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Research Paper

PHYTOCHEMICAL ANALYSIS, ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY ASSESSMENT OF ORANGE PEELS

Kaur M, Mehta A, Bhardwaj KK and Gupta R

Department of Biotechnology,
Himachal Pradesh University,
Summerhill, Shimla, 171 005,
India.

Abstract

In the present-day scenario perishable fruit peels are considered as a new era of pharmaceutical products as they are rich in phytochemicals and act as antioxidant agents. The present study was undertaken to analyze the phytochemical constituents qualitatively in the orange peel extracts. The 1,1-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging activity of methanolic extract and distilled water extract was found to be better than other extracts used in the study. Silver nanoparticles were also synthesized with the aid of orange peel extract. Characterization of silver nanoparticles was carried out using UV-Visible spectroscopy and X-Ray diffraction. Synthesized sliver nanoparticles showed significant antibacterial activity against *Bacillus* sp. followed by *Salmonella*, *Proteus*, *Pseudomonas*, *Escherichia*, *Shigella* and *Staphylococcus* sp.

Key words: Orange peel, Phytochemical constituents, Silver nanoparticles, Antibacterial activity, Antioxidant activity.

INTRODUCTION

Sweet orange originated from East Asia but is consumed all over world as an excellent source of vitamin C, a powerful natural antioxidant that builds the body immune system. The peel of citrus fruit is abundant source of flavanones and many polyethoxylated flavones which are very rare in other plants [1]. The phytochemicals are also known as secondary metabolites that are derived from primary metabolites and are used as drugs. They contribute to flavor, color and other characteristics of plant parts [2]. Phytochemical analysis is very useful in the evaluation of some active biological components of some vegetables and medicinal plants. Medically, the presence of phytochemicals explains the use of vegetables in ethno medicine for the management of various ailments [3]. These secondary plant metabolites are extractable by various solvents that exhibit varied biochemical and pharmacological actions in animals [4]. The antioxidant nature of the extract was assessed by using 1,1-diphenyl-1-picryl-hydrazyl

(DPPH) method. The biological synthesis of silver nanoparticles emerges as an eco-friendly and exciting approach in the field of nanotechnology. Silver nanoparticles (AgNPs) have been known to have inhibitory and bactericidal effects [5, 6]. Nanoparticles exhibit completely new or improved properties based on specific features such as size, distribution and morphology. Although chemical and physical methods may successfully make pure, well defined nanoparticles; these methods are rather expensive and potentially hazardous to the environment. Use of biological organisms, plant extract or plant biomass could be alternative to chemical and physical methods for production of nanoparticles in an eco-friendly manner. In small concentrations, silver nanoparticles (AgNps) are non-toxic to humans and are effective against microbes at low concentrations and have no side effects [7, 8].

MATERIALS AND METHODS

Plant material and preparation of extract

Orange peels were collected from fruit juice shops of Nalagarh, Distt. Solan of H.P. After collection, the peels were dried in natural sunlight for 3-4 days. The dried peels were pulverized using an electric blender and stored in airtight containers for further use. Four different solvents namely acetone, methanol, DMSO:methanol and distilled water were used for extraction. The extract was prepared by using the methods of Omm *et al.* and John *et al.* [9, 8].

Qualitative phytochemical screening

Phytochemical screening was carried out to analyze the presence of various phytoconstituents such as alkaloids, flavonoids, phenolics, amino acids, tannins anthraquinones, saponins, proteins, terpenoids and cardiac glycosides according to standard method described by Arora and Kaur [3] and Gayathri and Kiruba [6]. General reactions in this analysis revealed the presence or absence of these compounds.

Thin Layer Chromatography

The thin layer chromatography was used to determine the R_f value of separated compounds. Aliquots of the extract and standard (quercetin, 1 mg/ml in methanol) were applied on the TLC plate. The plate was then placed in a glass beaker containing n-butanol: acetic acid: water (2:2:6) as mobile phase. Appearance of bright yellow colored bands after spraying with ferric chloride indicates the presence of flavonoids [2, 8].

Anti-oxidant assay

DPPH free radical scavenging activity assay was performed by following the methodology of Senthamil *et al.* [10].

Assessment of antibacterial activity

Antibacterial activity of the extract was tested against *Escherichia, Salmonella, Staphylococcus, Bacillus, Pseudomonas, Proteus and Shigella* by using well diffusion method.

Minimum Inhibitory Concentration (MIC) against selected pathogenic strains

MIC was performed by using the method of Ashok *et al.* [4].

Biosynthesis of silver nanoparticles

Silver nanoparticles were synthesized by following the methods of Reenaa and Menon, [11].

Characterization of silver nanoparticles

Synthesized silver nanoparticles were characterized by UV-Visible spectrophotometer (GENESYS 10S UV-Vis v4.005 2L9S073217). Further, structure and size of synthesized silver nanoparticles was investigated by XRD on PAN analytical X'Pert PRO X-ray diffractometer [12].

Antibacterial activity of synthesized silver nanoparticles

Antibacterial activity of synthesized silver nanoparticles was tested by using well diffusion method [5].

RESULTS AND DISCUSSIONS

Phytochemical screening of different solvent extracts

Present study of phytochemical screening of orange peels revealed the presence of alkaloids, flavonoids etc. Besides the secondary metabolites, the presence of saponins was noticed in methanol, DMSO:methanol (1:1 v/v) and distilled water extracts. By contrast, the acetone extract exhibited the absence of saponins but showed the presence of other common metabolites as reported in other extracts. Methanol extract of orange peels showed the presence of secondary metabolites such as tannins and saponins. The results of phytochemical analysis are shown in Table 1.

Gayathri and Kiruba, reported the presence of alkaloids, flavonoids, terpenoids and phenols from dry leaf powder extracts of *Citrus aurantium* [6]. Another study on phytochemical profile of *Citrus limonum* peel extract showed the presence of flavonoids, phenols and cardiac glycosides [13].

TLC analysis of different solvent extracts

The number of spots observed and their corresponding R_f values were calculated as shown in Fig. 1 and Table 2. Three spots with R_f values 0.93, 0.83 and 0.76 were observed for acetone extract (AE) of citrus peels. Three spots with R_f values of 0.97, 0.83 and 0.71 were observed for methanol extract (ME), while single spot with R_f values of 0.95 and 0.97 was observed for DMSO:methanol (D:ME) and distilled water extract (DW) respectively. This indicated the presence of different flavonoids. Quercetin standard showed a single band with R_f value of 0.83. AL-Daody *et al.* reported the presence of flavonoids by the appearance of bright yellow colored bands from *Cyperus rotundas* [2].

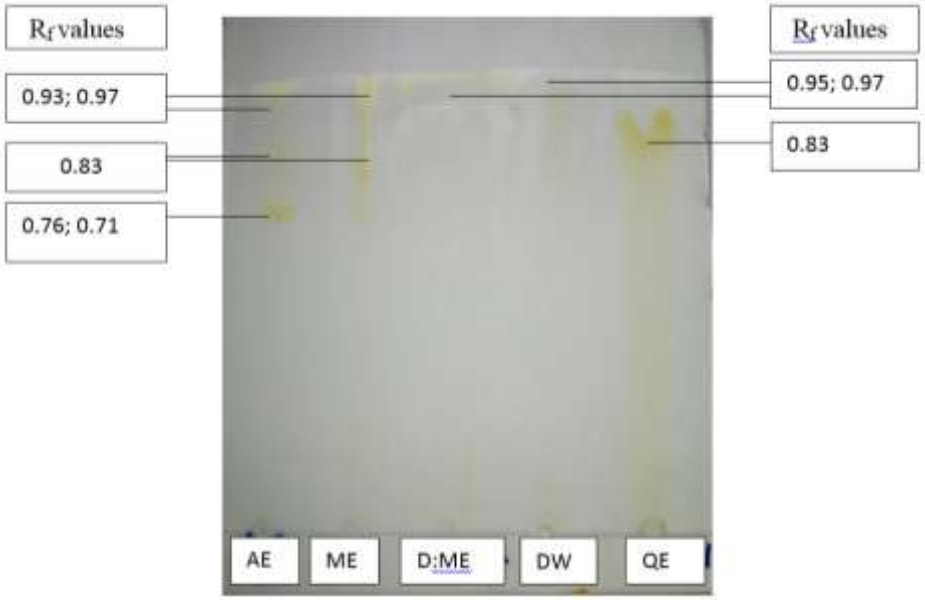

Fig. 1 TLC analysis of flavonoids.

Antioxidant assay of peel extracts

Fig. 2 represents the antioxidant activity of orange peels in different solvent extracts with control ascorbic acid. Methanol and distilled water extracts showed better DPPH radical scavenging activity when compared to the other extracts. The phytochemical constituents like alkaloids, glycosides, tannins and flavonoids present in the extract, are probably responsible for the antioxidant activity [14]. Hence, it can be viewed that this antioxidant activity might be due to the presence of any of these constituents.

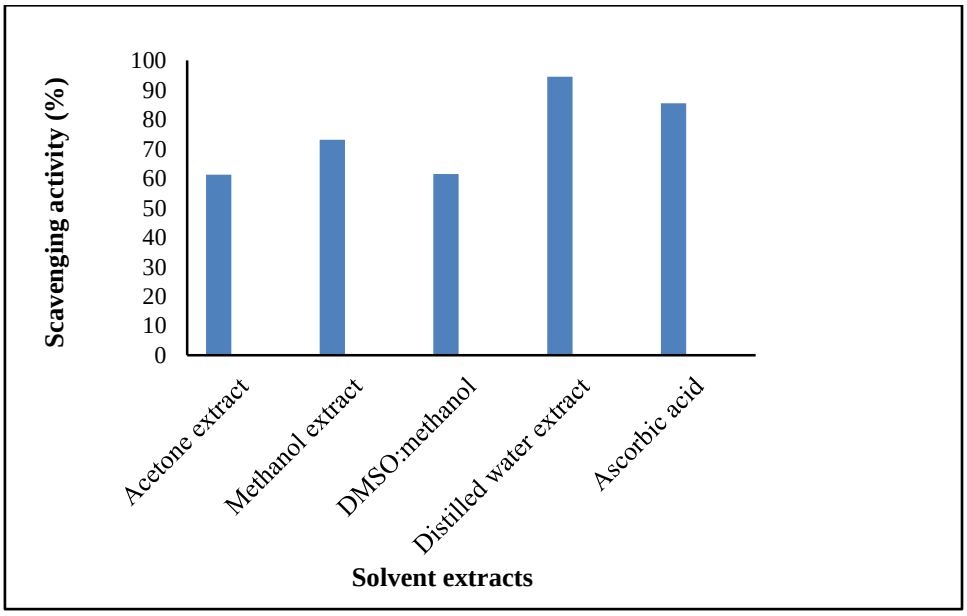

Fig. 2 Percent scavenging activity of different solvent extracts of orange peels.

Assessment of antibacterial activity

Acetone, methanol, DMSO:methanol and distilled water extracts of orange peels were tested for antibacterial activity against seven pathogenic strains (*Escherichia, Salmonella, Staphylococcus, Bacillus, Pseudomonas, Proteus* and *Shigella*). The results of this study showed that the methanol extract of orange peel demonstrated good activity on all the tested bacteria at all concentrations, while acetone and DMSO:methanol (1:1 v/v) extracts also showed good antibacterial activity, but their activity was lowered when used in diluted forms against some bacterial strains. However distilled water extract of orange peels did not show any antibacterial activity against any bacterial strain. Antibacterial activity of methanol extract of orange peels against pathogenic bacterial strains is shown in the Fig. 3 and Fig. 4.

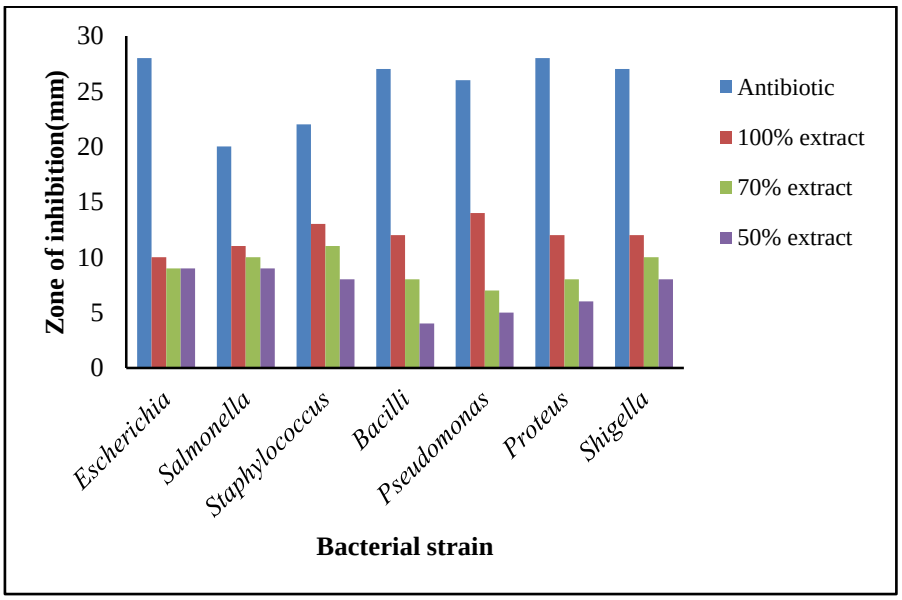

Fig. 3 Antibacterial activity of methanol extract of orange peels against pathogenic bacterial strains.

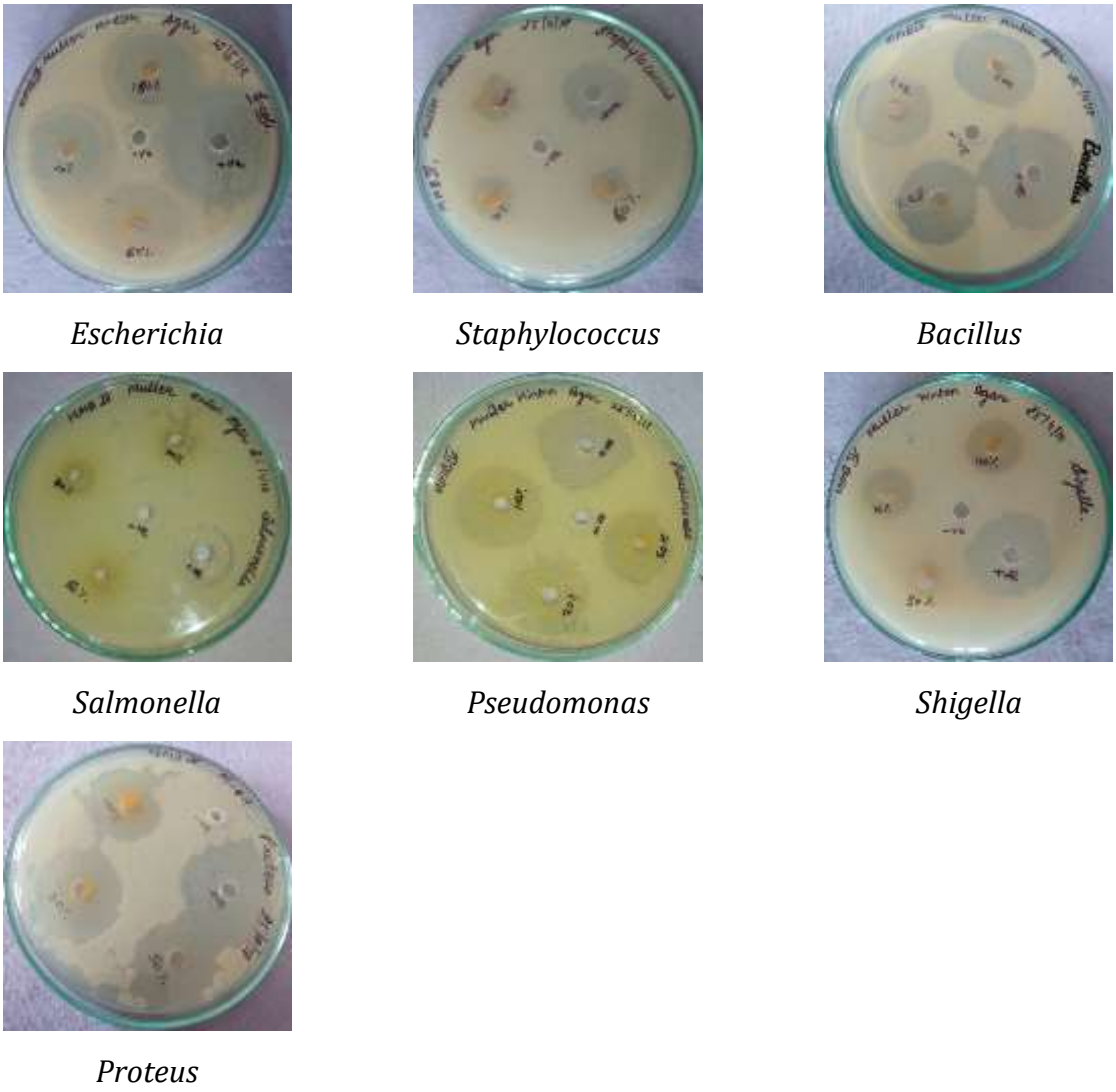

Fig. 4 Zone of inhibition shown by methanol extract of orange peels against pathogenic bacterial strains.

Minimum inhibitory concentration of methanol extract of orange peels

Pseudomonas was most sensitive to the peel extract with MIC value of 0.109 mg/100 µl, while MIC values of peel extract for *Escherichia, Salmonella, Shigella, Bacillus Staphylococcus,* , and *Proteus* were found to be 3.5 mg/100 µl, 3.5 mg/100 µl, 3.5mg/100 µl, 0.437 mg/100 µl, 0.218 mg/100 µl, and 0.218 mg/100 µl respectively. A study done on antimcirobial activity of *Citrus limon* showed that MIC for *Staphylococcus* and *Proteus* was found to be 0.024 mg/100 µl [11].

The absorption spectrum showed that the synthesized nanoparticles had a plasmon absorption band of 325 nm after 24 hrs as shown in Fig. 5 (a), while a study conducted to synthesize silver phytoparticles, revealed the plasmon absorption band of 425-435 nm after 48 hrs [5, 6]. Variations in results may be due to the presence of some

bioorganic compounds that might be deposited on the surface of the synthesized particles.

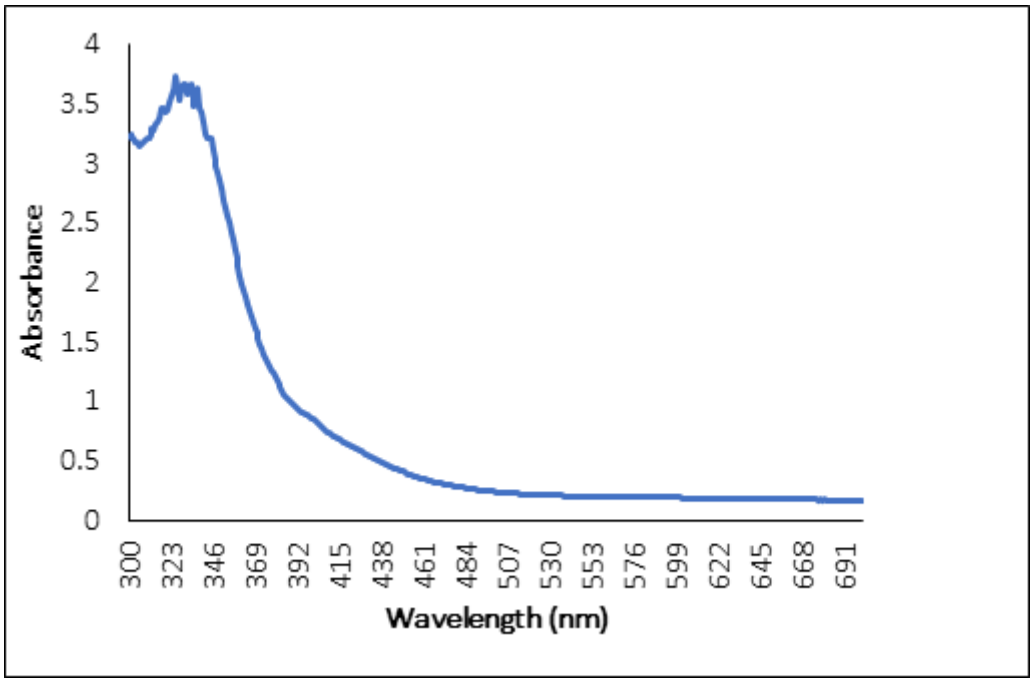

Fig. 5 (a) UV- Visible spectrum of silver nanoparticles synthesized from orange peel extract.

X-Ray Diffraction pattern recorded for the synthesized silver nanoparticles has been shown in Fig. 5 (b). The results of the XRD analysis showed 2θ intense values with various degrees (28.1305°, 32.4930°, 38.3788°, and 46.4652°) which showed the crystalline nature of the particles.

The size of the particles was calculated and the average crystalline size came out to be 12.32 nm. A previous study on synthesis of silver nanoparticles using plants extract and analysis of their antimicrobial property showed that average particle size of the silver was found to be 26 nm, 26 nm, 59 nm, 20 nm and 24 nm corresponding to *O. tenuiflorum*, *S. cumini*, *C. sinensis*, *S. tricobatum* and *C. asiatica* respectively [10].

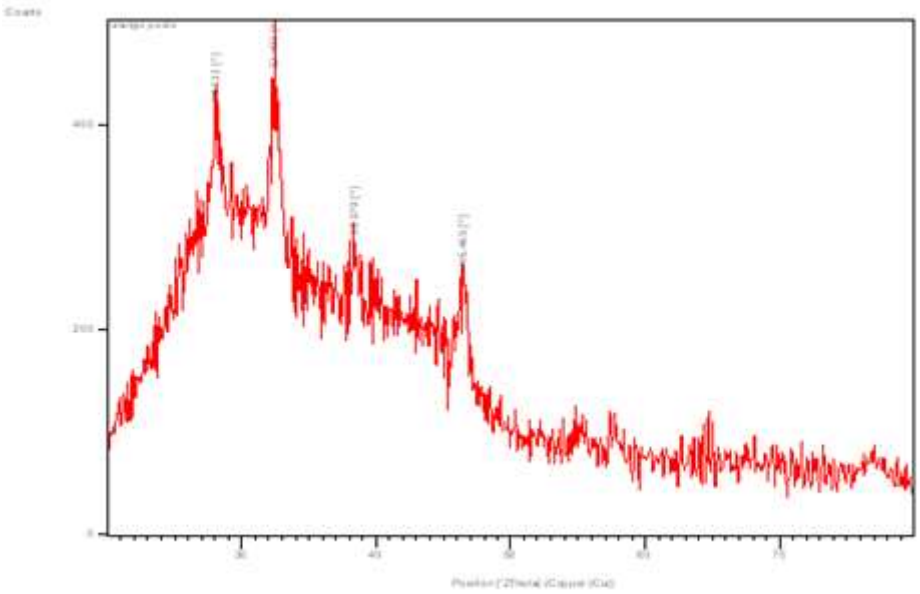

Fig. 5 (b) XRD pattern of the silver nanoparticles synthesized from orange peel extract.

Antibacterial activity of silver nanoparticles

The zone of growth inhibition formed by synthesized AgNps was much more than that formed by the methanol extract of orange peels as shown in Fig. 6. A study on synthesis of silver nanoparticles from different citrus fruit peel extracts showed that silver nanoparticles of *Citrus sinensis* extract showed good antibacterial activity against all the bacterial strains used in that study [5].

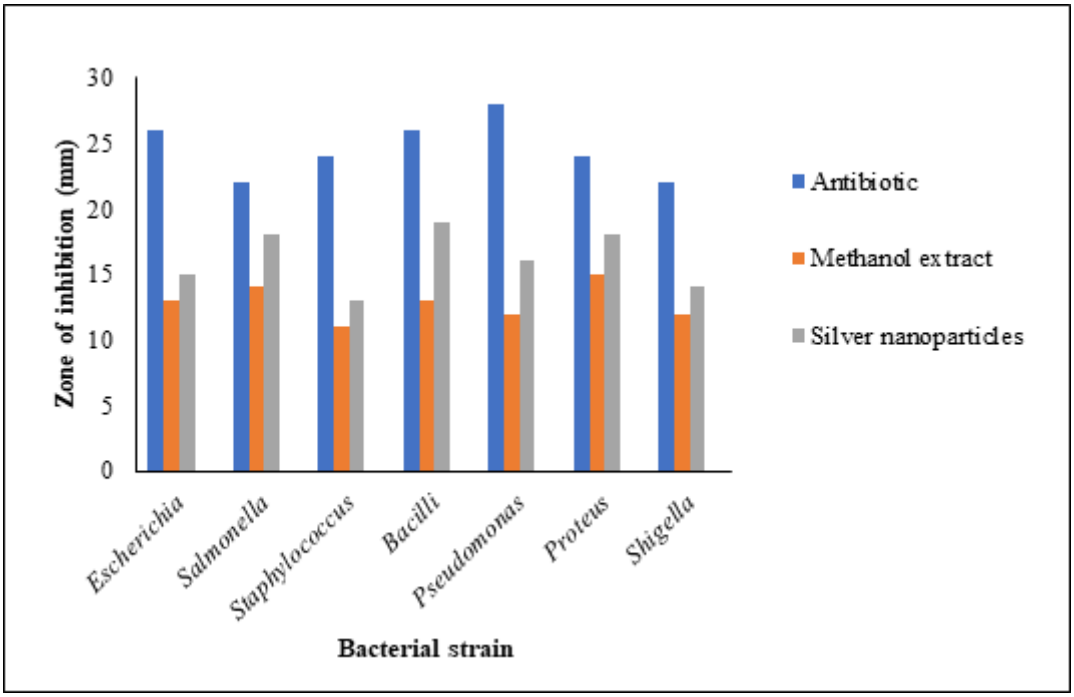

Fig. 6 Antimicrobial activity of synthesized silver nanoparticles against pathogenic bacterial isolates.

Table 1 Phytochemical screening of different solvent extracts of orange peels.

Phytochemicals	Acetone	Methanol	DMSO:methanol (1:1 v/v)	Distilled water
Alkaloids	+	+	+	+
Flavonoids	+	+	+	+
Phenols	-	+	-	-
Amino acid	-	-	-	-
Tannin	+	+	+	+
Anthraquinones	-	-	-	-
Saponins	-	+	+	+
Protein	-	-	-	-
Terpenoids	-	+	+	+
Cardiac glycosides	-	+	-	-

'+' – Present '-' – Absent

Table 2 R_f Values of different solvent extracts.

Sample	**R_f Value**
Acetone extract	0.93; 0.83; 0.76
Methanol extract	0.97; 0.83; 0.71
DMSO:methanol extract	0.95
Distilled water extract	0.97
Standard (Quercetin)	0.83

CONCLUSION

The study reveals that orange peel is a rich source of active compounds like alkaloids, flavonoids with various medicinal and pharmacological properties making them to be utilized as an attractive, alternate and cheap source of functional ingredients for the formulation of functional foods and nutraceuticals. Further studies are also required to unravel and characterize active components present in orange peel.

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CONFLICTS OF INTERESTS

The author(s) declare(s) that there is no conflict of interests regarding the publication of this article.

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