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Anti-oxidant and Anti-microbial Activities of Silver Nanoparticles of Essential Oil Extracts from Leaves of *Zanthoxylum ovalifolium*

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Authors' contributions

This work was carried out in collaboration among all authors. Author BST wrote the protocol and performed the experimentation. Author SA designed the study. Author BSRK wrote the first draft of the manuscript. Author SRK designed the study, managed the analyses of the study, managed the literature searches and performed statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The present study was aimed to synthesis and characterization of silver nanoparticles (AgNPs) using *Zanthoxylum ovalifolium* leaf essential oil extracts and to screen the anti-oxidant and antimicrobial potential of the same.
Place and Duration of Study: The studies were carried out at Department of Botany, AVK College for Women, Hassan and Department of Biochemistry, Aurora's Degree & PG College, Hyderabad from July 2017 to June 2018
Methodology: The essential oil from leaves of *Zanthoxylum ovalifolium* was obtained by

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hydrodistillation and analyzed by GC and GC-MS. Synthesis of silver nanoparticles of essential oil extract was carried out and characterized by using UV-VIS spectroscopy and transmission electron microscopy (TEM). The total phenolic and flavonoid contents were estimated in all the extracts. Furthermore, all the extracts were evaluated for anti-microbial activity against two gram-positive and two gram-negative bacteria and four pathogenic fungi using agar disc diffusion technique. Subsequently the minimum inhibitory concentration (MIC) was also determined.

Results: The major compounds identified were Limonene, isofenchol, Geijerene, isothujanol, Borneol, dihydrocarveol, isobornyl acetate, pregeijerene, β-elemene, trans-caryophyllene and Germacrene D. The TEM analysis of nanoparticles synthesized showed a size of 8 to 14 nm with a λ_{max} of 450nm. All the extracts were evaluated for *in vitro* anti-oxidant activity where the AgNP of essential oil extract showed maximum activity of 89.61% and 84.92% respectively for both DPPH free radical scavenging and Hydrogen peroxide free radical scavenging assays at a concentration of 100µg/ml. Among all the bacteria tested, *B. subtilis* was most susceptible at 100µg/ml with zone of inhibition of 22.5mm. While among all the fungi tested, *A. niger* inhibited more effectively by the AgNP of essential oil extract at 100µg/ml with a zone of inhibition of 16.2mm.

Conclusion: The results obtained were remarkable suggesting that AgNP of essential oil extract possess excellent anti-oxidant and anti-microbial activity and can be an alternative bio-friendly source for various pharmaceutical industries.

Keywords: Zanthoxylum ovalifolium; essential oil composition; AgNP synthesis; anti-oxidant and anti-microbial activities.

1. INTRODUCTION

Silver nanoparticles (NPs) have become an important aspect of today's research as it has many applications in various sectors such as energy, medicine, agriculture and environment sciences. The NPs of different metals including Cu, Pb, Ca, Pt, Ag, Au etc. have been synthesized and evaluated but silver nanoparticles (AgNP) have wide range of applications in various domains especially in pharmaceutical sciences which includes treatment of skin diseases like acne, dermatitis and ulcerative colitis, cell labeling, coating of surgical and medical devices, molecular imaging of cancer cells [1]. Various antibacterial formulations and devices such as household antiseptic sprays and antimicrobial bandages have also been developed from AgNPs [2]. With the advent of repeated usage of antibiotics, microorganisms have developed resistance towards many of the drugs and therefore, this problem can now be recognized as a promising global challenge to all the scientists worldwide. Therefore, there is a need for new and novel alternative antimicrobial agents which offer good efficacy and which are enviro-friendly. It is well known that synthesis of NPs using plant extract is faster and more stable when compared to those synthesized using microbes and other biological systems [1].

Essential oils (EOs) are complex mixture of natural compounds of plants comprising mostly of volatile constituents with characteristic aroma and are employed in drug, food and perfumery industries [3]. These essential oils have received much attention and this attracted many scientists to use essential oils in combination with nanoparticles which are thought to exert synergistic anti-oxidant and antimicrobial activities thus leading to the development of novel lead products for the treatment. The anti-oxidant, antimicrobial and cytotoxic activities of AgNP extracts of essential oils have been studied and reported [4-6].

Zanthoxylum ovalifolium belonging to the family Rutaceae is an erect shrub or small tree which grows up to 8 metres tall. The plant is distributed in India, Nepal, Myanmar, Indonesia, Papua New Guinea and Australia. Concerning the Phytochemistry, the plant is known to possess alkaloids, terpenoids, glycosides, phenols and flavonoids [7]. As evident from the literature, the plant exhibits potential antioxidant, antiinflammatory, natural pesticide, antimicrobial, anthelmintic, hepatoprotective, antiproliferative and antifungal activities [7]. It would be essential to assess the phytochemical composition of this plant mainly to ensure that the bioactive compounds are novel and may offer a possible role to play as effective and alternative therapeutic agents. Therefore, the aim of the present study is to analyze the composition of oil from leaves of *Z. ovalifolium*, to assess the anti-oxidant activity of extracts, antimicrobial activity of extracts and we present here the results of our investigative study.

2. MATERIALS AND METHODS

2.1 Chemicals and Solvents

All the chemicals and solvents were procured from Sigma Aldrich, Bengaluru and of analytical grade.

2.2 Plant Material

The leaves of *Zanthoxylum ovalifolium* were collected near Jayapura of Chikmagalore dist. Karnataka and a voucher specimen has been deposited at Yuvaraja's College, Mysuru, Karnataka (YCMBOT3524).

2.3 Essential Oil Extraction

Leaves were air dried and subjected to hydrodistillation in a Clevenger apparatus for 4 hr. The distilled oil thus obtained was dried over anhydrous sodium sulphate and stored under nitrogen atmosphere until further use. The oil content was expressed as ml/100g of dried leaves. The oil thus obtained was pale yellow in colour with a strong pungent odour and was subsequently analyzed by GC and GC-MS.

2.4 GC Analysis

Analysis was carried out, on a Varian-gas chromatograph equipped BP-1 capillary column (30m X 0.2mm i.d., film thickness 0.25μm). The carrier gas was helium and employed at a flow rate of 1.0 ml/min with 8 p.s.i inlet pressure. The temperature was programmed from 60ºC to 220ºC at a ramp rate of 5ºC/min and a final hold time of 6 min. The temperature of injector and detector was maintained at 250ºC and 300ºC respectively. 0.2μl of sample was injected with 1:100 split ratios.

2.5 GC-MS Analysis

Analysis was performed on an Agilent 6890 GC equipped with HP-5 capillary column (30m X 0.25mm X 0.25μ) and a 5973 N mass selective detector. The oven temperature was programmed from 50ºC to 280ºC at a ramp rate of 4ºC/min with a final hold time of 5 min. The temperature of Inlet and interface was maintained at 250ºC and 280ºC respectively and helium at a flow rate of 1.0 ml/min (constant flow) was used as a carrier gas. 0.2μl of sample was injected under split of 20:1. EIMS: electron energy, 70ev. The temperature of Ion source and quadrupole was maintained respectively at 230ºC and 150ºC.

2.6 Identification of Compounds

Identification of individual components was carried out by comparing the retention indices (RI) of the peaks determined on a BP-1 column using a saturated mixture of C_8-C_{22} n-alkanes as a reference with linear interpolation and also with those of literature [8-9]. Further identification was accomplished by GC-MS by comparing their mass spectra with mass spectral databases such as Wiley and NIST which are resident in the system [9-10].

2.7 Biosynthesis of Silver Nano Particles (AgNPs)

The biosynthesis of AgNPs was carried out according to the method of [11]. A 50 ml of 5×10^{-3} M aqueous solution of silver nitrate was prepared in an Erlenmeyer flask and to which 1 ml of essential oil extract (0.2 g/ml) was added at room temperature and kept in dark for 24 h until a brown color was developed. The AgNPs thus formed was analyzed by UV-VIS spectroscopy and Transmission Electron Microscopy (TEM).

2.8 UV–VIS Spectroscopy and Tem Analysis

The samples were diluted with HPLC grade water and the bio-reduction of silver nitrate ($AgNO_3$) to AgNPs was monitored periodically on UV-VIS spectrophotometer (Shimadzu UV1900) where a spectrum of silver and nanoparticles was recorded. All the readings were recorded at a wavelength range of 200–800 nm. The suspension containing AgNPs of essential oil extract of *Z. ovalifolium* was subjected to TEM analysis on a JEOL-JEM 2100- 200 KV electron microscope [12]. Sample preparation was carried out by placing a drop of the suspension of AgNP solution on carbon-coated copper grids and allowed to dry for 10 min. until water get evaporated and subsequently determined the shape and size of silver nanoparticles.

2.9 Estimation of Total Phenolic Content

The total phenolic content of all the extracts was determined using a method of Ainsworth [13] with slight modification. 0.5 ml of plant extracts, essential oil and AgNPs amounting to a concentration of 100 µg/ml was mixed with 2 ml of Folin-Ciocalteau reagent (1:10 diluted with deionized water) and neutralized with 7.5% w/v Na_2CO_3 solution. The reaction mixture was incubated for 30 min. at room temperature and

the color developed was measured at 760nm against reagent blank prepared in the same manner but without plant extracts. Gallic acid was used as the reference standard. Total phenolics were determined as milligrams of gallic acid equivalents per gram of sample by computing it with calibration curve. All the experiments were performed in quintuplicate.

2.10 Determination of Total Flavonoid Content

Total flavonoid content was measured with the aluminum chloride colorimetric assay of [14]. 1ml of plant extracts, essential oil and AgNPs accounting to a concentration of 100µg/ml was mixed with 0.2ml of 10% aluminum chloride, 0.2 ml of 1M potassium acetate followed by dilution with 5.6 ml of deionized water. The samples were incubated at room temperature for 30 minutes and the absorbance of the reaction mixture was measured at 420 nm. Quercetin was served as a reference standard sample. The total flavonoids were determined as milligrams of quercetin equivalents per gram of sample from the calibration curve of quercetin. All the experiments were performed in quintuplicate.

2.11 *In vitro* Antioxidant Activity

This was carried out by 3 assays.

2.11.1 Hydrogen peroxide free radical scavenging assay

The ability of *Z. ovalifolium* extracts to scavenge hydrogen peroxide was investigated according to the method developed by Ruch et al., 1989 [15] with slight modifications. 43 mM of hydrogen peroxide in phosphate buffer (1M, pH 7.4) was prepared and used for the assay. Different concentration of sample (10-500µg/ml) was added to a hydrogen peroxide solution (0.6 ml, 43 mM) and after 10 min the absorbance was measured at 230 nm against a blank prepared with phosphate buffer without hydrogen peroxide. Ascorbic acid was used as a reference standard compound. The free radical scavenging activity was determined by evaluating the % inhibition by using the following formula

% inhibition = (Control- Test) /control ×100

2.11.2 DPPH scavenging assay

The free radical scavenging ability of extracts of *Z. ovalifolium* was investigated using **1**,1-diphenyl-2picrylhydrazyl (DPPH) radical scavenging method as per the reports published in the literature [16]. Ascorbic acid served as a standard compound. The reaction mixture contained 2 ml of 1.0 mmol/L DPPH solution in methanol and 1.0 ml of extract (10-500 µg/ml) and standard compound. The mixture was incubated for 20 min at 37°C. The decrease in the absorbance was measured at 517 nm against a reagent blank. The scavenging ability was calculated using the following formula

Free radical scavenging activity (%) = $(A_s - A_t) / A_s \times 100$

where,

A_s = Absorbance of standard compound
A_t = Absorbance of sample

2.11.3 β-CAROTENE bleaching assay

The β -carotene bleaching assay was carried out according to the method developed by Wettasinghe et al., 1999 [17]. β -carotene solution 2 ml at a concentration of 200µg/ml in chloroform) was taken in a round bottom flask and mixed with 20 µl linoleic acid and 200 µl Tween 20. The mixture was then evaporated for 10 min at 40°C followed by the addition of HPLC grade water (100 ml). The mixture was vortex and an aliquot of 5 ml of the resulting emulsion was transferred into test tubes containing different concentrations of *Z. ovalifolium* extracts (10-500µg/ml). The mixture further was vortex and placed in a water bath at 50°C for 2 h. The absorbance was repeatedly measured every 15 min at 470 nm using a UV–VIS spectrophotometer (Shimadzu UV1900). All experiments were performed in quintuplicate. The total antioxidant activity was calculated based on the following formula

Anti-oxidant activity % = $1 - (A_0 - A_t) / (A_0^0 - A_0^t)$

Where,

A_0 = Absorbance of control
A_t = Absorbance of sample

2.12 Antimicrobial Activity

For the present study, two gram +ve bacteria, *Staphylococcus aureus* (ATCC 25923) and *Bacillus subtilis* (ATCC 10707), two gram –ve bacteria, *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) and fungi such as *Aspergillus niger* (ATCC 10553),

Fusarium oxysporum (ATCC 10960), *Sclerotium rolfsii* (ATCC 15206), *Rhizoctonia solani* (ATCC 16120) were obtained from Department of Microbiology, and Department of Botany, Mysore University, Mysuru. The bacterial and fungal strains were cultured and maintained respectively on nutrient agar and potato dextrose agar media. The antimicrobial activity was investigated by disc diffusion method [18-19]. The diluted bacterial strains (2ml) were added to each Petri plate containing 20ml of sterilized culture medium and uniformly spread with a sterilized spreader over the surface. Sterile paper discs of diameter of 6mm were made with a cork borer and impregnated with different concentrations of extracts (10-100μg) in dimethyl sulphoxide (DMSO, 0.5%). The discs treated with extracts were placed in the seeded agar plates and the plates were incubated at 37ºC for 24h and 30ºC for 48h respectively for bacteria and fungi. The zones of inhibition were subsequently measured. The commercially available standard antibiotic, streptomycin (dissolved in DMSO to give a concentration of 1-10μg) and Bifonazole (dissolved in DMSO to give a concentration of 1-10μg) were also tested as positive control samples where acetone served as negative control. All the experiments were performed in quintuplicate.

2.13 Minimum Inhibitory Concentration (MIC)

Determination of MIC was carried out by broth microdilution method [18-19]. All the experiments were performed in Mueller Hinton Broth and Potato Dextrose Broth for bacteria and fungi respectively supplemented with Tween 80 (0.5% v/v). All the bacterial and fungal strains were suspended in the broth to attain a density of 108 c.f.u./ml (0.5 McFarland turbidity standard). Various concentrations of extracts (2-250 μg/ml) were prepared in a 96 well microtitre plate and incubated at 37ºC for 24 h and 30ºC for 48 h respectively for bacteria and fungi. Control tests were also performed in parallel using DMSO and reference standard compounds for favourable comparison.

3. RESULTS AND DISCUSSION

The synthesis of AgNPs of essential oil extracts of Z. ovalifolium was carried out and when 0.2 g of the extract was added to 50 ml of 5 mM aqueous silver nitrate (AgNO3) resulted in the formation of the brown solution after the overnight incubation at 40^{o}C in the dark which indicated the biosynthesis of AgNPs (Fig. 1). Subsequently the solution was scanned in the range of 190–800 nm in a UV-VIS spectrophotometer which showed a maximum absorption at 440 nm (Fig. 2) followed by TEM analysis which revealed the size of AgNPs between 30 and 50 nm which was depicted in Fig. 3.

Fig. 1. AgNP formation from essential oil extract of *Z. ovalifolium*

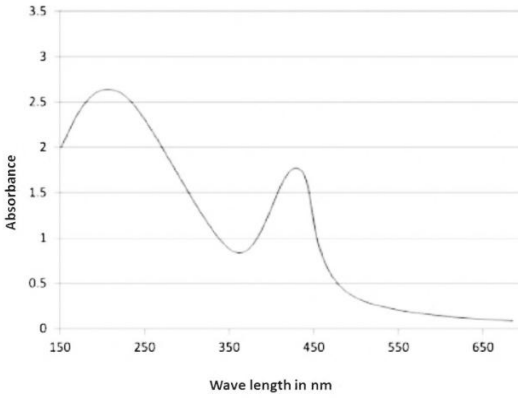

Fig. 2. UV-VIS Absorption cure of AgNP of essential oil extract of *Z. ovalifolium*

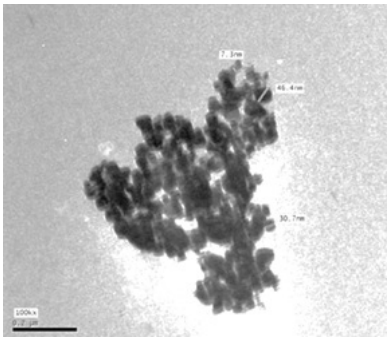

Fig. 3. TEM analysis of AgNP of essential oil extract of *Z. ovalifolium*

The results of chemical composition of *Zanthoxylum ovalifolium* plant extract alone and with AgNPs was carried out by GC and GC-MS and presented (Table 1). It showed that hydro distillation of leaves yielded 0.24% essential oil (EO) on a fresh weight basis. The GC–MS and GC analysis using Retention indices (RI) revealed that a total of 21 components were identified and represented 93.6% of the crude EO. These included α-Terpinene, p-Cymene, β-Myrcene, Borneol, isothujanol, isofenchol, isobornyl acetate, β-elemene and 15-hexadecanolide. Major portion of oxygenated fraction of the oil was comprised by alcohols i.e. 26.761%. dihydrocarveol, 3-borneol, iso-thujanol, terpinene-1-ol, iso-pulegol, and iso-fenchol were the alcohols which were observed in predominant percentages in the essential oil of *Zanthoxylum ovalifolium*. The quantitative determination of the total phenolics and total flavonoids was estimated and presented in Table 2. The total phenolic content AgNP of essential oil extract showed maximum phenolic and flavonoid contents of 34.15mg of gallic acid/gm and 15.65 mg of quercetin /gm respectively.

Table 1. Essential oil composition of *Zanthoxylum ovalifolium*

S. no.	Compound name	RI[a]	% composition
1	α-pinene	937	0.65
2	Sabinene	977	2.16
3	β-pinene	981	1.92
4	α-phellendrene	1009	1.01
5	Delta-3-carene	1012	0.79
6	α-terpinene	1020	1.12
7	Limonene	1024	3.18
8	terpinolene	1089	1.93
9	isofenchol	1101	3.13
10	sabinene hydrate	1116	0.98
11	Geijerene	1143	7.23
12	isopulegol	1146	1.44
13	isothujanol	1157	4.08
14	terpinen-1-ol	1166	0.87
15	Borneol	1180	8.21
16	dihydrocarveol	1195	8.26
17	isobornyl acetate	1271	7.82
18	Pregeijerene	1285	5.89
19	β-Bourbonene	1386	1.26
20	β-elemene	1389	4.55
21	trans-caryophyllene	1406	2.97
22	α-humelene	1446	1.54
23	Germacrene D	1480	9.24
24	Delta cadinene	1536	0.43
25	Spathulenol	1564	0.84
26	caryophyllene oxide	1574	0.98
27	β-Bisabolol	1672	0.87

Table 2. Quantitative determination of the total phenolic content

Extract	Total Polyphenol content (mg of gallic acid/gm)	Total flavonoid content (mg of quercetin /gm)
Essential oil	32.47 ± 0.68	13.08 ± 0.76
Ethanol extract	28.74 ± 0.92	10.26 ± 0.35
Ethyl acetate extract	23.23 ± 1.02	9.37 ± 0.77
AgNP extract	34.15 ± 1.12	15.65 ± 1.23

Mean ± S.E.M = Mean values ± Standard error of means of five experiments

The antioxidant efficacy of extracts were evaluated and depicted in Table 3-5. Among all the extracts tested, AgNP extract of essential oil at 100 µg/ml showed maximum activity of 89.6% and 84.9% for DPPH and H_2O_2 scavenging activities respectively, while the reference standard ascorbic showed exhibited 100% activity at 80µg/ml for the assays. These results indicate that AgNPs have antioxidant potential comparable with standard ascorbic acid. Interestingly, the results of β-carotene bleaching assay demonstrated a higher antioxidant activity of AgNPs extract with IC_{50} of 56µg/ml than the other extracts. Noticeable increase in the activities was observed with increase in concentration of extracts in a dose dependent manner.

The antibacterial activity of extracts of *Z. ovalifolium* was carried out against four organisms by disc diffusion method and the results were presented in Table 6. All the extracts showed noticeable antibacterial activity, as evidenced by their zones of inhibition. Moreover, the activity of AgNP extract of essential oil was higher when compared to other extracts, showing a strong inhibition against *B. subtilis* and *S. aureus* at 100 g/ml with inhibition zones of 22.5 and 18.1 mm respectively. Increase in the inhibition with increase in concentration of the extracts from 20 to 100 µg/ml was observed. The AgNP extract of essential oil could inhibit the complete visible growth of *B. subtilis* at a minimum concentration of 116.9µg/ml. The MIC values for streptomycin ranged from 11.8 to 27.6 µg/ml for all the organisms tested. The susceptibility of bacteria to the extracts was in the order *B. subtilis*>*S. aureus*>*E. coli*>*P. aeruginosa*.

Table 3. DPPH anti-oxidant scavenging activity of extracts of *Z. ovalifolium*

Concentration (µg/ml)	DPPH radical scavenging activity (%)				
	Ethyl acetate extract	Ethanol extract	Essential oil	AgNP extract	Ascorbic acid
10	6.73 ± 0.61	9.71 ± 0.43	10.12 ± 0.79	14.72 ± 0.98	21.23±0.57
20	12.11 ± 0.84	17.34 ± 0.92	22.25 ± 0.92	27.21 ± 0.89	33.45 ± 0.98
40	23.08 ± 1.06	28.38 ± 1.02	32.18 ± 1.14	40.08 ± 1.01	48.19 ±1.17
60	35.76 ± 1.22	43.67 ± 1.15	46.38 ± 1.62	58.47 ± 1.23	78.26 ± 1.28
80	44.93 ± 1.99	54.18 ± 1.98	64.57 ± 2.26	67.19 ± 2.17	100
100	57.28 ± 2.25	68.26 ± 2.07	77.72 ± 2.48	89.61± 1.24	100

Test extracts: significant from normal control, P < 0.05

Mean ± S.E.M = Mean values ± Standard error of means of five experiments

Table 4. H_2O_2 free radical scavenging activity of extracts of *Z. ovalifolium*

Concentration (µg/ml)	Hydrogen peroxide free radical scavenging activity (%)				
	Ethyl acetate extract	Ethanol extract	Essential oil	AgNP extract	Ascorbic acid
10	5.35 ± 0.52	9.17 ± 0.37	9.04 ± 0.68	11.22 ± 0.72	24.45±0.71
20	9.11 ± 0.79	18.34 ± 0.82	19.65 ± 0.93	23.16± 0.93	38.51 ± 0.96
40	17.15 ± 1.12	26.26 ± 1.22	30.23 ± 1.23	38.28 ± 1.21	51.32 ±1.08
60	29.51 ± 1.38	40.67 ± 1.35	48.19 ± 1.75	65.81 ± 1.25	88.16 ± 1.37
80	36.34 ± 1.91	51.23 ± 1.78	60.39 ± 2.29	73.37± 1.09	100
100	52.81 ± 2.34	64.16 ± 2.37	72.28 ± 2.37	84.92± 2.19	100

Test extracts: significant from normal control, P < 0.05

Mean ± S.E.M = Mean values ± Standard error of means of five experiments

Table 5. β-carotene bleaching activity various extracts of *Z. ovalifolium*

Sl. no.	Sample/Extract	IC_{50} (µg/ml)
1.	Ethyl acetate extract	84.76 ± 2.15 (69.288 – 90.193)
2.	Ethanol extract	72.88 ± 1.56 (63.724 – 81.156)
3.	Essential oil	61.28 ± 1.32 (54.189 – 69.342)
4.	AgNP extract	56.17 ± 1.27 (47.211 – 62.394)

Test extracts: significant from normal control, P < 0.05

Mean ± S.E.M = Mean values ± Standard error of means of five experiments

Table 6. Antibacterial activity of extracts of *Z. ovalifolium*

Organism	Extract	Concentration (µg/ml)						Streptomycin	
		20	40	60	80	100	MIC	DD	MIC
E. coli	E.O	2.1±0.12	3.4±0.13	5.1±0.14	7.4±0.28	9.6±0.18	169.4±3.24	17.2±0.56	22.3±0.28
	AgNP Ext	3.2±0.16	5.8±0.22[b]	8.6±0.18	9.9±0.21	11.3±0.28	154.2±2.67		
P. aeruginosa	E.O	3.3±0.15[a]	4.3±0.29	5.6±0.19[c]	8.9±0.32	11.7±0.29	193.7±4.06[f]	20.5±0.61	27.6±0.33
	AgNP Ext	4.5±0.19	6.2±0.31	8.8±0.27	10.5±0.26	13.4±0.34	171.3±3.73[f]		
S. aureus	E.O	5.4±0.24	6.9±0.33	8.2±0.26	11.2±0.37[d]	14.8±0.32	133.6±2.95	31.6±0.88	15.3±0.31
	AgNP Ext	7.3±0.[28a]	9.1±0.35[b]	12.3±0.23	15.7±0.33	18.1±0.31	121.8±3.11		
B. subtilis	E.O	6.6±0.23	8.4±0.31	12.2±0.31	14.5±0.34[d]	18.9±0.38[e]	124.4±2.79	39.9±1.02	11.8±0.39
	AgNP Ext	8.9±0.27	12.2±0.27	16.6±0.38[c]	18.7±0.41	22.5±0.42[e]	116.9±3.29		

Mean diameter of zone of inhibition in mm (mean ± S.E) (Excluding the diameter of the disc – 6 mm)
Numbers with the same letters in each column means differences are not significant (P > 0.05)

Table 7. Antifungal activity of extracts of *Z. ovalifolium*

Organism	Extract	Concentration (µg/ml)						Bifonazole	
		20	40	60	80	100	MIC	DD	MIC
A. niger	E.O	5.1±0.14	5.9±0.23	7.3±0.19	10.1±0.28	14.8±0.37	128.4±3.34[f]	37.2±1.43	12.8±0.24
	AgNP Ext	6.2±0.19	6.6±0.37[b]	8.7±0.19	12.2±0.32	16.2±0.39	119.7±3.18		
F. oxysporum	E.O	4.3±0.25	5.3±0.29	6.8±0.26[c]	8.1±0.31	11.9±0.23	143.1±3.09	30.9±1.66	17.7±0.38
	AgNP Ext	5.5±0.29[a]	6.2±0.33	7.4±0.31[c]	10.2±0.37[d]	13.6±0.41[e]	131.9±3.27		
R. solani	E.O	3.4±0.31[a]	4.5±0.31	5.2±0.21	7.3±0.39[d]	9.8±0.38	165.6±2.89	23.4±1.18	22.6±0.41
	AgNP Ext	4.8±0.22	5.6±0.35[b]	7.3±0.30	8.7±0.35	11.7±0.28	141.5±3.21[f]		
S. rolfsii	E.O	2.6±0.28	3.4±0.16	4.2±0.22	6.1±0.33	7.9±0.36[e]	198.2±4.06[f]	19.9±1.22	29.1±0.35
	AgNP Ext	3.9±0.17	4.3±0.25	5.4±0.25	8.1±0.37[d]	10.2±0.35	166.8±3.34		

Mean diameter of zone of inhibition in mm (mean ± S.E) (Excluding the diameter of the disc – 6 mm)
Numbers with the same letters in each column means differences are not significant (P > 0.05)

The antifungal activity of the extracts was also evaluated using the disc diffusion technique and the results were depicted in Table 7. As evident from the data, *A. niger* was inhibited effectively with a zone of inhibition of 16.2mm for AgNP extract of essential oil at 100µg/ml. Subsequently MICs were also determined and are presented. The AgNP extract used against *A. niger* showed strong antifungal potential with MIC value of 119.7100µg/ml. The MIC values for Bifonazole ranged from 12.8 to 29.1µg/ml for all the organisms tested.

The medicinal value and biological activity of various botanicals are usually due to their incredible phytochemical profiles and this composition is totally dependent on geographical and environmental factors. In this connection, Z. ovalifolium from from jayapura area, Karnataka displayed a different composition which could be helpful in investigating chemotype of the species. Phenols and Flavanoids present in plants play a pivotal role and are well known to contribute to the total antioxidant potential [20]. The phenolic compounds were shown to possess potential antioxidant activity as they can react very effectively with active oxygen radicals, scavenge them and inhibit lipid oxidation at an early stage [21-22]. The antimicrobial activity of silver nano particles have been reported in a number of reports of reports [23-26] where it was showed a very good inhibitory activity of the synthesized nano particles against gram +ve and gram –ve bacteria. In the present study, the AgNPs extract of essential oil of *Z. ovalifolium* was effective against all the tested bacterial and fungal strains and showed that gram-positive bacteria are more sensitive than gram-negative bacteria. These results are in contrast with those of earlier reports available in the literature [27-28]. The mild activity of gram-negative bacteria can be ascribed due to presence of an outer membrane with which these bacteria are coated [29]. The potential anti-microbial susceptibility of the organisms under investigation might be due to the varying membrane permeability of the AgNP extract [30].

4. CONCLUSION

The results obtained are remarkable showing maximum anti-oxidant and antimicrobial activities of the AgNP extracts of essential oil of *Z. ovalifolium* and need to be explored as a viable, better alternative source of natural antioxidant and antibacterial agent. Further work is underway to identify the active phyto-constituents in the extracts and oil which might help to develop promising lead products which could be useful for pharmaceutical and food industries.

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CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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