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ORIGINAL ARTICLE

Efficacy of Biochemically Synthesized Plant-Based Silver Nanoparticles as Antibacterial and Antioxidant Agents

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ABSTRACT
The plant species selected in the current research work was Citron, Citrus medica. Extracts of this plant are well known to possess numerous biological properties, especially antibacterial and antioxidant properties. In view of this, the current study was structured to determine the antioxidant and antibacterial properties of biochemically synthesized silver nanoparticles (NPs) from peel extract of Citrus medica. Multi-drug-resistant pathogenic bacteria were identified in clinical samples obtained from a local hospital in the city of Bangalore. The antibacterial efficacy of silver nanoparticles, which were produced biochemically using peel extracts from Citrus medica fruit, was assessed using the disc diffusion technique. The DPPH free radical scavenging technique for antioxidant assay was conducted using a spectrophotometric technique.The results of the antibacterial activity analysis in this study demonstrated that silver NPs, synthesized using the peel extract of Citrus medica fruit, exhibited efficient antibacterial effects against various pathogenic Gram-negative bacteria (Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli) and a Gram-positive bacterium (Staphylococcus aureus), as indicated by the observed zone (Zone of Inhibition). Additionally, the bio-chemically synthesized plant-based silver nanoparticles displayed effective free radical scavenging properties, demonstrating their antioxidant activity. The present findings indicate that biochemically produced silver NPs can serve as potent antibacterial agents against pathogenic bacteria that are resistant to many antimicrobial agents, and also exhibit notable capabilities in scavenging free radicals.
Key words: *Citrus medica, Silver nanoparticles, Pathogenic bacteria., Anti-bacterial, Antioxidants.*

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INTRODUCTION

In the last few years, the advancement of nanosciences has resulted in a significant increase in scientific research focused on the study of nanoparticles. Additionally, a growing interest has been observed in utilizing plants for the environmentally friendly production of NPs through green biosynthesis [1, 2, 3]. Compared to the chemical and physical methods, biological pathways are simple, fast, economical, as well as, above all, efficient and eco-friendly [4, 5, 6]. The compounds observed in the extracts have the ability to operate as effective decreasing as well as capping agents [7, 8, 9]. They inhibit the excessive development of nanoparticles and reduce their tendency to aggregate in colloidal synthesis. Additionally, these molecules have the ability to impact and customize the characteristics of the produced nanoparticles, enhancing their effectiveness in a highly suitable manner for various uses [10,11]. Plants that possess pharmacological effects are of great interest and offer significant potential for the synthesis of green AgNPs. Citrus is a medicinal plant that possesses a diverse range of pharmacological characteristics. Considering a worldwide production of 124.3 million tons per year, it is the broadly cultivated fruit crop worldwide [12, 13, 14, 15]. Citrus, comprising over 70 species, is the most extensive genus within the Rutaceae family [16, 17, 18, 19]. Due to their abundant bioactive compounds, like flavonoids, polyphenols, as well as vitamins [20, 21], these fruits possess notable anti-inflammatory, anti-tumor, and antioxidant properties, along with the cardiovascular as well as neuro protective effects [22, 23]. They serve as a significant store of minerals that help decrease water retention as well as enhance bone density and skeletal strength. Multiple researches have demonstrated that Citrus fruit cultivars have the potential to enhance human health by acting as antibacterial agents and antioxidants [24, 25], anti-inflammatory [26], and anti-tumor products [27]. It is very rich in bioactive substances, like citric acid [28], phenolic compounds [29], flavonoids [30], carotenoids [31], and ascorbic acid [32]. Thus, this study intended to biochemically synthesize silver NPs by utilizing the aqueous extract that was derived from the peel of *Citrus medica*. With regard to this background information, this work was designed to assess the antioxidant and antibacterial properties of silver nanoparticles that were generated biochemically utilizing peel extracts from *Citrus medica*. Gram-negative bacteria (*P. aeruginosa, K. pneumonia, E. coli*), and Gram-positive bacteria (*S. aureus*) were utilized to prove the AgNPs' antibacterial properties, and the antioxidant activity of these AgNPs and *Citrus medica* peel extract were examined with the DPPH free radical scavenging technique.

MATERIAL AND METHODS

Chemicals Used in the Experimental Plan

Silver nitrate ($AgNO_3$), and DPPH, were obtained from Sigma Co., USA; bacterial nutrient agar and broth were obtained from Merck, Germany. The reagents of all compounds were utilized in their initial condition.

Pathogen Collection

Multi-antibiotic-resistant bacterial isolates like *K. pneumonia, E. coli, P. aeruginosa,* and *S. aureus* were aseptically isolated from clinical samples of a local hospital in Bangalore [33] and the bacterial isolates were confirmed by several microscopic observations like Gram's staining. Motility, capsule, as"well as spore production were assessed following the protocol outlined by Collins and Lyne [34]. The bacterial pathogens had been additionally verified using specialized biochemical tests, as outlined by Barrow and Feltham [35].

Preparation of the *Citrus medica* Peel Aqueous Extract

The *Citrus medica* peel had been extracted by refluxing 20g of cleaned and dried peel in 200ml of deionized water at a temperature of 60°C while shaking for a duration of 15 mins. A transparent, light-yellow solution was acquired. Once the extract solution had cooled to room temperature, it was filtered using Whatman No.1 filter paper (Whatman, Fisher Scientific, Pittsburgh, PA, USA) to remove any particles in the suspension. The resulting extract had been then split into 2 portions: one portion had been stored at 4 to 8°C for future usage, whereas the other portion was subjected to vacuum oven drying at 40°C for 48 h to obtain the powdered *Citrus medica* peel extract.

Biochemical Synthesis of Silver Nanoparticles from *Citrus medica* Peel Extract

The biochemical synthesis had been performed as per the technique explained by Naseem *et al*. [36], with some adjustments. A 10ml solution of *Citrus medica* peel extract was combined with a 90 ml solution of newly manufactured silver nitrate ($AgNO_3$) in a 250ml flask. The mixture had been continuously stirred with a hot plate magnetic stirrer at a rotation speed of 200rpm. The stirring took place at a temperature of 60° C in a dark environment. The mixed solution grew turbid and reddish-brown after 30 min, and the colloidal suspension's yellow color changed to brown, signifying the creation of silver NPs. After centrifuging the suspension for 20 min at 15,000 rpm to produce a dark brown precipitate, it was twice washed with double-sterilized water as well as once with methanol to eradicate the *Citrus medica* peel extract and purify the AgNPs. The final powder precipitate had been dried to produce silver NPs. To obtain the optimal situations for *Citrus medica* peel extract (*Citrus medica* PE-AgNPs) production of AgNPs, we varied the concentration of peel extract, the duration of contact, as well as the concentration of $AgNO_3$. We also used UV spectroscopy to look at the form of the controlled AgNPs.

Characterization of Silver Nanoparticles UV Spectral Analysis

After 30 min, the reaction medium's UV spectra were measured to monitor the reduction of pure Ag^+ ions. A small portion of the material (400–700 nm) was collected for UV spectrum examination.

Antibacterial Activity Determination

Using peel extracts from *Citrus medica* fruit, biochemically produced silver NPs were tested for their antibacterial activity with the disc diffusion technique [37]. Nutrient agar medium plates had been prepared, sterilized, as well as solidified and then were used for inoculating the selected Gram-negative and positive bacterial cultures using the spread plate method. After being dipped in a solution of silver nanoparticles (100 μg/ml), the sterile discs were put in a nutrient agar plate and incubated for 28 h at

37ºC. The zones of inhibition for silver nanoparticles, silver nitrate, and *Citrus medica* PE-AgNPs were measured. Zone diameter mean values were reported after the experiments were conducted three times.

Antioxidant Assay

Using a modified version of Lakhedari *et al.*'s technique [38], the *Citrus medica* peel extract and antioxidant activity of AgNPs were assessed with the DPPH free radical scavenging technique. Methanol was utilized as the control and Vitamin C was utilized as the standard. DPPH radical-scavenging is a simple decolorization technique. The decrease of DPPH and the shift in color from deep violet to yellow occur when the antioxidant component is added to 1,1-dipheny l-2-Picrylhydrazyl methanolic solutions in their oxidized form. To achieve different concentrations (0.025, 0.050, 0.125, 0.250, 0.5 & 1 mg/ml) of the synthesized AgNPs–*Citrus medica* peel extract solution, 2 ml of a standard DPPH (0.1mM) solution was added to 1ml of methanol-diluted solution. Additionally, standard methanolic solutions of Vitamin C were also added. The mixture was then shaken and allowed to sit at room temperature in the dark. Following a one-hour incubation period, the free radical scavenging activity was assessed by utilizing a UV spectrophotometer to measure each solution's absorbance (A) at 517 nm and the equation mentioned below to estimate the percentage inhibition (I%):

I% =[(A control sample A test sample)/A control sample)] 10

RESULTS AND DISCUSSION

UV Spectral Analysis Biochemically Synthesized AgNPs Using *Citrus medica* Peel Extract

A UV spectrophotometer was utilized to detect color changes in the aqueous solution during the biochemical synthesis of AgNPs using *Citrus medica* peel extract. This was in contrast to the control sample, which consisted of extract as well as silver nitrate solution, which did not exhibit any absorption band under the same conditions, as depicted in Fig. 1. The peak at 520nm indicates SPR ('Surface Plasmon Resonance'), which is caused by free electrons in metal feeling thrilled during the synthesis of AgNP [39, 40]. This revealed that when the silver ion was exposed to the bioactive components of the plant extract, it was decreased to elemental silver and then to silver NPs, changing the optical features of the silver nitrate solution" [41].

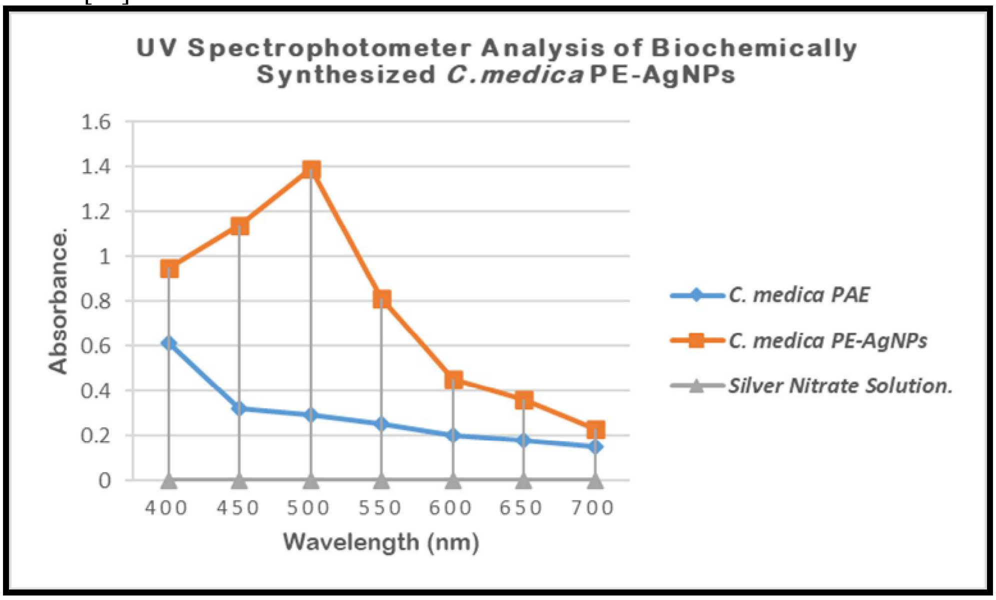

Figure 1:. UV spectrophotometer analysis of Biochemically synthesized AgNPs using *Citrus medica* peel extract in comparison with the control sample (extract and silver nitrate solution)

Antibacterial Activity

By employing the good agar diffusion technique, the sample's antibacterial activity was assessed in vitro against 4 bacterial strains: three Gram-negative bacteria (*E. coli, K. pneumonia, and P. aeruginosa*); and one Gram-positive bacteria (*S.aureus*). The various diameters of the zones of inhibition are noted inTable 1. Four levels of activity were identified by Ponce *et al.* [42] based on the bacterial sensitivity toward the test sample: (i) resistance D<8mm, (ii) sensitive 9mm D 14mm, (iii) very sensitive 15mm D 19mm, and (iv)extremely sensitive D>20 mm.

With regard to this investigation, Table 1's results showed that biochemically synthesized silver NPs using *Citrus medica* fruit peel extract have efficient antibacterial activities on the test isolates, as specified by the diameter of their zone. This was observed after the discs were incubated with all of the tested bacteria for 24 h. The zone was 21mm for *E. coli*, 18mm for *K. pneumonia*, 20mm for *P. aeruginosa* and *S. aureus*- 16 mm. In contrast, the zone of inhibition for peel extract of Citrus medica alone was 8.5mm for *E. coli,* 8mm for *K. pneumonia*, 7mm for *P.aeruginosa,* and *S. aureus*-6.5 mm. In contrast, the test reveals that the silver nitrate solution is ineffective against the isolates that were examined (Table 1 and Figure 2).

Table 1: Zone of inhibition activity of the biochemically synthesized silver NPs using peel extracts of *Citrus medica* fruit against the test bacteria

Test Bacterial Pathogens	Zone of Inhibition (mm)		
	Solution-Silver Nitrate	*Citrus medica* Peel extract	*Citrus medica* Peetl extract" / Silver nanoparticles
Klebsiella pneumonia	**0**	**8**	**18**
Escherichia coli	**0**	**8.5**	**21**
Pseudomonas aeruginosa	**0**	**7**	**20**
Staphylococcus aureus	**0**	**6.5**	**16**

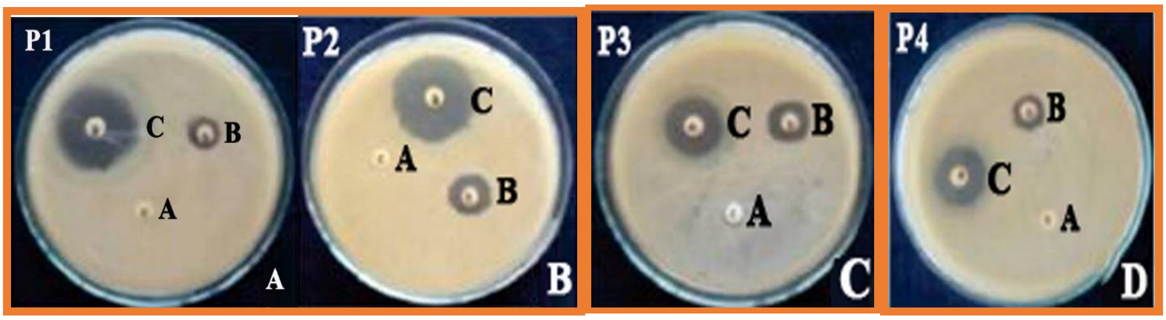

Figure 2: Antibacterial activity of biochemically synthesized silver NPs"from peel extracts of *Citrus medica* fruit against the test bacterium *E. coli* (P1), *K. pneumonia* (P2), *P. aeruginosa* (P3), *S. aureus* (P4). C—*Citrus medica* PE-AgNPs; B-*Citrus medica* PE; A-Silver Nitrate Solution

On the contrary, Table 1's results demonstrated "that the *Citrus medica* aqueous extract lacked antibacterial activity because the levels of activity fell within the resistance D < 8 mm range and the low concentration of silver nitrate (1 mM) failed to form a zone of inhibition around the well containing the silver nitrate [43, 44]. Agains *E. Coli,* the highest antibacterial capacity was attained, with maximal inhibitory zone widths of 21 mm, as opposed to 16 mm for *S. aureus*. However, Table 1's results also showed that biochemically produced AgNPs with an aqueous *Citrus medica* peel extract demonstrated a significant bactericidal strength against both Gram-positive and Gram-negative bacteria, with zone spanning from 21 to 16 mm. As per this result, Gram-negative strains were more susceptible than Gram-positive strains [45]. Gram-negative bacteria have a thin peptidoglycan layer and an additional lipopolysaccharide outer membrane, which suggests the incidence of a periplasmic membrane layer. These factors are most likely accountable for this. This configuration might make it easier for released ions and nanoparticles to enter the cell. Nonetheless, covalently linked teichuronic and teichoic acids are present in the thick peptidoglycan coating of Gram-positive bacteria's cell walls. This coating may operate as a barrier against the inhibitory effects of AgNPs and Ag^{+} [46]

Antioxidant Activity

DPPH free radical scavenging was used to assess the antioxidant activity of the AgNPs as well as the *Citrus medica* peel aqueous extract, and Vitamin C had been used as the standard to construct the calibration range. There is a variation in the antioxidant potential between the extracts and the silver NPs, as Figure 3 illustrates. Both samples reacted directly as well as lowered the broad range of free radicals of DPPH [47]; the scavenging rate raised as the tested sample's concentration increased (with *Citrus medica* peel aqueous extract at 25%, 30%, 42%, 51%, 65%, and 70% at 25, 50, 125, 250, 500, and 1000 μg/ml) and with *Citrus medica* peel extract AgNPs at 36%, 45%, 63%, 72%, 81%, and 88% at 25, 50, 125, 250, 500, and 1000 μg/m). The difference in antioxidant capacity between the extract and the silver nanoparticle produced using Citrus medica peel extract could be attributed to the chemical structure [48].

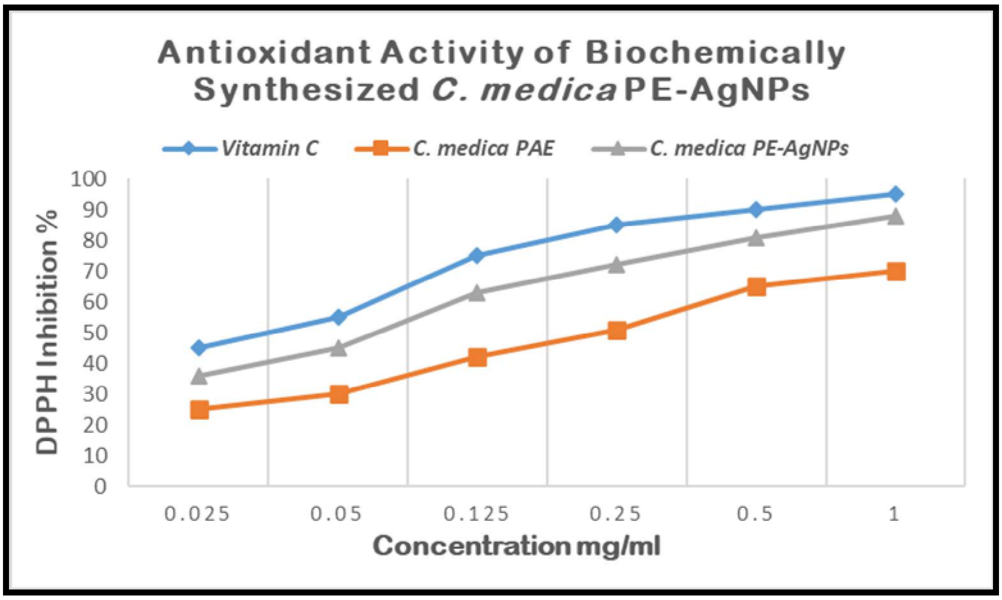

Figure 3: Antioxidant Activity of Biochemically Synthesized *Citrus medica* PE-AgNPs;

The antioxidant activity of silver NPs that were biochemically produced using *Citrus medica* peel extracts showed that the particles were effective at scavenging free radicals and that their antioxidant activity increased with concentration. (Figure 2). We can conclude that this work emphasizes the therapeutic value of $AgNO_3$ synthesized by *Citrus medica* peel extract as a source for antioxidant drug development for medical care, since both biochemically synthesized silver nanoparticles using peel extracts and *Citrus medica* peel aqueous extract demonstrated a considerably different performance in comparison with Vitamin-C as a standard solution.

According to the antimicrobial activity data, silver nanoparticles that were biochemically generated using *Citrus medica* fruit peel extracts showed significant antibacterial activity against the test isolates, as specified by the size of their zone. Our findings demonstrated that the biochemically produced silver NPs utilizing *Citrus medica* fruit peel extracts had an additional mechanism to get rid of bacteria not present in *Citrus medica* fruit peel extracts alone. This study's results are consistent with those made by Hindi *et al.* [49].

A portion of the process underlying silver ions' ability to reduce bacteria was understood. Previous research has shown that the silver ions' positive charge is essential to their antibacterial activity because it creates an electrical attraction between the negatively charged cell membrane of microorganisms and the positively charged NPs [50, 51. 52]. On the contrary, as per Sondi and Salopek-Sondi [53], the antibacterial activity of silver NPs against Gram-negative bacteria depended on their concentration and was directly linked to the development of pits in the bacteria's cell walls. The bacterial membrane then became permeable due to an accumulation of silver nanoparticles, which led to the death of the cell. It is inadequate to describe the antibacterial properties of positively charged silver nanoparticles, though, because those investigations comprised both negatively and positively charged silver ions. As per Amro *et al.* [54], the gradual release of lipopolysaccharide molecules as well as membrane proteins may result in the creation of irregularly shaped pits in the outer membrane and alter membrane permeability because of metal depletion. Furthermore, Sondi and Salopek-Sondi [53] hypothesize that a comparable mechanism could be responsible for the *E. coli* membrane structure's disintegration when the bacteria is treated with silver NPs [53]. Silver-generated free radicals were recently reported by Danilczuk *et al.* [55] through the ESR investigation of silver NPs, suggesting that the antibacterial effects of silver NPs may be related to the creation of free radicals as well as subsequent membrane damage caused by free radicals.

The biochemically produced silver NPs' antioxidant activity from *Citrus medica* peel extracts demonstrated the particles' efficient scavenging of free radicals. Our findings concur with those of earlier research [56, 57, 58]. Prior research has indicated that the growth of reducing power may be the cause of the antioxidant activity. Strong reducing agents, or reductones, are thought to react not only with peroxides directly but also with some precursors to stop the generation of peroxides [59]. It has been proposed that silver NPs serve as electron donors, combining with free radicals to create more stable compounds capable of stopping the radical chain reaction. Moreover, a significant relationship was found between the reducing power of silver nanoparticles and their ability to scavenge radicals.

CONCLUSION

Bioactive molecules like citric acid, ascorbic acid, phenolic acids, minerals, and flavonoids are abundant in *Citrus medica* peel extract. To achieve this, we employed a simple, safe, and environmentally friendly method for the biochemical production of silver NPs with peel extract from *Citrus medica*. We also improved a number of experimental parameters, such as the extract's content, and the length of the contact time, silver nitrate may be reduced to silver ions by the phytochemicals found in *Citrus medica* peel aqueous solution, like phenolic and flavonoid compounds. These ions then aggregate to form silver NPs at the nanoscale range, where they have a greater capacity to effectively stabilize AgNP synthesis. Because they encircle the AgNPs' core, these biomolecules have been employed as a naturally occurring decreasing and stabilizing agent. The study's findings indicate that the silver nanoparticles made from *Citrus medica* peel extract have a very intriguing capacity to lessen pathogenic bacteria, highlighting the particles' potential use in medicine and as antibacterial and antioxidant agents against strains of bacteria resistant to antibiotic drugs.

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CONFICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

ETHICAL APPROVAL

This study does not involve experiments on animals or human subjects.

REFERENCES

1. Albukhaty, S., Al-Karagoly, H., Allafchian, A.R., Jalali, S.A.H., Al-Kelabi, T. and Muhannad, M. [2021]: Production and characterization of biocompatible nanofibrous scaffolds made ofsitosterol loaded polyvinyl alcohol/tragacanth gum composites. Nanotechnology, 33: 85-102.
2. Yougbare, S., Chang, T.K., Tan, S.H., Kuo, J.C., Hsu, P.H., Su, C.Y. and Kuo, T.R. [2019]: Antimicrobial Gold Nanoclusters: Recent Developments and Future Perspectives. Int. J. Mol. Sci. 20:29-34.
3. Soltys, L., Olkhovyy, O., Tatarchuk, T. and Naushad, M. [2021]: Green Synthesis of Metal and Metal Oxide Nanoparticles: Principles of Green Chemistry and Raw Materials. Magnetochemistry. 7:145-152.
4. Singh, P. and Mijakovic, I. Rowan Berries: [2022]: A Potential Source for Green Synthesis of Extremely Monodisperse Gold and Silver Nanoparticles and Their Antimicrobial Property. Pharmaceutics. 14: 82-90.
5. Safat, S., Buazar, F., Albukhaty, S. and Matroodi, S. [2021]: Enhanced sunlight photocatalytic activity and biosafety of marine-driven synthesized cerium oxide nanoparticles. Sci. Rep. 11: 14734.
6. Tan, K.B., Sun, D., Huang, J., Odoom-Wubah, T. and Li, Q. [2020]: State of arts on the bio-synthesis of noble metal nanoparticles and their biological application. Chin. J. Chem. Eng., 30: 272–290.
7. Saif, S., Tahir, A., Asim, T. and Chen, Y. [2016]: Plant Mediated Green Synthesis of CuO Nanoparticles: Comparison of Toxicity of Engineered and Plant Mediated CuO Nanoparticles towards *Daphnia magna*. Nanomaterials, 6:205-212.
8. Javed, R.; Zia, M., Naz, S., Aisida, S.O., Ul Ain, N. and Ao, Q. [2020]: Role of capping agents in the application of nanoparticles in biomedicine and environmental remediation: Recent trends and future prospects. J. Nanobiotechnol. 18:172-180.
9. Karagoly, H., Rhyaf, A., Naji, H., Albukhaty, S., AlMalki, F.A., Alyamani, A.A., Albaqami, J. and Aloufi, S. [2022]: Green synthesis, characterization, cytotoxicity, and antimicrobial activity of iron oxide nanoparticles using *Nigella sativa* seed extract. Green Processing Synth. 11:254–265.
10. HaghighiPak, Z., Abbaspour,H., Karimi, N. and Fattahi, [2016]: A. Eco-Friendly Synthesis and Antimicrobial Activity of Silver Nanoparticles Using Dracocephalum moldavica Seed Extract. Appl. Sci. 6:69-80.
11. Hawar, S.N., Al-Shmgani, H.S., Al-Kubaisi, Z.A., Sulaiman, G.M., Dewir, Y.H. and Rikisahedew, J.J. [2022]: Green synthesis of silver nanoparticles from *Alhagi graecorum* leaf extract and evaluation of their cytotoxicity and antifungal activity. J. Nanomater. 10:58-70.
12. Klimek-Szczykutowicz, M., Szopa, A. and Ekiert, H. [2020]: *Citrus limon* (Lemon) Phenomenon—A Review of the Chemistry, Pharmacological Properties, Applications in the Modern Pharmaceutical, Food, and Cosmetics Industries, and Biotechnological Studies. Plants, 9: 119-130.
13. Mahato, N., Sharma, K., Sinha, M., Baral, E.R., Koteswararao, R., Dhyani, A., Cho, M.H. and Cho, S. [2020]: Bio-sorbents, industrially important chemicals and novel materials from citrus processing waste as a sustainable and renewable bioresource: A review. J. Adv. Res. 23: 61–82.
14. Lala, M., Bhattacharjee, S., Ghosh, C., Sen, A. and Sarkar, I. [2022]: In-silico studies on wild orange (*Citrus macroptera* Mont.) compounds against COVID-19 pro-inflammation targets. J. Biomol. Struct. Dyn. Pp. 1–13.
15. Smeriglio, A., Denaro, M., di Gristina, E., Mastracci, L., Grillo, F., Cornara, L. and Trombetta, D. [2022]: Pharmacognostic Approach to Evaluate the Micromorphological, Phytochemical and Biological Features of *Citrus lumia* Seeds. Food Chem., 375: 131-145.

16. Bora, H., Kamle, M., Mahato, D.K., Tiwari, P. and Kumar, P. [2020]: Citrus Essential Oils (CEOs) and Their Applications in Food: An Overview. Plants, 9: 357-361.
17. Favela-Hernández, J.M.J., González-Santiago, O., Ramírez-Cabrera, M.A., Esquivel-Ferriño, P.C. and Camacho-Corona, M.D.R. [2016]: Chemistry and Pharmacology of Citrus sinensis. Molecules, 21: 247-251.
18. Farag, M.A., Abib, B., Ayad, L. and Khattab, A.R. [2020]: Sweet and Bitter Oranges: An Updated Comparative Review of Their Bioactives, Nutrition, Food Quality, Therapeutic Merits and Biowaste Valorization Practices. Food Chem. 331: 127-136.
19. Loizzo, M.R.; Tundis, R.; Bonesi, M.; Sanzo, G.D.; Verardi, A.; Lopresto, C.G.; Pugliese, A.; Menichini, F.; Balducchi, R.; Calabrò, V. [2016]: Chemical profile and antioxidant properties of extracts and essential oils from *Citrus limon* (L.) burm. Cv. Femminello comune. Chem. Biodivers. 13: 571–581.
20. Mahato, N., Sinha, M., Sharma, K., Koteswararao, R. and Cho, M.H. [2019]: Modern Extraction and Purification Techniques for Obtaining High Purity Food-Grade Bioactive Compounds and Value-Added Co-Products from Citrus Wastes. Foods, 8:523-532.
21. Zayed, A., Badawy, M.T. and Farag, M.A. [2020]: Valorization and extraction optimization of Citrus seeds for food and functional food applications. Food Chem. 355: 129-140.
22. Min,K.Y., Lee, K.A., Kim, H.J., Kim, K.T., Chung, M. S., Chang, P.S., Park, H. and Paik, H.D. [2014]: Antioxidative and anti-inflammatory activities of *Citrus unshiu* peel extracts using a combined process of subcritical water extraction and acid hydrolysis. Food Sci. Biotechnol. 23: 1441–1446.
23. Shi, Y.S., Zhang, Y., Li, H.T., Wu, C.H., El-Seedi, H.R., Ye, W.K., Wang, Z.W., Li, C.B., Zhang, X.F. and Kai, G. [2020]: Limonoids from Citrus: Chemistry, anti-tumor potential, and other bioactivities. J. Funct. Foods, 75:104-115.
24. Otang, W.M. and Afolayan, A.J. [2016]: Antimicrobial and antioxidant efficacy of *Citrus limon* L. peel extracts used for skin diseases by Xhosa tribe of Amathole District, Eastern Cape, South Africa. S. Afr. J. Bot. 102:46–49.
25. Ehiobu, J.M., Idamokoro, M.E. and Afolayan, A.J. [2021]: Phytochemical content and antioxidant potential of leaf extracts of Citrus limon (L.) Osbeck collected in the Eastern Cape Province, South Africa. S. Afr. J. Bot. 141:480–486.
26. Basli, A., Sonia, T., Nawel, I., Bachra, K. and Khodir, M. [2016]: In-Vitro Antioxidant and Anti-Inflammatory Activities of Peel and Peeled Fruits Citrus limon.Curr. Nutr. Food Sci. 12: 279–287.
27. Dosoky, N.S. and Setzer, W.N. [2018]: Biological Activities and Safety of Citrus spp. Essential Oils. Int. J. Mol. Sci. 19: 1966-1970.
28. Penniston, K.L., Nakada, S.Y., Holmes, R.P. and Assimos, D.G. [2008]: Quantitative assessment of citric acid in lemon juice, lime juice, and commercially-available fruit juice products. J. Endourol. 22: 567–570.
29. Xi, W., Lu, J., Qun, J. and Jiao, B. [2017]: Characterization of phenolic profile and antioxidant capacity of different fruit part from lemon (Citrus limon Burm.) cultivars. J. Food Sci. Technol., 54:1108–1118.
30. Del Río, J.A., Fuster, M.D., Gómez, P., Porras, I., García-Lidón, A. and Ortuño, A. [2004]: Citrus limon: A Source of Flavonoids of Pharmaceutical Interest. Food Chem. 84: 457–461.
31. Goodwin, T.W. [1980]: Nature and distribution of carotenoids. Food Chem., 5:3–13.
32. DiMatteo, A., Simeone, G.D.R., Cirillo, A., Rao, M.A. and Di Vaio, C. [2021]: Morphological characteristics, ascorbic acid and antioxidant activity during fruit ripening of four lemon (*Citrus limon* (L.) Burm. F.) cultivars. Sci. Hortic. 276: 109-121.
33. Gram, C. [1884]: On the isolated staining of Schizomycetes in section and dry preparations. Advances in Medicine, 2:185-189.
34. Collins, C. H. and Lyne, P. M. [1970]: Microbiological methods. Microbiological methods., (3rd. Edition).
35. Barrow, G. and Feltham, R. K. A. [1993]: Manual for the identification of medical bacteria. SteeleCa, 3rd Ed., Cambridge University Press, Cambridge. pp331.
36. Naseem, K., Zia Ur Rehman, M., Ahmad, A.,Dubal, D. and AlGarni, T.S. [2020]: Plant Extract Induced Biogenic Preparation of Silver Nanoparticles and Their Potential as Catalyst for Degradation of Toxic Dyes. Coatings, 10: 1235-1244.
37. Cruickshank, R. [1968]: Medical Microbiology: A Guide to Diagnosis and Control of Infection, Edinburgh/London: E. and S. Livingstone Ltd, pp. 888.
38. Lakhdari, A., Sakhri, L., Khane, Y., Lakhdar, A.M., Kemassi, A. and Bouras, N. [2020]: Evaluation of drying effect on the composition of the essential oil isolated from aerial parts of *Pituranthos chloranthus* from southern Algeria and their biological activities. Biocatal. Agric. Biotechnol., 30:1018-1044.
39. Kreibig, U. and Vollmer, M. [1998]: Theoretical considerations. In Optical Properties of Metal Clusters; Springer: Berlin/Heidelberg, Germany, pp: 13–201.
40. Chandran, S.P., Chaudhary, M., Pasricha, R., Ahmad, A. and Sastry, M. [2006]: Synthesis of gold nanotriangles and silver nanoparticles using *Aloe vera* plant extract. Biotechnol. Prog. 22: 577–580
41. Singh, P., Kim, Y.J., Singh, H., Wang, C., Hwang, K.H., Farh, M.E.A. and Yang, D.C. [2015]: Biosynthesis, characterization, and antimicrobial applications of silver nanoparticles. Int. J. Nanomed., 10:2567–2577.
42. Ponce, A.G., Fritz, R., del Valle, C.E. and Roura, S.I. [2003]: Antimicrobial activity of essential oils on native microbial population of organic Swiss chard. Lebensm. Wiss. Technol., 36: 679–684.
43. Pandian, K., Ram, S., Deepak, V., Kalishwaralal, K., Viswanathan, P. and Gurunathan, S. [2016]: Mechanism of Bactericidal Activity of Silver Nitrate—A Concentration Dependent Bi-Functional Molecule. Braz. J. Microbiol. 41:805–809.

44. Mai-Prochnow, A., Clauson, M., Hong, J. and Murphy, A.B. [2016]: Gram positive and Gram-negative bacteria differ in their sensitivity to cold plasma. Sci. Rep. 6: 386-390.
45. Feng, Q.L., Wu, J.,Chen, G.Q.,Cui, F.Z., Kim, T.N. and Kim, J.O. [2000]: A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*. J. Biomed. Mater. Res. 52: 662–668.
46. Guimarães, P.M.R., Teixeira, J.A. and Domingues, L. [2010]: Fermentation of lactose to bio-ethanol by yeasts as part of integrated solutions for the valorisation of cheese whey. Biotechnol. Adv. 28:375–384.
47. Kumar, B., Smita, K., Seqqat, R., Benalcazar, K., Grijalva, M. and Cumbal, L. [2016]: In Vitro evaluation of silver nanoparticles cytotoxicity on Hepatic cancer (Hep-G2) cell line and their antioxidant activity: Green approach for fabrication and application. J. Photochem. Photobiol. B Biol., 159: 8–13.
48. Ortiz, R., Antilén, M., Speisky, H., Aliaga, M.E., López-Alarcón, C. and Baugh, S [2012]:. Application of a microplate-based ORAC-pyrogallol red assay for the estimation of antioxidant capacity: First action JAOAC Int., 95: 1558–1561.
49. Hindi, N. K. K., Chabuck, Z. A. G. and Hindi, S. K. K. [2014]: Antibacterial Evaluation of Aqueous Extract Of Four Citrus Species In Hlla, Iraq. International Journal of Pharmacological Screening Methods, 4(**1**): 45-60.
50. Hamouda, T., Myc, A., Donovan, B., Shih, A. Y., Reuter, J. D. and Baker, J. R. [2001]: A novel surfactant nanoemulsion with a unique non-irritant topical antimicrobial activity against bacteria, enveloped viruses and fungi. Microbiological Research, 156(**1**): 1-7.
51. Dibrov, P., Dzioba, J., Gosink, K. K., and Häse, C. C. [2002]: Chemi-osmotic mechanism of antimicrobial activity of Ag+ in *Vibrio cholerae*.Antimicrobial agents and chemotherapy 46(**8**):2668-2670.
52. Dragieva, I., Stoeva, S., Stoimenov, P., Pavlikianov, E. and Klabunde, K. [1999]: Complex formation in solutions for chemical synthesis of nanoscaled particles prepared by borohydride reduction process. Nanostructured materials, 12(**1-4)**: 267-270.
53. Sondi, I. and Salopek-Sondi, B. [2004]: Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. Journal of colloid and interface science, 275(**1**): 177-182.
54. Amro, N. A., Kotra, L. P., Wadu-Mesthrige, K., Bulychev, A., Mobashery, S., and Liu, G. Y. [2000]: High-resolution atomic force microscopy studies of the Escherichia coli outer membrane: structural basis for permeability. Langmuir, 16(**6**): 2789-2796.
55. Danilczuk, M., Lund, A., Sadlo, J., Yamada, H. and Michalik, J. [2006]: Conduction electron spin resonance of small silver particles. Spectrochemica Acta- Part A: Molecular and iomolecularSpectroscopy, 63(**1**):189-191.
56. Gao, X., Zhang, J. and Zhang, L. [2002]: Hollow sphere selenium nanoparticles: their in-vitro anti hydroxyl radical effect. Advanced Materials, 14(**4**): 290-293.
57. Nie, Z., Liu, K. J., Zhong, C. J., Wang, L. F., Yang, Y., Tian, Q. and Liu, Y. [2007]: Enhanced radical scavenging activity by antioxidant-functionalized gold nanoparticles: a novel inspiration for development of new artificial antioxidants. Free Radical Biology and Medicine, 43(9): 1243-1254.
58. Raghunandan, D., Bedre, M. D., Basavaraja, S., Sawle, B., Manjunath, S. Y. and Venkataraman, A. [2010]: Rapid biosynthesis of irregular shaped gold nanoparticles from macerated aqueous extracellular dried clove buds (*Syzygium aromaticum)* solution. Colloids and Surfaces B: Biointerfaces, 79(**1**): 235-240.
59. Xia, D. Z., Yu, X. F., Zhu, Z. Y. and Zou, Z. D. [2011]: Antioxidant and antibacterial activity of six edible wild plants (*Sonchus* spp.) in China. Natural product research, 25(**20**):1893-1901.