out according to using NIRS methods.[17,45,71,46]
Table (3) shows that Miswak has the highest ash content, *P. oceanica* has the highest fibre and carbohydrate contents, and black mulberry leaves have the highest protein, fat and moisture contents. The proximate analysis of miswak typically coincided with that of, 67,72,70 illustrated that Miswak root sticks contain 27.1% ash of considerable amounts of chlorides. However, the algae of concern coincided with those of,68 reported that *P. oceanica* does not contain fats. The proximate analysis of black mulberry leaves was highly in agreement with the results of ,14, who found that the fibre and protein ratios of *Morus nigra* L. were 12.32 ± 1.18 and 19.76 ± 2.12, respectively.

Characterization of the CuNPs
The properties, spectroscopic analysis, and T.E.M. micrographs of the tested nanosized plant samples are shown in Fig. 2. Our spectroscopic analysis and T.E.M. micrographs revealed that the diameter of the miswak nanosized particles ranged from 4.36 to 28.12 nm, the diameter of the black mulberry nanoparticles ranged from 4.48nm to 15.46nm, the diameter of the *S. linifollium* nanoparticles ranged from 15.26 to 35.14nm, and *the diameter of the P. oceanica* particles ranged from 3.93 to 11.22 nm. The average sizes agreed with those of ,[73], who reported that synthesized particles with average sizes ≤100 nm were found to have bacteriostatic and/or bactericidal effects and were size- and dose dependent.

Impact of the examined plant extracts and their Nanosized particles on reducing bacterial growth
The experiment was completed utilizing the serial dilution technique according to .[44] The experiment was repeated before and after the plant material nanosizing process. The data shown in Tables 4 and 5 are as follows:

For Staphylococcus aureus
The differences between the *S. aureus* inhibition ratios before and after nanosizing were highly significant at all dilutions. Treatment with 500 µl of *S. linifolium* at the conc500 µl resulted in the greatest inhibition of *S. aureus* growth, followed by treatment with 500 µl of black mulberry and then Miswak at the conc500 µl. Our findings agree with both of,[74,] who reported that black mulberry leaf extract had a potent antibacterial effect on *S. aureus*. Nanosized Miswak particles were the most effective growth inhibitor (10 CFU in 50 µl), followed by *P. oceanica (*14 CFU in 50 µl), and then Miswak and *P. oceanica* (21 CFU at100 µl). Our results are consistent with those of,[75,76] who reported that the antibacterial activity of ZnO and MgO increased with decreasing particle size.

Table 2: Phytochemical features of the tested plant materials

Materials	Flavonoids g/ml ± S.D.	Total Phenol Content (TPC) mgGAEs/g D.W. ± S.D.	Antioxidant activity% (A.A.) g/mL ± SD
Miswak root sticks	48.27±1.137	66.13± 2.03	39.2±1.02
Black mulberry	55.66± 2.07	70.94± 1.537	47.87± 1.075
S. linifolium	211± 1.53	329.82± 1.4029	83.6± 0.907
P. Oceanica	30.17±2.07	36.94±1.507	31.73± 0.940

Table 3: Proximate analysis of the tested materials

Materials	Ash content ± S.D.	Fibre content± SD	Protein content± SD	Fat content± SD	Moisture%± SD	Carbohydrates± SD

Miswak	25.096±1.116	28.835±1.085	9.845±0.609	4.77±0.957	8.89±0.774	9.05±0.995
Bl. mulberry	18.67± 0.665	12.91±0.62	19.09± 0.940	8.66±0.821	9.83±0.621	12.99±0.678
S. linifolium	38.46± 0.593	32.32±0.768	5.20±1.110	1.94± 0.05	6.87± 0.577	11.21± 0.931
P. Oceanica	19.66± 0.900	42.69± 0.695	7.38± 0.848	0.07±0. 01	5.5± 0.319	17.8± 0.699

For Enterobacter cloacae

In the case of *E. cloacae*, before the nanosizing process, the best inhibition ratio was observed for miswak, followed by *S. linifolium* at a conc. of 1000 µl. Moreover, after the nanosizing process, the efficacy of the tested materials in bacterial growth inhibition greatly increased. Moreover, 50 µl of the Miswak nanoparticles was still the best inhibitor treatment, followed by 100 µl of *P. oceanica* and 50 µl of both *S. linilolium* and *P. oceanica*. The bacterial inhibition process before and after nanosizing was highly significant. Our findings indicated that the nanosizing process not only increased the efficacy of bacterial inhibition but also that the antibacterial properties of the tested particles were completely altered. Recorded results closely agree with those of [77] who showed that plant particle size significantly impacts how pathogen cells interact with plant particles. Furthermore[71] clarified that aqueous extracts from the rhizomes of *Posidonia oceanica* have antimicrobial activity, and *S. linifolium* can be considered a good antibacterial agent due to its high content of phlorotannins, which are dominant in brown algae.[71]

Effect of antibacterial plant part extracts on bacterial growth before and after the nanosizing process

Results demonstrated in Tables (4 and 5) showed the determination of the efficacy of the tested antibacterial agents before and after the nanosizing process. Moreover, Figures (3 and 4) illustrated the efficacy ratios of the tested plant extracts before and after the nanosizing process on both tested bacteria at serial dilutions of 10^{-6}.

The efficacy ratios (E.R.s) of the treatments depended on the type of treatment and the Gram type of the tested bacteria. The (ER%) of the treatments against *S. aureus* and *E. cloacae* before nanosizing was dependent on their concentrations in the case of black mulberry leaves, *S. Libyalinifolium* and *P. oceanica* but not the efficacy ratio of Miswak was concentration dependent only in the case of *E. cloacae*. The lower concentration of 500 µl achieved the best results *for S. linifolium*, followed by black mulberry leaf extract, *P. oceanica*, and *S.* aureus (Table, 6). However, in the case of *E. cloacae*, the nanosized *P. oceanica* had the best inhibition ratio, except for treatment with *P. oceanica*, which had the best efficacy ratio at 500 µl. Our findings agree with,[78] who reported that the type of bacterial strain and the concentration of the AgNP particles influenced the inhibition zones of *S. aureus* and *E. coli*. The nanosizing process increased the ratio of the treatment's efficacies at the lowest concentrations for both tested bacteria. Treatment with nanosized Miswak had the greatest inhibitory effect on both tested bacteria, with 96.84% in the case of *S. aureus* and 95.185% in the case of *E. cloacae* at a concentration of 50 µl and dilution10^{-6}. Our results also revealed that 50 µl of the nanosized particles of (P.O.) algae was highly effective at inhibiting *S. aureus*. with an ER 95.58% greater than that of inhibiting *E. clocoae*, with an efficacy ratio of 94.07% at 100 µl, which was inhibited at 100 µl. with an ER% of 94.07%. Our findings are highly consistent with those of, [25] who reported that *Posidonia oceanica* (P.O.) extract acts as a bacteriostatic agent against gram-negative-negative bacteria and a bactericidal agent against gram-positive-positive bacteria. The nanosizing process changed the antibacterial properties of the studied particles from moderately effective to highly effective; for example, the efficacies of Miswak and *P. oceanica* completely changed from 35.02% to 96.84% and from 36.28% to 93.69%, respectively, in the case of Miswak against *S. aureus* growth and from 18.92 to 95.58% and from 10.72% to 93.37%, respectively, Table (6), in the case of *P. oceanica* against *S. aureus* growth. However, in the case of *E. cloacae*, the behaviour of these treatments was the same except for the nanosized miswak at a concentration of 100 µl, where its efficacy against *E. cloacae* growth decreased. Our findings corresponded with,[79] who reported that the antibacterial efficacy was affected by the bacterial cells' physiological status and morphology and crystal growth habits. Their results indicated that flower-shaped nanoparticles were significantly more photocatalytically inactive than rod-shaped nanoparticles, followed by spherical nanoparticles, against *E. coli* and *S. aureus*. The antibacterial activity of the nanoparticles increased with decreasing crystallite size. Also,[80] founded that nanosized copper particles are considered nanoparticles with very stable chemical and physical properties that play a valuable antimicrobial role due to their extremely high surface areas with unusual crystals and that these nanoparticles can change the structure of cell membranes after penetrating bacterial cell walls, causing cell death. Their efficiency was due to their nanoscale size and large ratio of surface area to volume. Our results revealed that *S. aureus* was more vulnerable to the tested nanosized treatments than was *E. cloacae* and, closely with ,[81,82] who reported that gram-positive bacteria lack an important layer, which makes gram-negative bacteria more resistant or less susceptible to antibacterial agents than gram-positive bacteria.

Fungal experiment

The fungal experiment was carried out using an aflatoxigenic strain of *Aspergillus flavus*, considered the most ubiquitous feed contaminant. Aflatoxin B1 is the most hazardous health mycotoxin for animals and humans. The fungicides and detoxifier agents tested before and after nanosizing were evaluated. Results, as shown in Table (7), illustrated the inhibition ratio before and after nanosizing process data as followed as Miswak achieved the best fungal growth inhibition ratio (66.49%) at concentration 500µl and (65.97%) at 1000 µl concentration. Then, *P. oceanica* was recorded (62.83%) at a conc. of 500µl. After the nanosizing process, there was no significant ameliorate fungal growth inhibition, although *P. oceanica* attained the highest ratio (56.02%) at a concentration of 50µl.

The study reported that *P. oceanica* extract acts as an antifungal agent against *Aspergillus niger* and *Penicillium chrysogenum*, which was in harmony with our results.[25] Tables (4, 5 and 7), showed the antibacterial activity of the nanoparticles was greater than their antifungal activity, which may be due to differences in the structure and cell wall of prokaryotic bacterial cells and *eukaryotic* fungal cells, where the cell wall of bacteria, even if present, is composed of peptidoglycan mines. In contrast, the cell wall of fungi is composed of chitin, cellulose, or hemicellulose.[83]

On the contrary, results shown in Table (8) revealed that the *P. oceanica* had the highest AflB1 inhibition ratio (97.64%) at a concentration of 1000 µl before nanosizing and after nanosizing (98.23%) at a conc. 100µl. Moreover, the nano-treatment decreased the concentration of aflatoxin B1 applied from 1000 µl to 100µl and simultaneously increased the efficiency of this treatment in inhibiting aflatoxin B_1 production. Furthermore, the Nano processing process increased the efficiencies of all the tested particles by approximately 2.783-fold in the case of Miswak, 1.174-fold in black mulberry, 1.103-fold in *S. linifolium* and 1.080-fold in *P. ocianica* at low concentrations of 50µl and 500 µl. On the other hand, the nano-Fenton process augmented the aflatoxin inhibition efficiencies of Miswak by 1.31-fold, black mulberry by 1.04879-fold, *S. linifolium* by 1.08-fold and *P. oceanica* by 1.01-fold at concs, 100 µl and 1000 µl, respectively, which indicated that the efficiency of the particles was greater at 50 µl than at 100 µl. Our findings did not coincide with those of, [84] who reported that the inhibition of *Pseudomonas aeruginosa* and *Botrytis cinerea* was enhanced with increasing concentrations of N.P.s, which may be due to differences among pathogens, plant particles and nanoparticle types. Another finding unequivocally showed that the AgNPs significantly prevented the growth and development of the plant pathogen *Botrytis fabae*, lowering the rhizosphere and the number of bacteria (cfu/ml). The

study advised he use of AgNPs as an antibacterial agent in the agricultural sector.[32]

As shown in Tables 7 and 8, our results revealed that the tested treatments were more effective at reducing or inhibiting aflatoxin production than reducing *A. flavus* growth. Our findings are consistent with those of ,[85] who reported that the inhibitory effect on fungal growth was not directly related to mycotoxin inhibition. Our data on aflatoxin detoxification also agreed with those of ,[86] who reported that macroalgae possess a high biosorption capacity.

Proximate analysis of the tested treatments

The proximate analysis of the tested plant parts and algae was carried out according to using NIRS methods.[17,45,71,46]

Proximate analysis of the tested treatments

The proximate analysis of the tested plant parts and algae was carried out according to using NIRS methods.[17,45,71,46]

Table 4: Effects of the tested treatments before and after the nanosizing process on *S. aphyllia aureus* growth

Treatments		Concentration in (µl)	Serial dilutions 10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Before nanosizing process	*Staphyoccus aureus*	Control(C)	446[a]	430[a]	391[a]	381[a]	332[a]	317[a]
	C+ Miswak	500	394[d]	348[e]	304 [f]	274 [e]	228 [de]	206 [d]
		1000	363[h]	346 [f]	317 [e]	258 [f]	224 [e]	202 [d e]
	C+ Bl. mulberry	500	338[i]	244 [h]	239 [g]	222 [h]	208 [f]	177 e
		1000	396 [c]	372[c]	344 [c]	337 [c]	230 [c]	209 [d]
	C+ S. *linifolium*	500	372 [g]	259[g]	234 [g]	187 [i]	115 [h]	109 [f]
		1000	390 e	372 [c]	337 [d]	251 [g]	196 [g]	180 [e]
	C+P. *Oceanica*	500	381 [f]	354 [d]	321 [e]	312 [d]	270 [c]	257 [c]
		1000	413 [b]	397 [b]	360 [b]	350 [b]	300 [b]	283 [b]
After nanosizing process	C+ Miswak	50	82 [o]	46 [m]	26 [m]	22 [o]	19 [n]	10 [j]
		100	59 [p]	55 [i]	42 [k]	40 [m]	36 [l]	21 [i]
	C+ Bl. mulberry	50	214 [k]	120 [j]	56 [j]	50 [l]	41 [k]	26 [hi]
		100	91 [n]	87 [k]	75 [i]	71[k]	55 [j]	32 [h]
	C+ S. *linifolium*	50	218 [j]	211 [i]	125 [h]	83 [j]	63 i	44 [g]
		100	137 [l]	89 [k]	80 [i]	72 [k]	31 [m]	27 [hi]
	C+P. *Oceanica*	50	104 [m]	39 [o]	32 [l]	22 [o]	19 [n]	14 [j]
		100	55 [q]	43 [n]	39 [k]	32 [n]	24 [n]	21 [i]
	L.S. $D_{0.05}$		1.5622	1.7736	5.57412	4.179	4.78552	6.6978

The data were statistically analyzed via a one-way randomized complete blocks design (RCBD) Values with the same letters are not significantly different at least significantly ($L.S.D_{0.05}$).

Table 5: Effects of the tested treatments before and after the nanosizing process on *Enterobacter cloacae* growth

Treatments		Concentration in (µl)	Serial dilutions 10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Before nanosizing process	*Enterobacter cloacae*	Control	474 [a]	448 [a]	389[a]	357 [a]	340[a]	270[a]
	C+ Miswak	500	362 [c]	339 [b]	294 [b]	268 [b]	254 [b]	200[b]
		1000	316 [f]	293[g]	253 [f]	232 [f]	204 [f]	89[h]
	C+ Bl. mulberry	500	346 [d]	325[d]	281 [d]	256 [c]	242 [c]	174 [cd]
		1000	319 [f]	300[ef]	227 [g]	207 [g]	196 [g]	153 [f]
	C+ S. *linifolium*	500	349 [d]	329[c]	285 [c]	247 [d]	235 [d]	172 [d]
		1000	285 [g]	254[h]	220 [h]	201 [h]	189 [h]	149 [g]
	C+P. *Oceanica*	500	340 e	320e	276 [e]	253[c]	207[f]	164 [e]
		1000	373 [b]	339[b]	293[b]	240 [e]	222 [e]	176 [c]
After nanosizing process	C+ Miswak	50	124 [i]	105[i]	91 [m]	79 [i]	43 [i]	13 [n]
		100	230 [h]	126 [j]	109 [j]	100 [j]	94 [i]	67 [i]
	C+ Bl. mulberry	50	162 [i]	134[i]	115 [i]	105 [i]	72 [j]	32 [k]
		100	90 [m]	85[m]	73 [n]	45 [n]	28 [n]	15 [mn]

C+ S. *linifolium*	50	135 ^{jk}	106^{l}	90 ^{m}	82 ^{l}	34 ^{m}	26 ^{l}
	100	137 ^{j}	113^{k}	98 ^{i}	89 ^{k}	62 ^{k}	48 ^{j}
C+P. *Oceanica*	50	130 ^{k}	127^{j}	105 ^{k}	92 ^{k}	75 ^{j}	26 ^{i}
	100	83 ^{n}	66^{n}	58 ^{o}	50 ^{m}	42 ^{i}	16 ^{m}
L.S. $D_{0.05}$		5.321	3.095	1.607	4.685	4.383	2.055

The data were statistically analyzed via a one-way randomized complete blocks design (RCBD).

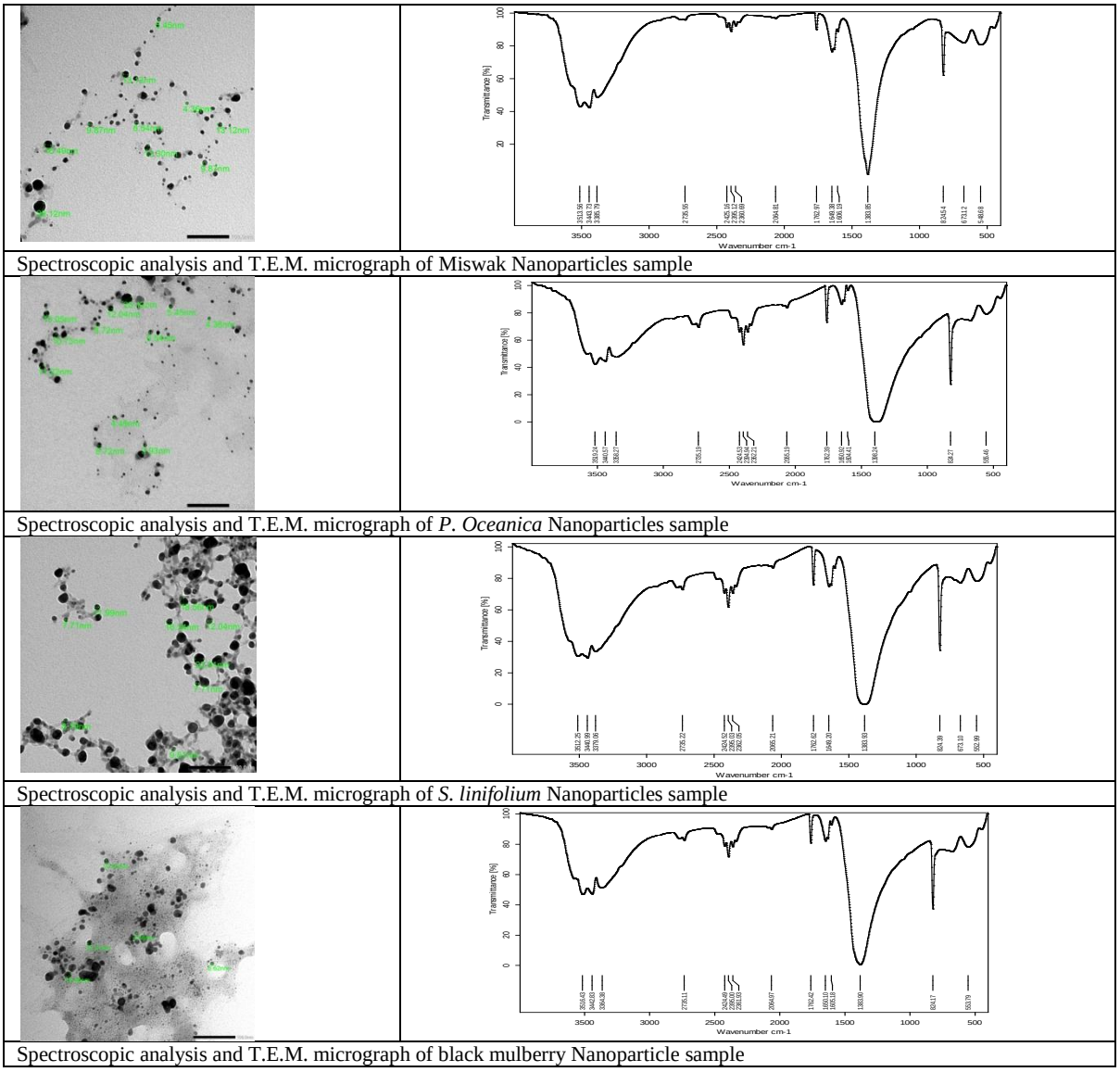

Figure 2: Spectroscopic analysis and T.E.M. micrograph of the tested nanosized plant samples.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

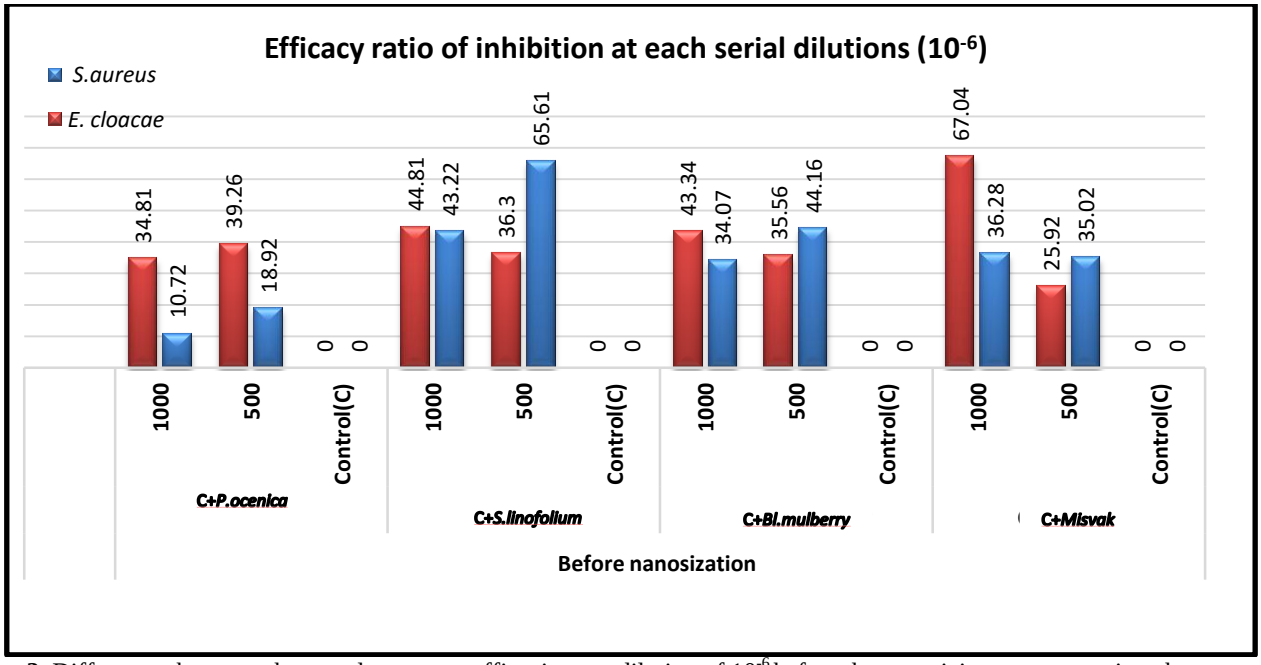

Figure 3. Differences between the tested treatment efficacies at a dilution of 10^{-6} before the nanosizing process against the two studied bacteria

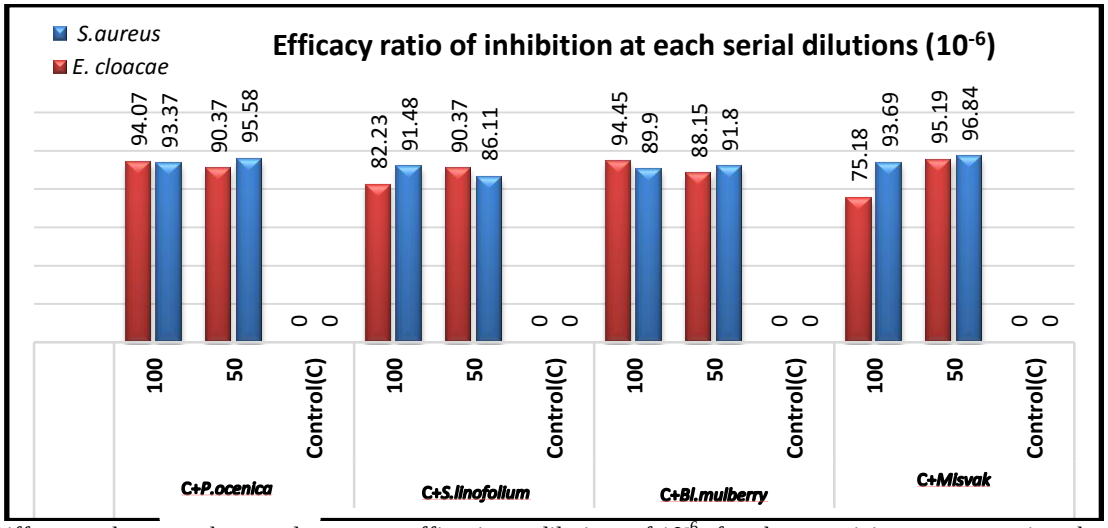

Figure 4. Differences between the tested treatment efficacies at dilutions of 10^{-6} after the nanosizing process against the two studied bacteria

Table 6: Efficacy ratios of the tested plant extracts before and after the nanosizing process on both tested bacteria

Treatments		Concentration in (µl)	Serial dilutions E.R% 10^{-1}	E.R% 10^{-2}	E.R% 10^{-3}	E.R% 10^{-4}	E.R% 10^{-5}	E.R% 10^{-6}
Before nanosizing process	*Staphylloccus aureus*	Control(C)	0	0	0	0	0	0
	C+ Miswak	500	11.66	19.07	22.25	28.08	31.32	35.02
		1000	18.61	19.53	19.69	32.28	32.53	36.28
	C+ Bl. mulberry	500	24.21	41.86	41.94	42.25	42.77	44.16
		1000	11.21	13.49	12.02	13.06	30.72	34.07
	C+ *S. linifolium*	500	16.59	39.77	40.15	50.92	65.36	65.61
		1000	12.56	13.49	13.81	34.12	40.96	43.22
	C+*P. Oceanica*	500	14.57	17.67	17.90	18.11	18.67	18.92
		1000	7.40	7.64	7.93	8.14	9.64	10.72

After	C+ Miswak	50	81.61	89.30	93.35	94.22	94.28	96.84
nanosizing		100	86.77	87.21	89.26	89.50	89.16	93.69
process	C+Bl. mulberry	50	52.02	71.86	85.68	86.88	87.65	91.80
		100	79.60	79.77	80.82	81.36	83.43	89.90
	C+ *S. linifolium*	50	51.12	51.16	68.03	78.21	81.02	86.11
		100	69.28	79.30	79.54	81.10	90.66	91.48
	C+*P. Oceanica*	50	76.68	90.93	91.81	94.22	94.28	95.58
		100	87.67	90	90.02	91.60	92.77	93.37
Treatments		Concentration in	Serial dilutions					
		(µl)	E.R% 10^{-1}	E.R% 10^{-2}	E.R% 10^{-3}	E.R% 10^{-4}	E.R% 10^{-5}	E.R% 10-^{6}
Before	*E. cloacae*	Control(C)	0	0	0	0	0	0
nanosizing process	C+ Miswak	500	23.63	24.33	24.42	24.93	25.29	25.92
		1000	33.34	34.60	34.96	35.01	40	67.04
	C+ Bl. mulberry	500	27	27.45	27.76	28.29	28.82	35.56
		1000	32.70	33.03	41.64	42.02	42.35	43.34
	C+ *S. linifolium*	500	26.37	26.56	26.73	30.81	30.88	36.30
		1000	39.87	43.30	43.44	43.70	44.41	44.81
	C+*P. Oceanica*	500	28.27	28.57	29.04	29.13	39.12	39.26
		1000	21.31	24.33	24.68	32.77	34.70	34.81
After	C+ Miswak	50	73.84	76.56	76.61	77.87	87.35	95.185
nanosizing		100	51.48	72.87	71.98	72.0	72.35	75.18
process	C+ Bl. mulberry	50	65.82	70.09	70.44	70.59	78.82	88.15
		100	81.01	81.03	81.23	87.39	91.76	94.45
	C+ *S. linifolium*	50	71.52	76.34	76.86	77.03	90.0	90.37
		100	71.01	74.78	74.81	75.07	81.76	82.23
	C+*P. Oceanica*	50	72.57	72.77	73.01	74.23	77.94	90.37
		100	82.49	85.27	85.35	85.99	87.65	94.07

Values with the same letters are not significantly different, at least significantly different (L.S.D $_{0.05}$).

Table 7: Effects of the tested treatments before and after the nanosizing process on *Aspergillus flavus* growth

Treatments		Concentration in (µl)	Serial dilutions	
			Growth dry weight	ER% of growth inhibition
Before nanosizing process	*flavus growth*	Control(C)	1.91 ^{a}	------
	C+ Miswak	500	0.64 ^{k}	66.49
		1000	0.65 ^{k}	65.97
	C+ *Bl. mulberry*	500	0.78 ^{i}	59.16
		1000	0.91 ^{e}	52.36
	C+ *S. linifolium*	500	1.5 ^{c}	21.46
		1000	0.79 ^{hi}	58.64
	C+*P. Oceanica*	500	0.71 ^{j}	62.83
		1000	1.6 ^{b}	16.23
After nanosizing process	C+ Miswak	50	0.85 ^{g}	55.50
		100	0.91 ^{e}	52.37
	C+ *Bl. mulberry*	50	0.91 ^{e}	52.37
		100	0.87 ^{f}	54.45
	C+ *S. linifolium*	50	0.84 ^{g}	56.02
		100	1.49 ^{c}	21.99
	C+*P. Oceanica*	50	0.80 ^{h}	58.11
		100	1.03 ^{d}	46.07
	L.S. $D_{0.05}$		0.016529	

The data were statistically analyzed as a complete randomized design. Values with the same letters are not significantly different at an L.S.$D_{0.05}$

Table 8: Effects of the tested treatments before and after the nanosizing process on aflatoxin B1 inhibition

Treatments		Concentration in (µl)	Serial dilutions	
			$AflB_1$ production (ppb)	ER% of growth inhibition
Before nanosizing process	*flavus growth*	Control(C)	13.54 a	-------
	C+ Miswak	500	9.74	28.06
		1000	4.94 ^{c}	63.51
	C+ Bl. mulberry	500	3.5 ^{d}	74.15
		1000	1.44 ^{l}	89.36
	C+ *S. linifolium*	500	2.32 ^{g}	82.86
		1000	1.84 ^{i}	86.41
	C+*P. Oceanica*	500	2.52 ^{f}	81.39
		1000	0.32 p	97.64
After nanosizing process	C+ Miswak	50	2.89 ^{e}	78.65
		100	2.28 ^{h}	83.16
	C+ Bl. mulberry	50	1.75 j	87.07
		100	0.85 ^{o}	93.72
	C+ *S. linifolium*	50	1.16 ^{m}	91.43
		100	0.87 ^{n}	93.57
	C+*P. Oceanica*	50	1.64 ^{k}	87.89
		100	0.24 ^{q}	98.23
	L.S. $D_{0.05}$		0.01659	

The data were statistically analyzed as a complete randomized design. Values with the same letters are not significantly different at an L.S.D $_{0.05.}$

Conclusion

The antibacterial activities of the nanoparticles were greater than their antifungal activities, which may be partially due to the presence of copper ions, which act as sterilizers against bacteria, in addition to the differences between the structure and the cell wall of prokaryotic bacterial cells and *eukaryotic* fungal cells. The nanosized particles used in the tested treatments were less effective at reducing fungal growth than their natural structures without nanosizing, indicating that the nanosizing process was unnecessary for *A. flavus* growth inhibition. Miswak, blue mulberry leaves and *S. linifolium* nanoparticles exhibited high antibacterial activity and remarkable aflatoxin detoxification efficiency. *Posidonia oceanica* (P.O.) nanoparticles are considered the best antibacterial agent against the two tested bacteria and the best detoxifying agent for aflatoxin B1. These nano treatments, especially *Posidonia oceanica* (P.O.), are recommended as antibacterial and aflatoxin detoxifier agents.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

The authors would like to thank Prof. Dr. Soliman Mohmed, a professor of animal nutrition, for his kind help in providing Table 1, a copper supplement for animal diets.

References

1. Sumbana J, Santona A, Fiamma M, Taviani E, Deligios M., Chongo V, Sacarlal J, Rubino ,S.and, Paglietti ,B. Polyclonal emergence of MDR *Enterobacter cloacae* complex isolates producing multiple extended spectrum beta- lactamases at Maputo Central Hospital, Mozambique.2022. Rend. Fis. Acc. Lincei 33, 39–45. https://doi.org/10.1007/s12210-021-01039-4t.
2. Franco M, Stefa´nski T, Jalava T, Lehto M, Kahala M, Järvenpää E, Mäntysaari P, and Rinne M. Effect of Potato By-Product on Production Responses of Dairy Cows and Total Mixed Ration Stability. Diary. 2021; 2, 218–230. MDPI https://doi.org/10.3390/dairy2020019.
3. Schroeder BK, DuToit LJ, and Schwarts HF. This is the first report of *Enterobacter cloacae* causing onion bulb rot in the Columbia Basin of Washington State. Plant Dis.2009; 93:323.
4. Wang GF, Xie GL, Zhu B, Huang JS, Liu, B, Kawicha P, Benyon L, and Duan YP. Identification and characterization of *Enterobacter* complex causing mulberry (*Morus alba*) wilt disease in China. Eur. J. Plant Pathol. 2010; 126.465-478.

5. Santana-Méridas O, González-Coloma A, & Sánchez-Vioque R. Agricultural residues as a source of bioactive natural products. Phytochem Rev. 2012; 11.447–466.
6. Zhang ZF, and Nan ZB. Occurrence of lucerne seedborne *Enterobacter cloacae* sprouts decay in Gansu Province of China. Eur. J. Plant Pathol. 2013; 135.5-9.
7. Ashmawy NA, El-Bebany AF, Shams AH, and Shoeib AA. Identification and differentiation of soft rot and blackleg bacteria from potato using nested and multiplex PCR J. of Plant Dis and Prot. 2019; 127.141–153.
8. Haag AF. J Ross Fitzgerald JR. José R Penadés JR. *Staphylococcus aureus* in Animals. Microbiol Spectr. 2019. 7(3). DOI: 10.1128/microbiolspec.GPP3-0060-2019.
9. Nagase N, Sasaki A, Yamashita K, Isolation and species distribution of *Staphylococci* from animal and human skin. J of Veterinary Medical Sci. 2002 .64 (2).245-250. https://doi.org/10.1292/jvms.64.245.
10. Dharumadurai D, Shanmugapriy S, Thajuddin N, Annamalai P. Aflatoxins and aflatoxins in humans and animals. Cited from book: InTech: Aflatoxins - Biochem and Mol Biol. 2011. DOI: 10.5772/22717.
11. Zain EM. Impact of mycotoxins on humans and animals. J of Saudi Chem Soci. 2011; 15(2). 129-144. https://doi.org/10.1016/j.jscs.2010.06.006.
12. Mahato N, Sinha M, Sharma K, Wararao RK, and Hwan Cho M. Modern Extraction and Purification Techniques for Obtaining High Purity Food-Grade Bioactive Compounds and Value-Added Co-Products from Citrus Wastes. Foods.2019; 8 (11). https://doi10.3390/foods8110523.
13. Mohindru JJ, Garg UK. Green synthesis of copper nanoparticles using tea leaf extract. Int J of Engin Sci and Res Techn.2017; 6. 307-311.
14. Iqbal S, Younas U, Sirajuddin S, Chan KW, Sarfraz RA, and Kamaluddin Md. Proximate Composition and Antioxidant Potential of Leaves from Three Varieties of Mulberry (*Morus* sp.): A Comparative Study. Int J Mol Sci. 2012; 13(6). 6651–6664. MDPI. doi: 10.3390/ijms13066651PMCID: PMC3397487.
15. El-Sherbiny G M, Gazelly A M, Sharaf M H, Moghannemm S A E, Ismail, M KA and , El-Hawary AS. 2023. Exploitation of the antibacterial, antibiofilm and antioxidant activities of *Salvadora Persica* (Miswak) extract. J of Bioresource and Bioproducts. 2022. 8(1): 59–65. https://doi.org/10.1016/j.jobab.2022.11.006.
16. El-Sherbiny GM, Elbestawy MKM. A review: plant essential oils active against Helicobacter pylori. J. Essent. Oil Res.2022. 34. 203-215.
17. Kumari A, Parida AK Rangani J, and Panda. Antioxidant Activities, Metabolic Profiling, Proximate Analysis, Mineral Nutrient Composition of *Salvadora persica* Fruit Unravel a Potential Functional Food and a Natural Source of Pharmaceuticals. Front Pharmacol. 2017; 8: 61. doi: 10.3389/fphar.2017.00061 PMCID: PMC5306401. PMID: 28261096.
18. Hamed SM, Abd El-Rhman AA, Abdel-Raouf N, and Ibraheem IBM. Role of marine macroalgae in plant protection and improvement for sustainable agriculture technology. Beni-Suef Univ. 2017. J Basic Appl Sci 2017. 7. 104–110. https://doi.org/10.1016/j.bjbas.2017.08.002.
19. Keyimu XG, and Abuduli M. Seaweed Composition and Potential Uses. Int J of ChemTech Res. 2019; 12 (1). 105-111. http://dx.doi.org/10.20902/IJCTR.2019.120112.
20. Morsy GMT, Bekhet EK, and Mohamed EA. Phytochemical screening for antibacterial compounds of some seaweed from coastal area of Abu-Qir, Alexandria, phytochemical screening for antibacterial compounds of some seaweed from coastal area of Abu-Qir, Alexandria. Egyptian J. of Phycol.2020; 19: 47-57.
21. Nazal KM. Advanced Sorption Process Applications. Intech Open; London, UK 2019, Marine Algae Bioadsorbents for Adsorptive Removal of Heavy Metals.
22. Abudeshesh RM, Aboul-Nasr AM, Khairy MH, Atia MM, Sabra MA. Differential impacts of interactions between *Serendipita indica*, *Chlorella vulgaris*, *Ulva lactuca* and *Padina pavonica* on Basil (*Ocimum basilicum* L.). Plant Physio and Biochem.2024. 206. 108-218.
23. Shabaka SH. Checklist of seaweeds and seagrasses of Egypt (Mediterranean Sea). The Egyptian J of Aqua Res. 2018. 44 (3). 203-212.
24. Hamoutene D, Romeo M, Gnassia M. Cadmium effects on oxidative metabolism in a marine seagrass: *Posidonia oceanica*. Bulletin of Enviro Contami and Toxicol J.1996. 56(2). https://doi.org/10.1007/s001289900048.
25. Pivaa G, Fracassettib D, Tirellib A,Mascheronib E, Musattib A, Inglese P, Piergiovanni L, Rollini M. Evaluation of the antioxidant/antimicrobial performance of *Posidonia oceanica* in comparison with three commercial natural extracts and as a treatment on fresh-cut peaches (*Prunus persica* Batsch). Postharvest Biolo and Techn.2017; (124). 54-61.
26. Espinosa CD, Stein HH. Digestibility and metabolism of copper in diets for pigs and influence of dietary copper on growth performance, intestinal health, and overall immune status. J Animal Sci and Biotechnol. 2021; 12(13).
27. Samira S, GHORANNEVISS M, HANTEHZADEH MR, Synthesis and optical characterization of copper nanoparticles prepared by laser ablation. Bull Mater Sci. 2017; 40. 37–43. https://doi.org/10.1007/s12034-016-1348-y.
28. Jayanta S, Leepipatpiboon N. Hollow-fibre Microextraction Combined with Hydrophilic Interaction Liquid Chromatography–Mass Spectrometry for Analytical Determination of High Polarity Herbicides in Water - Chiang Mai J Sci. 2018; 45(6). 2381-2396 http://epg.science.cmu.ac.th/ejournal.
29. Ibrahim E, Zhang M, Zhang Y, Hossain A, Qiu W, Chen Y, Wang Y, Wu W, Sun GLiB. Green-synthesization of silver nanoparticles using endophytic bacteria isolated from garlic and its antifungal activity against wheat Fusarium head blight pathogen (*Fusarium graminearum)*. Nanomaterials. 2020; 10:219.
30. Sánchez-López E, Gomes D, Esteruelas G, Bonilla L, Lopez-Machado AL, Galindo R, Cano A, Espina M , Ettcheto M, Camins A , Silva AM, Durazzo A, Santini A, Garcia ML, Eliana B, and ,Souto EB. Metal-Based Nanoparticles as Antimicrobial Agents.. Nanomaterials (Basel). 2020; 10(2). 292. doi: 10.3390/nano10020292.
31. Thakur S, Sharma S, Thakur S, Rai R. Green synthesis of copper nanoparticles using *Asparagus adscendens* Roxb root and leaf extracts and their antimicrobial activities. Inter J of Curr Microbiol and Appl Sci. 2018; 7. 683-694.
32. Sabra MA, Alaidaroos BA, Jastaniah SD, Heflish AI, Ghareeb RY, Mackled MI, El-Saadony MT, Abdelsalam NR and Conte-Junior CA. Comparative Effect of Commercially Available Nanoparticles on Soil Bacterial Community and "*Botrytis fabae*" Caused Brown Spot: In vitro and in vivo Experiment. Front. Microbiol. 2022. 13:934031. doi: 10.3389/fmicb.2022.934031.
33. Rojas B, Soto N, Villalba M, Bello-Toledo H, Meléndrez-Castro M, and Sánchez-Sanhueza G. Antibacterial Activity of Copper Nanoparticles (CuNPs) against a Resistant Calcium Hydroxide Multispecies Endodontic Biofilm Nanomaterials (Basel). 2021; 11(9): 2254. doi: 10.3390/nano11092254.
34. Jung WK, Koo HC, Kim KW, Shin S, Kim SH, Park YH. Antibacterial Activity and Mechanism of Action of the Silver Ion in *Staphylococcus aureus* and *Escherichia coli*. J Appl Environ Microbiol. 2008; 74 (7) 2171-8. DOI: https://doi.org/10.1128/AEM.02001-07.
35. Amer MW, and Awwad MA. Green synthesis of copper nanoparticles by Citrus limon fruits extract,

characterization, and antibacterial activity. Chem Inter. 2021; 7(1), 1–8. https://doi.org/10.5281/zenodo.4017993.
36. Ermini ML, and Voliani V. Antimicrobial Nano-Agents: The Copper Age A.C.S. Nano. 2021; 15(4). 6008–6029 https:/ pubs.acs.org /doi.org/10.1021/acsnano.0c10756.
37. Pham TD, Lee BK . Cu doped TiO_2/G.F. for photocatalytic disinfection of *Escherichia coli* in bioaerosols under visible light irradiation: Application and mechanism. Appl. Surf. Sci. 2014; 296.15–23.
38. Engle TE, Fellner V , Spears JW. Copper status, serum cholesterol, and milk fatty acid profile in Holstein cows fed varying concentrations of copper. J Dairy Sci. 2001; 84(10):2308-13. DOI: 10.3168/jds. S0022-0302(01)74678-4. PMID: 11699463.
39. Suzuki T, and Iwahashi Y. The addition of carbon to the culture medium improves the detection efficiency of aflatoxin-producing synthetic fungi. Toxins. 2016; 8: 338-94.
40. Youssef NH. Comparison between certain chitosan wraps and cellophane wrapson fungal inhibition and mycotoxins migration. Res. on Crops .2019;20. 105-112. DOI: 10.31830/2348-7542.2019.141.
41. Windisch W, Schedle K, Plitzner C, Kroismayr A. Use of phytogenic products as feed additives for swine and poultry. J. of Animal Sci. 2008; 86.140–48.
42. Youssef NH, and Sabra MA. Antibacterial Effects of Fenugreek, Wheat and Hot Red Pepper Seeds and Their Germs Extract on Inhibiting *Staphylococcus aureus* and *Enterobacter cloacae* Growth. J. of Advan in Microbiol. 2021; 21(4): 1-16.
43. Youssef NH, Salaheddin AP, Mohamed ZB, Ahmed A, and Sabra MA. Use of novel microbial and phyto-biotic feed additives in mycotoxins degradation in vitro and their potential in vivo application in fish die. Malaysian J of Microbiol.2023; 19(4). 421-434. DOI: http://dx.doi.org/10.21161/mjm.220028.
44. Youssef NH, Qari SH, Behiry SI, Dessoky ES, El-Hallous EI, Elshaer MM, Kordy A, Maresca V, Abdelkhalek A, Heflish AA. Antimycotoxigenic Activity of Beetroot Extracts against *Altenaria alternata* Mycotoxins on Potato Crop. Appl. Sci. 2021;11, 4239.
45. Rahman MM, Refat B, Zhang H, Zhang W, and Yu P. Detect molecular spectral features of newly developed *Vicia faba* varieties and protein metabolic characteristics in ruminant system using advanced synchrotron radiation based infrared microspectroscopy. A preliminary study. Spectrochimica Acta Part A: Mol and Biomolecular Spectroscopy.2019; 206. 413–420.
46. Johnson JB, Walsh K, Naiker M. Application of infrared spectroscopy for the prediction of nutritional content and quality assessment of faba bean (*Vicia faba L.*). Legume Sci. 2020; 2(40):1 -13. https://doi.org/10.1002/leg3.40.
47. Wang J, Liu H, and Ren G. Near-infrared spectroscopy (NIRS) evaluation and regional analysis of Chinese faba bean (*Vicia faba* L.). The Crop J. 2014; 2(1), 28–37. https://doi.org/10.1016/j.cj.2013.10.001.
48. Alhakmani F, Kumar S, Khan SA. Estimation of total phenolic content in vitro antioxidant, and anti-inflammatory activity of flowers of (*Moringa oleifera).* Asian Pac J. Trop Biomed.2013; 3. 623-7.
49. Baba SA, and Malik SA. Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of (*Arisaema jacquemontii)* Blume. J of Taibah Uni for Sci. 2015 9. 449–454:
50. Csepreg K, Kocsis M, and Hideg É. On The Spectrophotometric Determination of Total Phenolic and Flavonoid Contents. Acta Biologica Hungarica. 2013; 64(4).500-509. DOI: 10.1556/ABioI.64.2013.4.10.
51. Tabasum S, Khare S, Jain K. Spectrophotometric Quantification of Total Phenolic, Flavonoid, and Alkaloid Contents of *Abrus precatorius* L. Seeds. Asian J. of Pharmaceutical and Clinical Res. 2016 ;9(2). 371-374.
52. Rhayanne T, Ramos M, Bezerra IC, Ferreira MRA and Soares LAL. Spectrophotometric Quantification of Flavonoids in Herbal Material, Crude Extract, and Fractions from Leaves of (*Eugenia uniflora*) Linn. Pharmacognosy Res. 2017; 9(3): 253–260. doi: 10.4103/pr.pr_143_16.
53. Bedlovi˘cová1 Z, Strapá˘c I, Baláž M and Aneta Salayová AA. Brief Overview on Antioxidant Activity Determination of Silver Nanoparticles. J. Mol 2020; 25. 3191. doi:10.3390/molecules25143191.
54. Ismail IA ,Qari SH, Shawer R , Elshaer MM, Dessoky ES, Youssef NH, Hamad NA, Abdelkhalek A, Elsamra IA. Behiry SI. The Application of Pomegranate, Sugar Apple, and Eggplant Peel Extracts Suppresses (*Aspergillus flavus*) Growth and Aflatoxin B1 Biosynthesis. Pathway. Horticul. 2021; 7. 558. https://doi.org/10.3390/horticulturae7120558.
55. Murugan K, and Iyer VV. Antioxidant and Antiproliferative Activities of Marine Algae (*Gracilaria edulis)* and (*Enteromorpha lingulata)*, from Chennai Coast. Inter. J.of Cancer Res.2012; 8(1)15-26. DOI: 10.3923/ijcr.2012.15.26.
56. Asnaashari M, Farhoosh R, Sharif A. Antioxidant activity of gallic acid and methyl gallate in triacylglycerols of Kilka fish oil and its oil-in-water emulsion. Food Chem. 2014; 159. 439–444.
57. Ramesh S, Chandra C, and Venkatesan G. Phytochemical and G.C.–M.S. analysis of leaf extract of *Mimosa pudica l.* Inter J of Current Res and Develop.2014;.2 (1): 78 - 87 .https://www.researchgate.net/publication/340663751.
58. Altemimi A, Lightfoot DA, Kinsel M, Watson DG. Employing response surface methodology for the optimization of ultrasound assisted extraction of lutein and β-carotene from spinach. Mol. 2015; 20.6611–6625. doi: 10.3390/molecules20046611.
59. Garcia SP, Morales SA, Segura CA, Fernandez GA. Phenolic-compound-extraction systems for fruit and vegetable samples. Mol. 2010; 15.8813–8826. doi: 10.3390/molecules15128813.
60. Amjad R, Mubeen B, Ali SS, Imam SS, Alshehri S, Ghoneim MM, Alzarea SI, Rasool R, Ullah I, Nadeem MS, Kazmi I. Green Synthesis and Characterization of Copper Nanoparticles Using (*Fortunella margarita)* Leaves. Polymers. 2021; 13. 4364. https://doi.org/10.3390/polym13244364.
61. Ahmed A, Usman M, Liu QY, Shen YQ, Yu B, Cong HL. Plant mediated synthesis of copper nanoparticles by using (*Camelia sinensis*) leaves extract and their applications in dye degradation. Ferroelectrics. 2019; 549, 61-69.
62. Angrasan JM, Subbaiya R. Biosynthesis of copper nanoparticles by Vitis vinifera leaf extract and its antibacterial activity. Inter J. of Current Microbiol and Appl Sci. 2014; 3. 768-774.
63. Arashisar S, Hisara O, Kayab M, Yanik T. Effects of modified atmosphere and vacuum packaging on microbiological and chemical properties of rainbow trout (*Oncorynchus mykiss*) fillets. Inter Of Food Microbiol. 2004; 97. 209–214.
64. Wiegand I, Kai Hilpert, Robert EW, Hancock. Agar and broth dilution methods were used to determine the minimal inhibitory concentration (M.I.C.) of antimicrobial substances. Nature Protocols. 2008; 3(2). 163–75. https://doi.org/10.1038/nprot.521.
65. McDonald, J. h. 2009. Handbook of biological statistics. Baltimore: Sparky house Publishing. Preacher, K. J. 2001. calculation for the chi-square test: an interactive calculation tool for chisquare tests of goodness of fit and independence [computer software]. available from http://quantpsy.org.
66. Rushdi MI, Abdel-Rahman IM, Saber H, Attia EZ, Abdelraheem WM, Madkoure HA, Hassan HM, Elmaidomyf AH, and Abdelmohsen UR. Pharmacological

and natural products diversity of the brown algae genus *Sargassum*. RSC Adv, 2020, 10, 24951-24972. DOI: 10.1039/D0RA03576A.
67. Wasimuzzama K, Atar M, Tausif S, Katekar SM, Rashmi T, Rukhsana RA. Pharmacognostic and Preliminary Phytochemical investigation of *Salvadora persica Linn* (Salvadoraceae). Res J. of Pharmacog and Phytochem.2010; 2(4) 319-323 Print ISSN: 0975-2331. Online ISSN: 0975-4385.
68. Benito GI, López RA, Martínez AA, Ballester AR, Falcó E, González CL, Sánchez G, Jesús LSJ, Borrás LI, Segura CA, and Martínez SM. In-Depth Characterization of Bioactive Extracts from *Posidonia oceanica* Waste Biomass. Mar Drugs. 2019; 17. 409-427. doi:10.3390/md17070409.
69. Jiménez AM, Rafe B, Anna T, Maria LC, Antonio DH, Núria M. Aeolian transport of seagrass (*Posidonia oceanica*) beach-cast to terrestrial systems. Estuarine, Coastal and Shelf Sci. 2017;196 (0272-7714).31-44. https://doi.org/10.1016/j.ecss.2017.06.035.
70. Halawany HS. A review on miswak (*Salvadora persica*) and its effect on various aspects of oral health. The Saudi Dental J. 2012; 24. 63–69.
71. Kamal M, Gheda SF, Abdel Hamid A, Mohamed TM. Antibacterial and antioxidant activities of Phlorotannins extracted from *Sargassum linifolium* brown alga. Delta J. of Sci. D.J.S. 2020; 41. 32-38. https://djs.journals.ekb.e.g.
72. Akhtar J, Siddique KBS, Mujeeb MA. Review on phytochemical and pharmacological investigations of Miswak (*Salvadora persica* Linn). J Pharm Bioallied Sci. 2011 Jan-Mar; 3(1): 113–117. doi: 10.4103/0975-7406.76488.
73. Agnihotri S, Mukherji S. Size-controlled silver nanoparticles synthesized over the range 5–100 nm using the same protocol and their antibacterial efficacy. R.S.C. Adv. 2014; 4. 3974–3983. Doi: 10.1039/C3RA44507K.
74. Chen J, Chen C, Liang G, Xu X, Hao Q. In situ preparation of bacterial cellulose with antimicrobial properties from bioconversion of mulberry leaves. J. Carbohydrate Polymers. 2019; 220 (15). 170-175.
75. Yamamoto O, Sawai J, Hotta M, Sasamoto T. Growth Inhibition of Bacteria by MgO-ZnO Solid-Solution Powders. J. of the Ceramic Society of Japan . 1998; 106 (12).1252-1254. 1998; DOI: 10.2109/jcersj.106.1252.
76. Seil JT, Webster T. Antimicrobial applications of nanotechnology: Methods and literature Inte. 2012; 7 (27). 67-81. DOI: 10.2147/IJN.S24805PubMedCC BY-NC 3.0.
77. Gratton SEA, Ropp PA, Pohlhaus PD, Luft CJ, Madden VJ, apier ME, and DeSimone JM. The effect of particle design on cellular internalization pathways. Appl Physical Sci 2008; 105(33).11613-11618. https://doi.org/10.1073/pnas.0801763105.
78. Harleen K, Kristi P, Rosario MS, Protima R, Erwan R. *Artemisia vulgaris* tincture-associated synthesis of silver. J. of Physics: Conference Series; Bristol. 2022; 2315 (1). 012027. Doi:10.1088/1742-6596/2315/1/12027.
79. Talebian N, Amininezhad SM, Doudi M. Controllable synthesis of ZnO nanoparticles and their morphology-dependent antibacterial and optical properties. J Photochem Photobiol B. 2013; 5(120). 66-73. doi: 10.1016/j.jphotobiol.2013.01.004 PMID: 23428888.
80. Yin IX, Zhang Jg, Zhao IS, Mei ML, Li Q , and CH Chu. The Antibacterial Mechanism of Silver Nanoparticles and Its Application in Dentistry. Int. J. Nanomedicine. 2020; 15. 2555–2562. doi: 10.2147/IJN.S246764.
81. Miller SI. Antibiotic Resistance and Regulation of the Gram-Negative Bacterial Outer Membrane Barrier by Host Innate Immune Mol mBio. 2016; 7(015). 41-16. doi: 10.1128/mBio.01541-16.
82. Breijyeh Z, Jubeh B, and Karaman R. Resistance of Gram-Negative Bacteria to Current Antibacterial Agents and Approaches to Resolve It. Mol. 2020; 25(6).1340.doi:10.3390/molecules25061340 PMCID: PMC7144564PMID: 32187986.
83. Madiga M, Martinko J, Stahl D, and Clark D. Brock Biology of Microorganisms. Madigan, Brock Biology of Microorganisms, Global Edition, 15th edition. 2018; (1064).
84. Bayat M, Chudinova E, Zargar M, Lyashko M, Louis K, and Kebede AF. Phyto-assisted green synthesis of zinc oxide nanoparticles and its antibacterial and antifungal. Res. on Crops 2019; 20(4). 725-730. DOI: 10.31830/2348-7542.2019. 107.3.
85. Sadhasivam S, Shapiro OH, Ziv C, Barda O, Zakin V, and Siono E . Synergistic Inhibition of Mycotoxigenic Fungi and Mycotoxin Production by Combination of Pomegranate Peel Extract and Azole Fungicide. Front Microbiol. 2019; 10. 1919. doi: 10.3389/fmicb.2019.01919.
86. Mahjoub NS, Adel J, Ghith and Alminderej FM. Production of cellulose from Aegagropila Linnaei macroalgae: Chemical modification, characterization, and application for the biosorption of cationic and anionic dyes from water. Project: Bio-polymers and treatment of coloured waters. Inter J of Biologi Macromol. 2019; 135.