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Biogenic Synthesis of Zinc Nanoparticles Utilizing *Hibiscus Sabdariffa* (Malvaceae) Flower Extract and Assessment of Antibacterial Efficacy

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Abstract: Zinc oxide nanoparticles (ZnO NPs) were synthesized using Hibiscus sabdariffa flower extract, and their antibacterial activity was assessed. The synthesis process and particle growth were investigated, with the formation of NPs confirmed by UV-visible spectroscopy, FTIR spectroscopy, and X-ray diffraction (XRD). Electron microscopy studied particle morphology and size. The antibacterial activity of the synthesized ZnNPs was evaluated against various pathogenic bacteria. Increasing the concentration of ZnNPs generally leads to an increase in antibacterial activity. In some cases, ZnNPs at a concentration of 5mm exhibited antibacterial activity comparable to or even higher than that of tetracycline. This green synthesis approach utilizing Hibiscus sabdariffa flower extract presents a promising alternative for the production of effective antibacterial agents.

Key points: Biogenic Synthesis, Zinc Nanoparticles, Hibiscus sabdariffa (Malvaceae), Antibacterial Efficacy.

Introduction

Many of the drugs used in the fight against microbial diseases have contributed to the emergence of resistant strains, which represents a great challenge for biomedical scientists worldwide. Although the discovery and development of antibiotics was a revolutionary achievement in the treatment of infectious diseases [1, 2], the overuse of antibiotics led to a serious problem in the field of biomedical research [3], there is an urgent need to develop new drugs and treatment protocols The emergence of natural substances as a promising alternative, either as direct antimicrobial agents or as a catalyst for the development of new antibiotics. And among these materials, there are the nano-particles derived from the botanical extracts. The action of natural plant compounds, such as flavonoids, proteins, terbinoids, polysaccharides, polyphenols, alkaloids, and ketones, as reducing agents in the synthesis of metallic nanoparticles [3,4]. As a result, nanoparticles have improved properties, which makes them attractive candidates for antibacterial applications [5]. The Hibiscus sabdariffa plant, which is a perennial herb belonging to the Malvaceae family, is a promising source of nano particles. This plant is characterized by erect stems up to 2-2.5 meters (7-8 feet), alternately lobed leaves, and large yellow or white flowers with a dark red spot at the base. The calyx of the flower is characterized by swelling and turning into a dark crimson red color at maturity, and it will last for about six months [6,7].

Hibiscus plant is known for its medicinal and nutritional importance, as it contains a variety of biologically active compounds, including organic acids, fatty acids, and trace elements[8,9].

The flavonoid compounds found in hibiscus have anti-inflammatory and antibacterial properties [10]. In addition, the plant contains natural chemical components such as aldehydes and alkaloids, which contribute to the reduction and conversion of metal ions into nanoparticles. Zinc oxide nanoparticles prepared using *hibiscus* extracts have shown broad-spectrum antimicrobial activity against a variety of microorganisms, including S. aureus), E. *coli*, and *C. albicans* [11,12,13]. Nano-zinc particles are characterized by high activity due to the ratio of large surface area to volume and unique chemical and physical properties. The hibiscus extract contains organic matter particles in addition to plant elements and natural quality [14]. The large volume of zinc particles and the high surface area led to an increase in the antimicrobial activity of disease-causing microorganisms, it is possible to invest in antimicrobial effects [15]. The use of hibiscus extracts as reducing and stabilizing agents in the environmentally friendly synthesis of ZnO nanoparticles. The interaction of flavonoids and phenolates and other metabolites found in the plant with zinc salts (such as zinc sulfate or zinc nitrate) to form spherical nanoparticles with an average size of 15-42 nm, achieves excellent stability as a result of coating with compounds derived from plants, as shown by zeta voltage measurements [16], and the appearance of zinc oxide nanoparticles manufactured using hibiscus extract has anti-inflammatory activity. The widespread microbe Listeria monocytogenes has the largest inhibition zone (concentration 1024 micrograms/ml), and *E. coli* and *Staph. aureus* active inhibition at lower concentrations (512 micrograms/ml), and includes the mechanisms of bacterial cell membrane disruption and oxidative stress caused by reactive oxygen molecules [17]. The effect of hibiscus nanofertilizers derived from hibiscus plants on the growth and productivity of hibiscus plants during the increase in height (up to 29.9 cm), fresh/dry weight, and anthocyanin content (35.2 mg/g dry weight at 12 parts per million of silicon + 2000 parts per million of zinc), and the height of total soluble sugars (0.45%) and citric acid content. And it is very important for the quality of the product, nano zinc with a concentration of 2000 parts per million with nano silicon (12 parts per million) showed the most important improvements[18.19], the appearance of nano zinc oxide particles stabilized by hibiscus plants anti-diabetic effects in mice by enhancing the expression of insulin receptors and pancreatic alginate activity through ELISA and RT-PCR. Small nanoparticles have superior efficiency compared to larger particles volume, and this is probably due to the best bioavailability[20,21], it can be used in disinfectants, drug delivery, and wastewater treatment due to its optical properties and antioxidants[23]. It was found that the antibacterial activity is active and has an active effect, as the inhibition rate was 40% for all the tested strains, and it was also found that ZnNPs are highly toxic to all the tested strains, and this was attributed to the fact that the activity of NPs may be related to intrinsic toxicity. The inhibition zone shows that zinc nanoparticles in *Mangifera indica* seeds significantly increase the inhibition rate of bacteria and reduce inflammation. The topic of manufacturing zinc nanoparticles from the extracts of the hibiscus plant [24,25]. the topic of employing the disease-causing bacteria *S. aureus, S. epidermidis, E. coli* and *P. vulgaris* to test their antibacterial ability. Composite zinc nanoparticles can be effective against bacteria and harmless to the ecosystem[26], and the purpose of the study is the production of zinc nanoparticles from the water extract of hibiscus and tests on some bacterial isolates that cause diseases.

Materials and methods

Preparation of plant extract and preparation of Zinc nanoparticles

- The plant extract was prepared from the flowers of *H. sabdariffa* flowers from the local markets and classified by the Natural History Museum. its were dried after extracting them from the fruits and crushed using an electronic pulverizer. 100gm of seed powder was weighed and a sufficient amount of water was added to it and brought to a boil at a boiling point. 80 m for 60 minutes with constant stirring and after cooling, then filter with Whatman No.1. To purify the extract for later use by preparing Zinc nanoparticles [27], 20 ml of the plant extract was heated at 50 °C for 10 min and 50 ml of 91 mM of zinc acetate solution (1 g of zinc acetate was dissolved in 50 ml of distilled water) was added drop wise to it under stirring. The reaction mixture became yellowish and cream coloured precipitate of zinc hydroxide was formed. The reaction mixture was left for 30 min for complete reduction to zinc hydroxide. Then the precipitate was collected by centrifugation at 16 000 rpm for 10 min at 4°C. The precipitate was vacuum dried at 30 °C and the sample (PZN30) was stored for further studies. Two other samples were prepared by heating PZN30 for 4 hours at 60°C (PZN60) and 100 °C (PZN100).

Effect of plant extract on synthesis of ZnO NP was investigated by varying the ratio of plant extract to zinc acetate (v/w) and was represented by yield of ZnO, calculated by using following formula

- Analytical Methods: UV Vis Spectrophotometers, SEM, and XRD were evaluated and measured to finally confirm them as ZnNPs nanoparticles:
- Ultraviolet-Visible spectrophotometer: 1.7g of the synthesized Zinc oxide nanoparticles were dissolved in 17 ml of Sulfoxide Dimethyl, and it was measured in a UV spectrophotometer, as both visual examination and absorbance measurements were monitored. The sample then varies as a result of the spectroscopic reading of Zinc oxide nanoparticles. This examination is used to verify the formation of Zinc oxide nanoparticles.

1- The structure of the synthesized Zn NPs was performed by XRD. The crystal size of the Zn NPs synthesis was calculated using Scherrer's constant ($D = 0.9\ \lambda/\beta \cos\theta$, where θ is the diffraction angle, $\lambda = 1.5406$ Å, and β is the peak width at half maximum. D-0.942, /B COS 0, where D is the average crystal field size perpendicular to the reflective planes, à is the X-ray wavelength. A laminated film of Zn NPs was formed on a glass plate and the XRD pattern was investigated in the range of 10 to 80 nano.

2- Scanning Electron Microscopy: This device relies on scanning the surface of the sample with a focused electron beam accurately for the purpose of obtaining microscopic images of the prepared Zinc oxide particles.

a. **Antibacterial capabilities**:

The diffusion method technique was used to test ZnNPs' antibacterial properties. Plates of nutrient agar were made, sanitized, and solidified. Bacterial isolates(*Klebsiella, Salmonella typhimurium, Staphylococcus aureus, Enterococcus faicalis* and *Protus*) were swabbed on these plates after they had solidified. The sterilized discs were dipped in Zinc nanoparticles solution (2, 2.5,5 mg\ml), and Digging was done in the culture media and the prepared concentrate was added to it on the nutrient agar plate and incubated for 24 hours at 37 C. Tetracycline 250 mg/ml was used as a control. The studies were performed three times, and the mean zone diameters were mm each time[28].

Statistical Analysis

Statistical analyzes were performed using SPSS v.22 and Excel 2013. Quantitative data were described using means and standard errors (mean ± standard error). The correlation coefficient (R) is calculated to determine the nature and strength of the relationship between variables. The significance level of the test is $P < 0.05$[29] .

Results and discussion

The present results indicated that adding 50ml of aqueous extract of the lower link seeds to 5 ml of Zinc sulfate produced 0.4gm of (ZnNPs), and this proved the color change of the extract from brown to milky white, after a whole day of incubation in the dark at room temperature, which indicates the generation of Zinc nanoparticles, this change in color has been previously observed by several investigato[30]. These authors suggested that the color change appeared due to the surface resolution of the plasmon.

3.1. UV/VIS Spectrophotometry

This was confirmed by taking UV visible spectrum analysis in the range of 100-900 nm using Shimadzu UV / VIS 2401PC. The maximum absorption showed a maximum absorption between 440 and 500 nm and a conscious Peak at 374nm (Fig. 1), which reduces the presence of secondary metabolites in Plants such as phenols and flavonoids, and Zinc ions in Zinc oxide solution. The plant extract acts as reducing agents and stabilizing agents, which can be considered as the intrinsic absorption peak of ZnNPs due to electron transitions from the valence band to the conduction band.

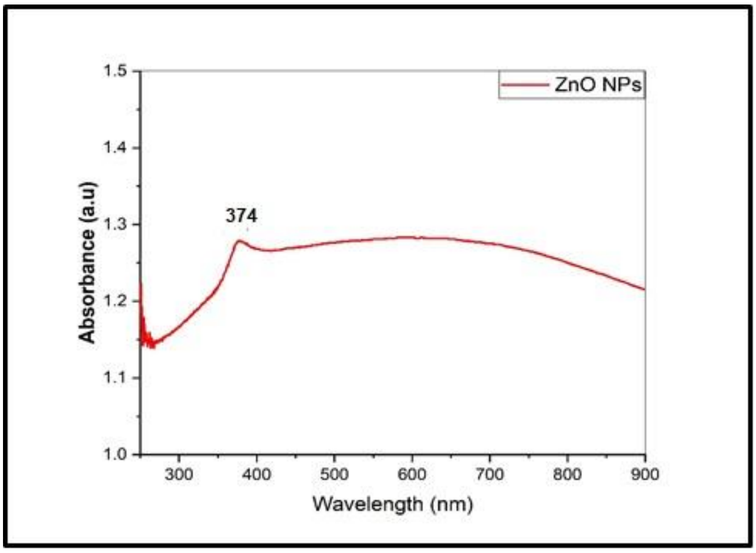

Figure 1: The UV-Vis absorption spectrum of Zinc nanoparticles synthized

3.2. Diagnosing nanoparticles with the XRD device

The XRD results clearly showed in Fig 2 that the ZnNPs formed by reducing Ag ions with aqueous extract were crystalline in nature and that the X-ray diffraction represented the mediation of the specific line of the XRD peaks that the prepared material consisted of nanoscale particles. Standard diffraction pattern of JCPDS No. 89-3722. Few unmapped peaks (28.04°, 31.52°, 45.67°, 55.91°, and 66.95°), these Bragg peaks may be due to the organic compounds presenting the extract and responsible for the reduction of Zinc ions and their stabilization of the resulting nanoparticle size average for the synthesized ZnNPs By biological, as being within the hexagonal phase of the particles, the diameter of ZnNPs nanoparticles was calculated using the Debye-Scherre formula, the average crystalline size of the formed nanoparticles was estimated and found to be 52.24 nm. Peaks matched with ICDD card number 01-079-0207(Raja et al., 2017; Tran et al., 2022). The shape of the nanoparticles was found to be hexagonal in nature with lattice parameters a (= b) equal to 3.3267 A 0 and c equal to 5.2114 A 0, which corresponds to the previously reported values (Souza et al.,2019), and the average size was found to be about 15 nm for Zn NPs from the aqueous extract of the flowers extract.

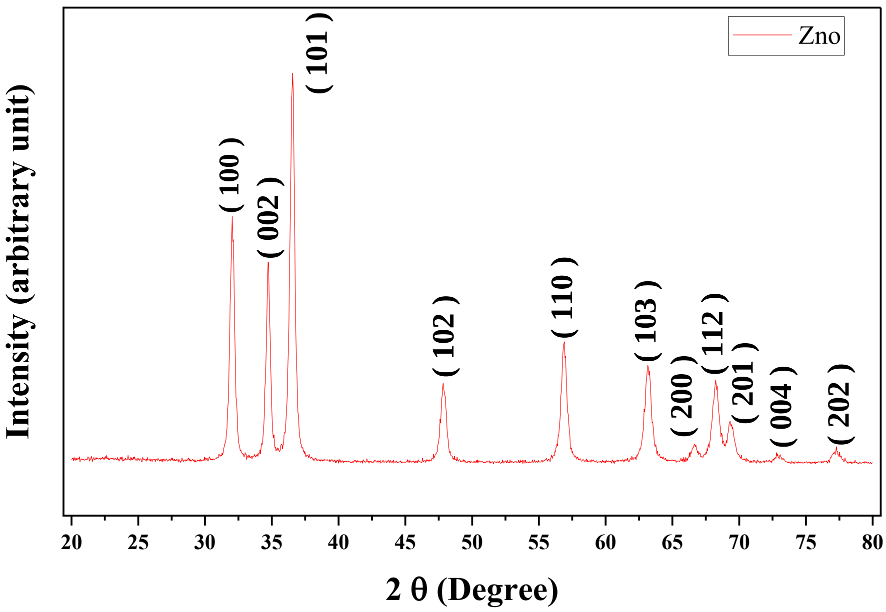

Figure 2: X-ray diffraction spectrum of synthesized Zinc nanoparticles.

3.3 .Diagnosing nanoparticles with the FESEM device

A scanning electron microscope was used to determine the surface shapes of nanoparticles with an average diameter calculation, the agglomerations of individual Zinc nanoparticles with the presence of many nanoscale aggregates Figure 4. Zinc nanoparticles showed agglomeration with the presence of some individual crystals, TEM analysis revealed that the size of ZN-NPs ranged between 30 and 53 nm (Figure 3), and the presence of irregular aggregations of particles is attributed to the presence of secondary metabolites in the extract, and this is consistent with What he found .

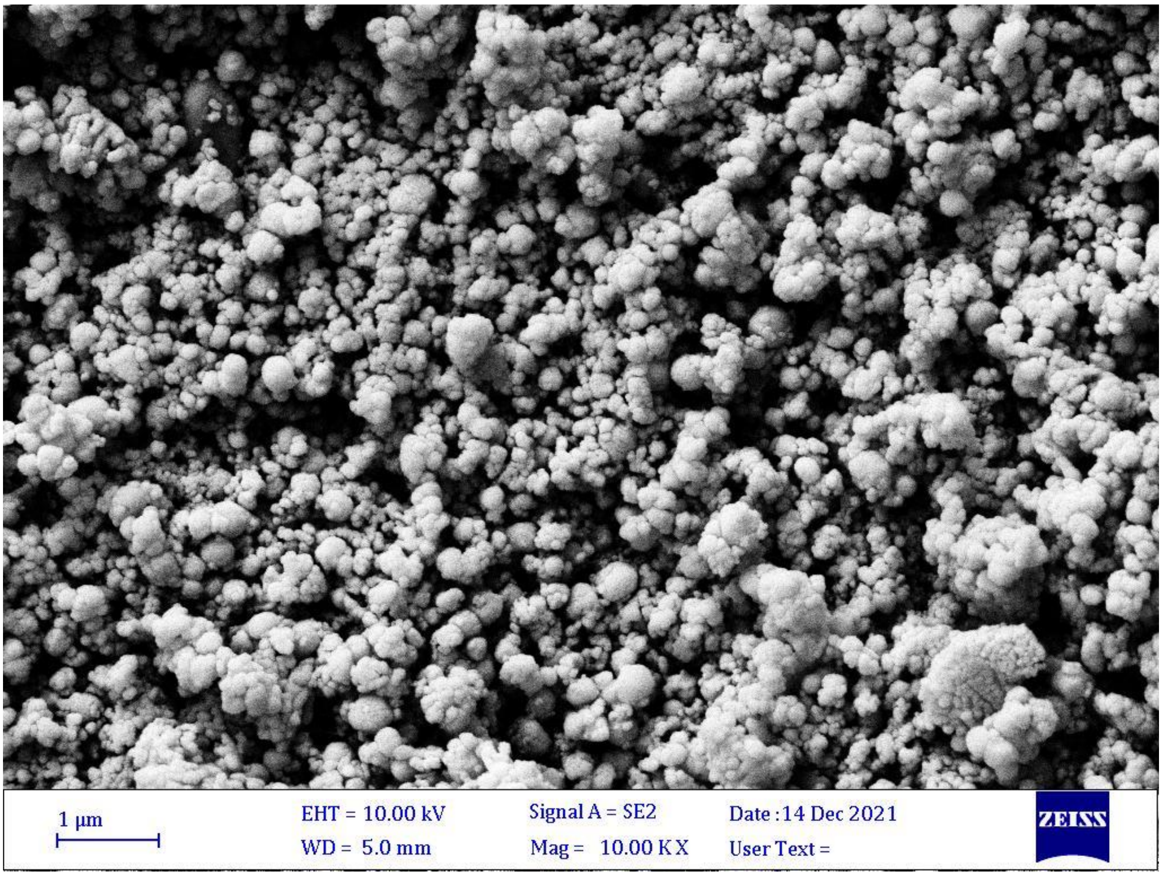

Figure. 3. FESEM image of ZnNPs nanoparticles

3.4. Effect of ZnNPs nanoparticles against pathogenic bacteria

The results of the study showed the antimicrobial activity of H. *sabdariffa* flowers extracts and Zinc by the method of diffusion around the pits as a simple and quick way to distinguish the antimicrobial activity of the tested samples and explained by increasing the net area using the same concentration, the results indicated that the leaf extract alone did not show the effect of A strong antimicrobial against pathogenic bacteria, Zinc nanoparticles showed a significant positive effect against the tested microorganisms (low visibility area). The plant ZnNPs showed the greatest antimicrobial activity against the tested microorganisms, which led to the death of bacterial . Table 1 shows the effect of different concentrations of nano-zinc particles (2, 2.5, 5 mm) on the inhibition zone of five different types of disease-causing bacteria, in addition to the effect of tetracycline antibiotic as a reference for comparison. In general, the table shows that increasing the concentration of zinc nanoparticles leads to an increase in the diameter of the inhibition zone, which indicates an increase in antibacterial activity. The appearance of pathogenic bacteria in different responses to zinc nanoparticles, which indicates differences in the mechanisms of resistance or sensitivity between different species.

It appears that nano-zinc particles, with a concentration of 5 mm, show an antibacterial effect similar to or even higher than that of tetracycline in some cases. Klebsiella showed a good response to nano zinc particles, with a slight increase in the stabilization area with increasing concentration. The appearance of *S. typhi* is similar to that of *Klebsiella*, with a gradual increase in the inhibition zone. The appearance of *Staph. aureus* is a strong response to zinc nanoparticles, with a significant increase in the stabilization zone with increasing concentration, and a clear superiority over tetracycline. The manifestation of intestinal colic is moderate response, with a significant increase in the inhibition zone with increasing concentration. And the appearance of moderate response, with a gradual increase in the stabilization zone. It is known that nano zinc has antibacterial properties, which can be attributed to several mechanisms, including the release of zinc ions that interfere with bacterial cellular processes (Sirelkhatim et al., 2015), the production of reactive oxygen species (ROS) that cause damage to bacterial cells, and the inhibition of bacterial adhesion and the formation of biofilms. It is possible that the active substances found in hibiscus flowers, such as organic acids and flavonoids, contribute to enhancing the antibacterial activity of zinc nanoparticles, either by increasing the settlements or by enhancing the effects on bacteria. Some bacteria, such as *Staph. aureus*, are resistant to tetracycline, which explains the superiority of nano-zinc particles in inhibiting growth. The results indicate that nanoparticle zinc particles from hibiscus flowers can be a promising alternative to traditional antibiotics, especially in light of the increasing problem of antibiotic resistance. Zinc nanoparticles can be employed in a variety of applications, such as treating bacterial infections, disinfecting medical surfaces, and developing antibacterial packaging materials.

TABLE 1: Antibacterial activity of *H.* *sabdariffa* flowers extracts,(P.= *Tetracycline* **antibiotic-control) (LSD 0.05= 0.0210)**

Bacteria isolats	Zone inhibition (Mm)			
	2		2.5	5
Klebsiella		1±0.231	±0.2112	12±1.01
Salmonella typhimurium		±0.2310	1±1.321	1.3±12
Staphylococcus aureus		1±0.231	14±0.22	1.515±
Enterococcus faecalis		±0.2310	12±1.03	2.4±14
Protus		±0.239	11±0.7	12±0.09
Tetracycline		11±0.12		

Zinc nanoparticles show good antimicrobial activity due to their large surface area to volume ratio and their distinctive chemical and physical properties, ZnNPs in the literature. ZnNPs may enter the cell by cellular proliferation and endocytosis; They come into contact with the cytoplasm and interfere with the functioning of mitochondria, promoting the release of ROS and Zn 2+. These released ions can penetrate the membrane and reach the DNA, causing nuclear damage such as irreversible chromosome damage, leading to cell death , Pasquet found that the release of ROS into the aqueous medium may be responsible for cell death. In addition, upon contact with visible light, ZnNPs NPs caused an increased rate of cell death, and there is a difference between the components of Gram-positive and Gram-negative cell walls, and that Gram-positive bacteria are more susceptible to attack than Gram- negative bacteria.

Zinc oxide nanoparticles (ZnO NPs) exhibit potent antibacterial activity through multiple mechanisms, primarily involving the generation of reactive oxygen species (ROS), the release of Zn^{2+} ions, and membrane disruption [31,32]. Their effectiveness is contingent upon the bacterial species, nanoparticle characteristics, and concentration. ZnO NPs induce oxidative stress by producing ROS such as hydrogen peroxide (H_2O_2), hydroxyl radicals (OH^-), and superoxide ions (O_2^-). These radicals inflict damage on lipids, proteins, and DNA, leading to cell death [33]. In beta-lactam-resistant Gram-negative bacteria, ROS levels increase up to 3.5-fold, causing lipid peroxidation and membrane leakage, dissolved Zn^{2+} ions from ZnO NPs bind to bacterial membranes, destabilizing their structure and increasing permeability. This disrupts enzyme function and nucleic acid synthesis. Gram-negative bacteria (such as *Escherichia coli* and *Pseudomonas aeruginosa*) are more susceptible due to electrostatic interactions between positively charged Zn^{2+} and their negatively charged outer membranes[34]. Direct contact with ZnO NPs causes physical damage to bacterial membranes. Studies show that 70% of *Bacillus subtilis* cells experience cytoplasmic membrane damage within 15min. of exposure. Transmission electron microscopy

(TEM) reveals membrane disintegration, protein denaturation, and intracellular content leakage in treated cells[35,36,37]. ZnO NPs also reduce biofilm formation in pathogens like *Serratia marcescens* by inhibiting biofilm-associated genes (such as *LuxS*) and eradicating pre-existing biofilms at sub-inhibitory concentrations 32 µg/mL.

Conclusion

Zinc oxide nanoparticles were synthesized using hibiscus flower extract, and their antibacterial efficacy was evaluated. The results confirmed that these nanoparticles exhibited antibacterial activity against a variety of pathogenic bacteria, with efficacy increasing with increasing concentration. In some cases, the nanoparticles demonstrated efficacy comparable to or even superior to tetracycline, a common antibiotic. This green synthesis approach, using hibiscus flower extract, suggests a promising alternative for producing effective antibacterial agents.

Acknowledgment and/or disclaimers

None to declare.

Conflict of interest

The author(s) confirm that there was no conflict of interest.

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