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Antibacterial and antioxidant studies of silver nanoparticles formulated from *Erythrina senegalensis* stem bark extract

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Abstract: *Erythrina senegalensis*, belongs to the family of Fabaceae and it has been used traditionally to treat microbial infections and diabetes mellitus. The aim of this study was to formulate silver nanoparticles (AgNPs) from the methanol stem bark extract of *Erythrina senegalensis* and to evaluate the antibacterial and antioxidant effects of the nanoparticles. The methanol extract was screened qualitatively for the presence of tannins, alkaloids, saponins and flavonoids using standard protocols. The silver nanoparticles were synthesized using green method and the particles were evaluated using scanning electron microscope (SEM) and X-Ray diffraction (XRD). The percent inhibition effects of the AgNPs and extract were analyzed using hydrogen peroxide and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays. The antibacterial effect of the methanol and the AgNPs were determined by disk diffusion method. The phytochemical screening revealed the presence of tannins and flavonoids. The DPPH assay revealed IC_{50} values of 3.60, 2.25 and 1.75mg/mL for the silver nanoparticles, methanol extract and ascorbic acid respectively. The findings from this study indicated that the extracts contain some secondary metabolites such as flavonoids which may be responsible for the bio-reduction of silver ion (Ag^+) to AgNPs as well as the antimicrobial and antioxidant activities.

Keywords: Antibacterial, antioxidant, *Erythrina senegalensis*, silver nanoparticles

INTRODUCTION

Over the years, there has been a rising concern about the pathogenesis of diseases caused by excessive free radicals and antibiotic resistance. There is a widespread anxiety concerning the diseases whose etiology have been associated to free radicals (Baliyan *et al*., 2022). Free radicals particularly the reactive oxygen species (ROS) have been found to be involved in the pathogenesis of cancer, diabetes and other neurodegenerative diseases (Lien *et al*., 2008; Oleh *et al*., 2018). In recent years, resistance of bacteria to antibiotics has become so rampant and this is an important clinical problem (Chawla *et al*., 2022). According to Dever and Dermody (1991), there are three major mechanisms through which antibiotic resistance occur: (i) changes in membrane permeability to antibiotics, (ii) enzymatic degradation of antibacterial drugs and (iii) alteration of bacterial proteins that are antimicrobial targets. Therefore, nanoparticles due to their nano-sizes have higher membrane permeability and can reduce resistance of bacteria to antibiotics. Nanoparticles especially those derived from silver have found numerous therapeutic applications and they have been reported to be useful in the management of various disease conditions caused by free radicals (Haajira *et al*., 2022). For example, paclitaxel nanoparticles that efficiently delivers the active ingredient to its site of action in the management of breast and ovarian cancers have been previously formulated. Also, ribonucleic acid (RNA) nanoparticles synthesized with folate and siRNA (small interfering RNA) have also been formulated for the management of nasopharyngeal cancer by bypassing the blood-brain barrier and delivering drugs to treat tumors in the brain (Airemwen and Halilu, 2022).

Erythrina senegalensis is a member of the Fabaceae family and has traditionally been used in humans as a muscle relaxant and in the treatment of gonorrhoea, dysentery and jaundice. The bark is used as an emmenagogue and is administered to women after childbirth. *Erythrina senegalensis* is also used to treat diabetes, renal problems, arterial hypertension, inflammation and pains (Rambo *et al*., 2019, Fofana *et al.,* 2022). The decoction of the stem bark has been used as febrifuge in the treatment of malaria and yellow fever. The extracts of the bark and roots are often used in the treatment of diabetes, gastrointestinal disorders and hemorrhoids (Nembo *et al.,* 2015, Fofana *et al.,* 2021). Previous studies have demonstrated that the extracts of this plant contain saponins, phenols, xanthoprotein, alkaloids, steroids, flavonoids, terpenoid, quinone and tannins. The plant extract has been shown to demonstrate antibacterial activity against *Streptococcus pyogenes*, *Staphylococcus aureus* and *Bacillus subtilis* (Bioltif *et al.,* 2020). The aim of this study was to formulate silver nanoparticles of *Erythrina senegalensis* and evaluate the antioxidant and antibacterial activity of the crude methanol extract and the silver nanoparticles.

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MATERIALS AND METHODS

Collection, identification and preparation of plant materials

Erythrina senegalensis was collected in December, 2021 along with its leaves, flowers and stem bark for identification purposes. It was identified by a botanist (Dr. Mshelia Halilu) and voucher specimen with number: CIU/PHAR/ FABA/001 was prepared and then deposited at the Herbarium of the Faculty of Pharmacy, Cyprus International University. The bark was air-dried for 14 days and then size reduced with wooden mortar and pestle. The powder was kept in a suitable packaging material until when needed.

Organoleptic and microscopic examination

The powder was evaluated for organoleptic characters (odour, taste, texture and colour) and microscopic characters according to the methods described by WHO (2011) and Mshelia *et al*., (2020)

Physicochemical evaluation

The water-soluble and alcohol soluble extractive values, acid insoluble ash, total ash and moisture contents were determined using the methods previously described by Halilu and Muhammad (2022).

Extraction of plant

The powder plant (10g) was extracted by maceration using 100 mL of methanol in a conical flask. It was stirred for 5 h and macerated for 24 h. It was filtered and the residue was rinsed twice with 50 mL each of methanol to ensure maximum extraction. The extract was concentrated using a rotary evaporator was used to concentrate the extract at reduced pressure. The semi-solid residue was allowed to dry at 50ºC for 24 h.

Phytochemical screening

The crude methanol extract was screened qualitatively for the presence of flavonoids (sodium hydroxide test), saponins (frothing test), phenolics (Ferric chloride test), tannins (lead acetate test) and alkaloids (Mayer's and Dragendorff's test) as described by Halilu *et al.,* (2023).

Thin layer chromatography (TLC) and qualitative antioxidant analysis using TLC

The method described by Fatiha *et al*., (2022) was adopted with slight modification. The plant extract was spotted on a TLC plate and then allowed to develop in two mobile phases (I) consisting of petroleum ether and ethyl acetate in the ratio of 8:2 respectively and (II) consisting of 7:2:1 petroleum: ethyl acetate; methanol respectively. The developed plates were dried and the compounds (spots) were then identified by viewing under ordinary light, followed by ultra-violet (UV) light at 254 nm and then finally sprayed with 10% H_2SO_4. The retention factor (R_f) values were calculated. To determine whether the plant extract had antioxidant activity, it was spotted on a TLC plate and then sprayed with DPPH solution. The appearance of a yellow spot on a purple background shows antioxidant activity due to free radicals.

Green synthesis of silver nanoparticles from the plant extract

The AgNPs were prepared by reacting 5 mM of $AgNO_3$ with the plant extract in ratio 9:1 and stirring continuously using a magnetic stirrer at 300 rpm for 16 h at 25°C. A change in colour of the solution from colourless to dark brown shows the bio-reduction of Ag^+ to AgNPs. The solution was then kept for 48 h in the dark at 25°C. The solution was centrifuged at 6000 rpm for 20 min and the supernatant was withdrawn with the aid of a Pasteur pipette. The wet nanoparticle was transferred to a clean watch glass and dried in an oven at 50ºC for 24 h to obtain dry powder particles (Hossain *et al*., 2023).

Characterization of silver nanoparticles

UV-Visible spectroscopy

The methanol extract and a 2mL of the solution containing the AgNPs were scanned separately between the range of 200-600 nm and the spectra were recorded.

Fourier transform infrared (FTIR) analysis

The nanoparticles were subjected to FTIR analysis and was scanned between 4000-1000 cm^{-1} (Haajira *et al*., 2022).

Scanning electron microscopy (SEM)

The SEM was conducted on the powder nanoparticles by the methodology previously reported by Omeche *et al.,* (2021).

X-Ray Diffraction (XRD) Analysis

The XRD analysis of the formulated silver nanoparticles was conducted using Rikagu generator (XRD Rikagu Rint 2000, Japan) at a voltage of 25 kV, 20 mA current intensity, 2Ɵ angle and $3°min^{-1}$ in the range of 4-50° (Jemal *et al*., 2017).

Evaluation of antioxidant activity of the extract using hydrogen peroxide and DPPH assay

The antioxidant activity of the methanol extract and the nanoparticle were investigated according to previous method used by Rakmai *et al*., (2018) and Fatiha *et al.,* (2022) respectively with some modifications.

Antibacterial studies

Antibacterial studies were done using the agar difference method. *Bacillus subtilis*, *Salmonella typhi*, *Escherichia coli* and *Staphylococcus aureus* plates were incubated at 37°C. Nanoparticles equivalent to 25mg, 50mg and 375 mg of the plant extract were soaked in 5mL of dissolution medium for 12h to give 5mg/mL, 10mg/mL and 75 mg/mL concentrations respectively. Released extracts were collected and put in the bacterial media. Gentamycin was used as the standard. It was then cultured at 37°C for

24h after which clear zones of inhibition were measured using a transparent rule (Lakshman *et al.,* 2021).

STATISTICAL ANALYSIS

The tests were conducted in triplicate. The results were recorded as mean ± SD. The data obtained were statistically analyzed using Paired t-test and GraphPad Instat 7.0 software (California, CA, USA). $P<0.05$ was considered significant.

RESULTS

Organoleptic and microscopic examination of powder sample

The organoleptic characterization of the powder showed a smooth texture, brown colouration, bitter taste and characteristic odour. The microscopic examination revealed the presence of some cell wall materials including calcium oxalate crystals, phloem parenchyma and cork cells (fig. 1). The chemo microscopy showed the presence of starch, cellulose, tannins and calcium carbonate.

Physicochemical evaluation of powdered stem bark

The physicochemical properties mainly establish the quality of the crude drug as shown in table 1. The amount of extractable active constituents was determined using organic solvents. The alcohol soluble extractive (21% w/w) was higher than the hydrophilic extractive (12.1%w/w). This indicates that alcohol was a better extracting solvent when compared with water. Halilu *et al*., (2020) reported that polar solvents tend to extract higher amount of phytochemicals than nonpolar solvents.

Extraction of plant and preliminary phytochemical screening

Some secondary metabolites such as saponins, tannins and flavonoids were discovered during the phytochemical screening of the extract (table 2). Findings from this research agrees with earlier reports of Bioltif *et al*., (2020).

Thin layer chromatography (TLC) and Qualitative TLC assay of antioxidant Activity by DPPH

Table 3 and fig. 2 show the separation profile of the extract in the solvent systems. The antioxidant compound in the plant extract was rapidly detected using qualitative TLC screening. After spraying with DPPH, a yellow spot appeared on the TLC plate against a purple background, indicating that the extract contains free radical scavenging compounds (fig. 2c). The R_f values from the TLC separation (table 3 and figs. 4a, b) indicated the presence of chemical group which are present in the extract.

Ultraviolet (UV) spectroscopy of solution of silver nanoparticles

Based on measurements of the electronic transition of σ-bonds, π-bonds and lone pair of electrons, UV analysis was used to detect the presence of chromophores and aromatic rings in the extract and nanoparticles. The spectrum showed maximum absorption at 400 nm.

Synthesis of silver nanoparticle

The sequence of colour change which occurred after the addition of silver nitrate to the plant extract is shown in fig. 3. There was a colour change from light yellow to dark brown which indicated the reduction of Ag^+ to silver nanoparticles.

FTIR result

The FTIR spectra of the methanol extract and the formulated silver nanoparticles are shown in figs and tables 4 and 5. The FTIR spectrum of the extract showed similar pattern with the silver nanoparticles. The most prominent peak being the OH group occurring between 3301 to 1414 cm^{-1} as seen in the spectra (Natarajan *et al*., 2009).

XRD Analysis

XRD result revealed diagnostic peaks of AgNPs at different intensities and degrees (fig. 6). The XRD showed three significant peaks between 20 to 50 degrees (Airewmen and Halilu, 2022).

SEM analysis

The SEM images in fig. 8 showed high density silver nanoparticles. The SEM analysis revealed face-centered crystalline and spherical structure. The result is similar to the findings of previous studies done by Alhajj *et al.*, 2023.

Antioxidant activity

DPPH scavenging activity against methanol extract and silver nanoparticles

The quantitative antioxidant assay results were expressed as IC_{50} and percentage inhibition (table 6 and fig. 8). The result revealed that ascorbic acid had higher antioxidant activity than the methanol extract and silver nanoparticles at all concentrations. On the basis of the percentage inhibition, the antioxidant effects demonstrated by all the samples were dependent on the concentration. From the graph of the percentage inhibition against the concentration, the IC_{50} of silver nanoparticles, methanol extract and ascorbic acid were 3.60 mg/mL, 2.25 mg/mL and 1.75 mg/mL respectively.

Hydrogen peroxide scavenging activity against extract and silver nanoparticles

The quantitative antioxidant assay results against hydrogen peroxide were expressed as IC_{50} and percentage inhibition (table 7 and fig. 9). The antioxidant effect of ascorbic acid was significantly higher than that of the methanol extract and silver nanoparticles and it was concentration dependent. The IC_{50} of silver nanoparticles, methanol extract and ascorbic acid were 3.15 mg/mL, 2.10 mg/mL and 1.65 mg/mL respectively.

Table 1: Physicochemical Evaluation

Parameter	% w/w (mean ± S.D)
Acid insoluble ash	01.0±0.02
Total ash	01.5±0.02
Moisture	07.2±0.02
Alcohol soluble extractive	21.0±0.03
Water soluble	12.1±0.02

Table 2: Phytochemical Evaluation

Secondary metabolite	Inference
Saponins	+
Tannins	+
Phenol	+
Flavonoids	+
Alkaloids	+

Key: + = Present

Table 3: Thin layer chromatography

Solvent system	Spot/R_f value
A. EtOAc: Petroleum ether	Five spots/ R_f = 0.68, 0.51, 0.37, 0.31, 0.2
B. EtOAc: Methanol: Petroleum ether	Six spots/ R_f = 0.75, 0.62, 0.5, 0.37, 0.32, 0.25

Table 4: FTIR analysis of *Erythrina senegalensis* methanol extract

Frequency (cm^{-1})	Functional group	Peak Appearance
3301	O H Alcohol	Weak
1414	CH_3 bend	Weak
1027	C-0-stretch	Strong

Table 5: FTIR analysis of solid silver nanoparticles

Frequency (cm^{-1})	Functional group	Peak Appearance
3348	OH alcohol	Strong
2912	-C-H Stretch	Weak
1582	NO_2 Stretch	Strong
1288	C-O-C Stretch	Strong

Table 6: Percentage inhibition of extract, silver nanoparticles and ascorbic acid against DPPH

Conc. (mg/mL)	Silver nanoparticles (%)	Extract (%)	Ascorbic (standard) (%)
0.625	16.15	21.45	33.27
1.25	27.25	36.26	54.20
2.5	46.28	55.18	70.13
5	65.58	73.25	82.26
10	78.29	82.56	89.68

Table 7: Percentage inhibition extract, silver nanoparticles and ascorbic acid against H_2O_2

Conc. (mg/ml)	Silver nanoparticles (%)	Extract (%)	Ascorbic (standard) (%)
0.625	21.26	26.54	38.58
1.25	31.29	41.28	58.49
2.5	50.84	59.87	74.26
5	65.98	78.43	87.86
10	80.73	88.47	94.17

Table 8: Results of antimicrobial activity of stem bark extract of *Erythrina senegalensis*

Organisms	*E. Senegalensis*	Nanoparticles	Gentamicin	$AgNO_3$	Distilled water
S. aureus	4.33±0.04	9.67±0.03	17.67±0.02	5.67±0.03	0
E. coli	6.33±0.02	9.00±0.01	24.50±0.01	5.33±0.03	0
B. subtilus	0.32±0.01	1.00±0.01	13.50±0.11	4.98±0.01	0
S. typhi	2.50±0.01	1.50±0.02	21.00±0.01	5.00±0.02	0

Results are expressed as Mean ±SD of three replicate readings.

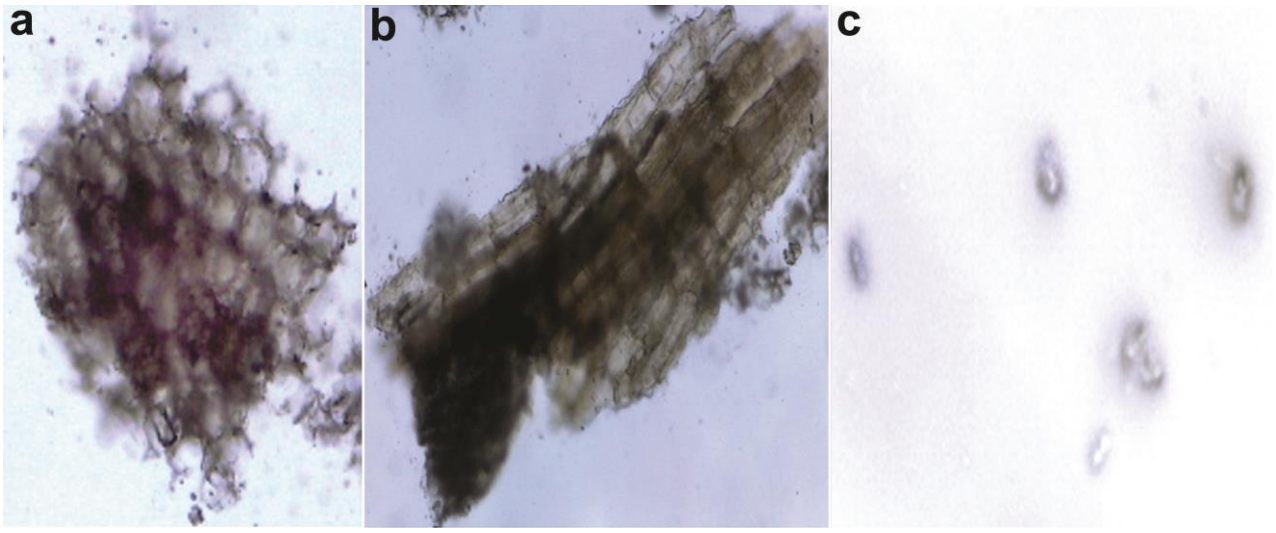

Fig. 1: (a) Cork cells (b) Phloem parenchyma (c) Prismatic crystals of calcium oxalate

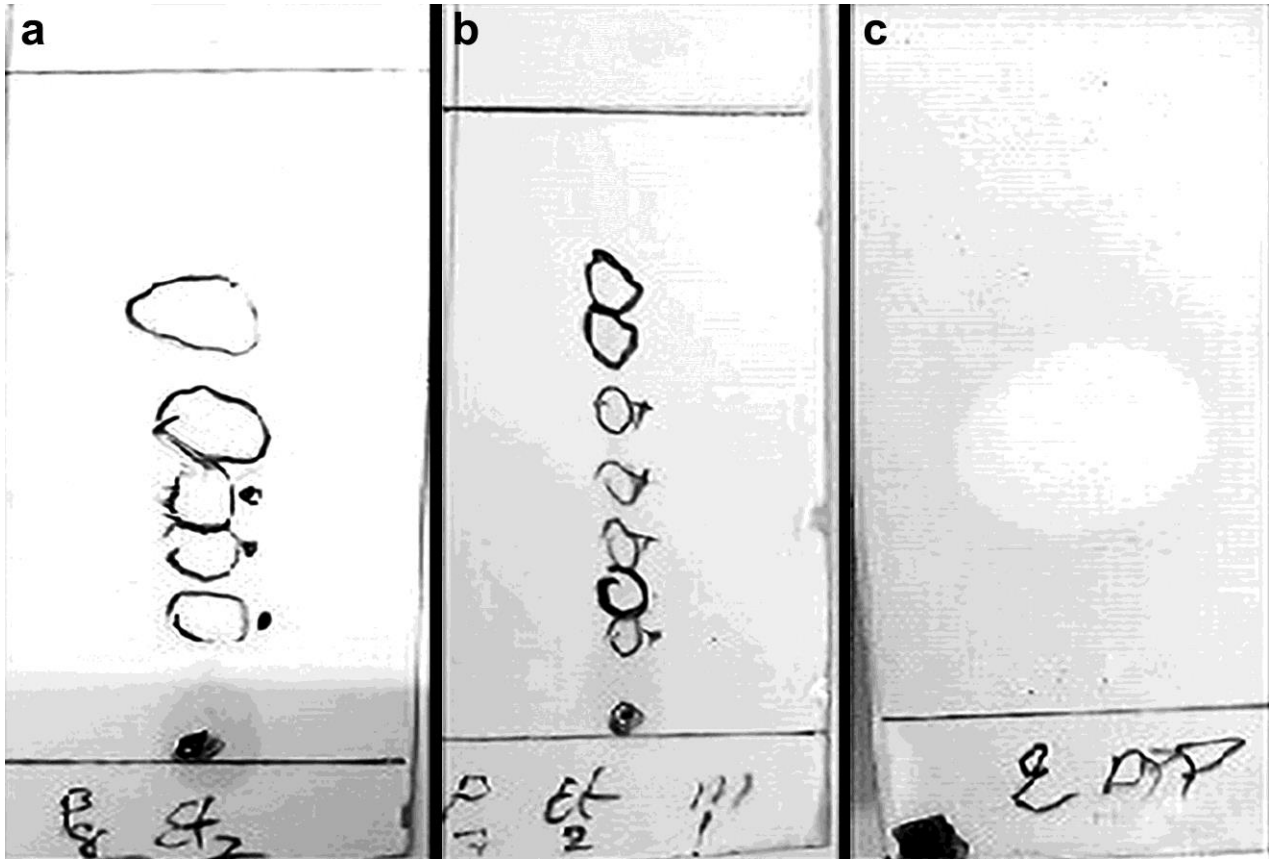

Fig. 2(a): Petroleum ether/ethyl acetate (8:2) (b): Petroleum ether/ethyl acetate/methanol (7:2:1) (c): Qualitative screening of antioxidant activity using DPPH

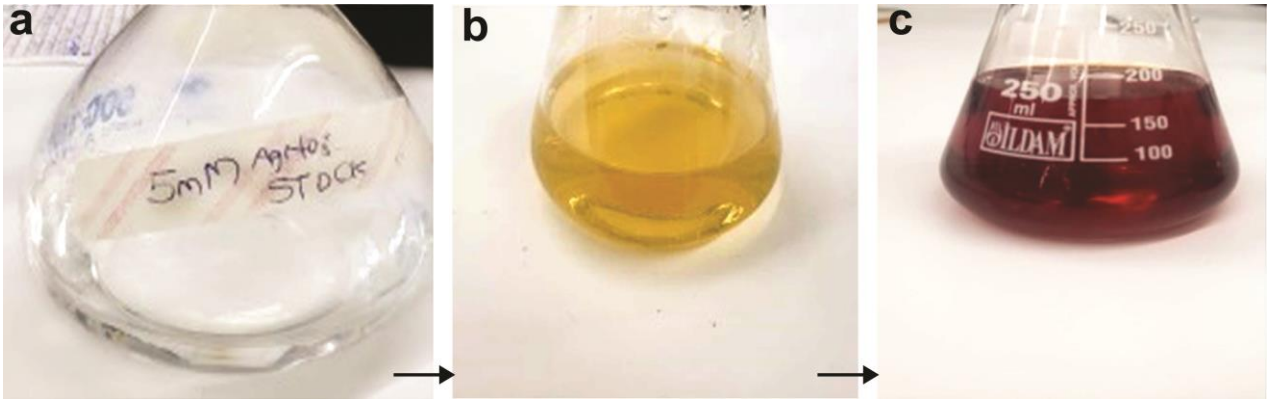

Fig. 3: Formulation of silver nanoparticles and sequence of colour change with time (a) 5mM $AgNO_3$ solution (b) Just after mixing $AgNO_3$ solution with the extract and (c) After 6 h.

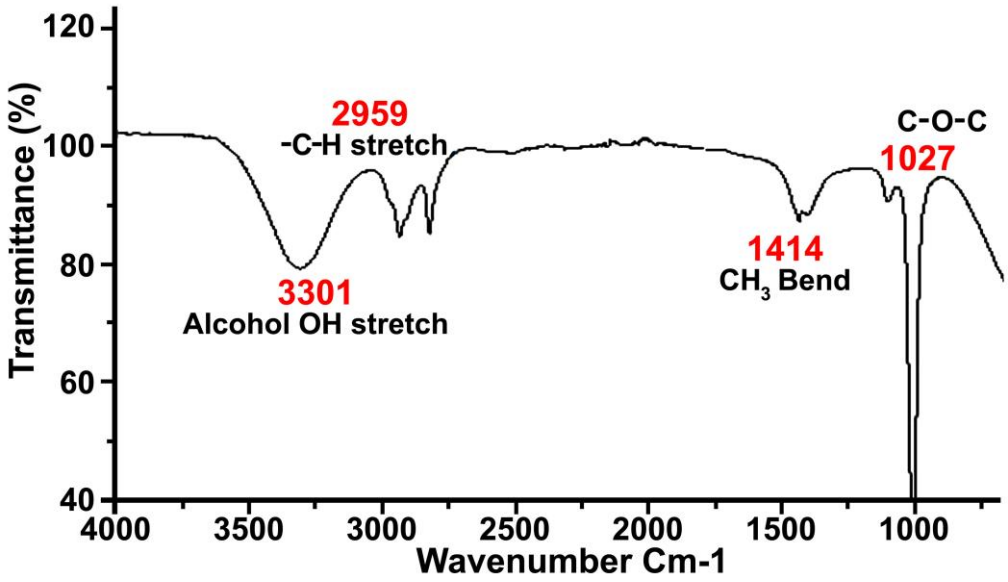

Fig. 4: FTIR spectrum of *Erythrina senegalensis* extract

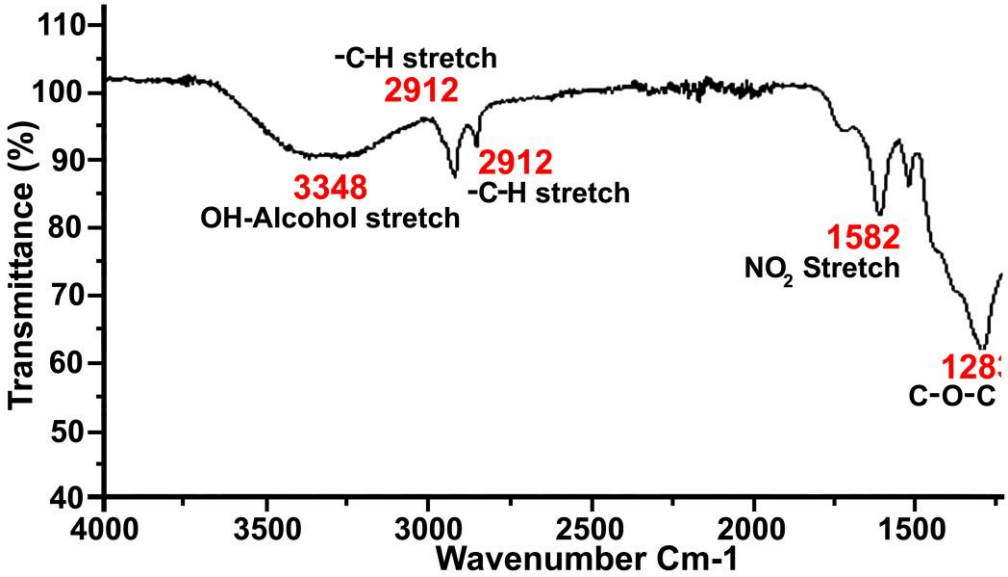

Fig. 5: FTIR spectrum of solid silver nanoparticles

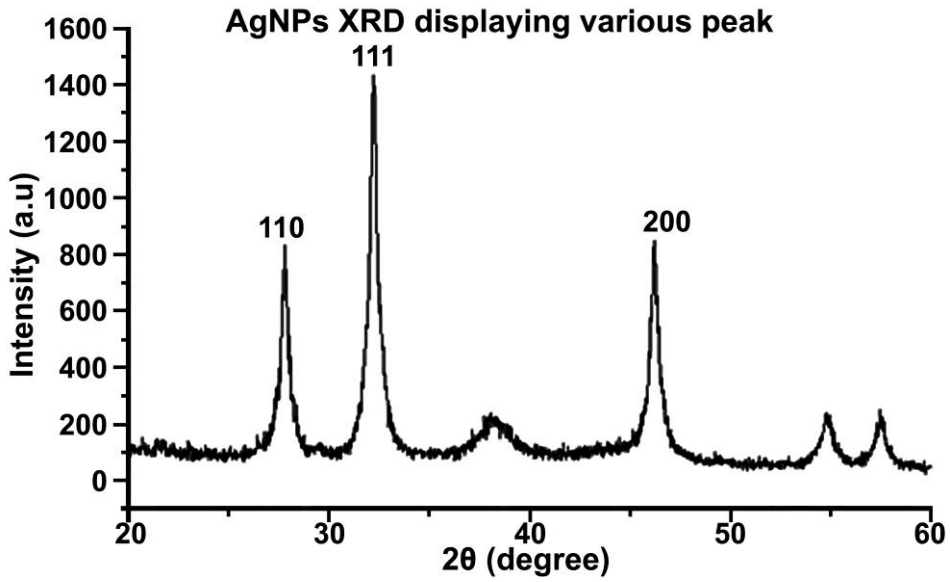

Fig. 6: XRD graph of the silver nanoparticles

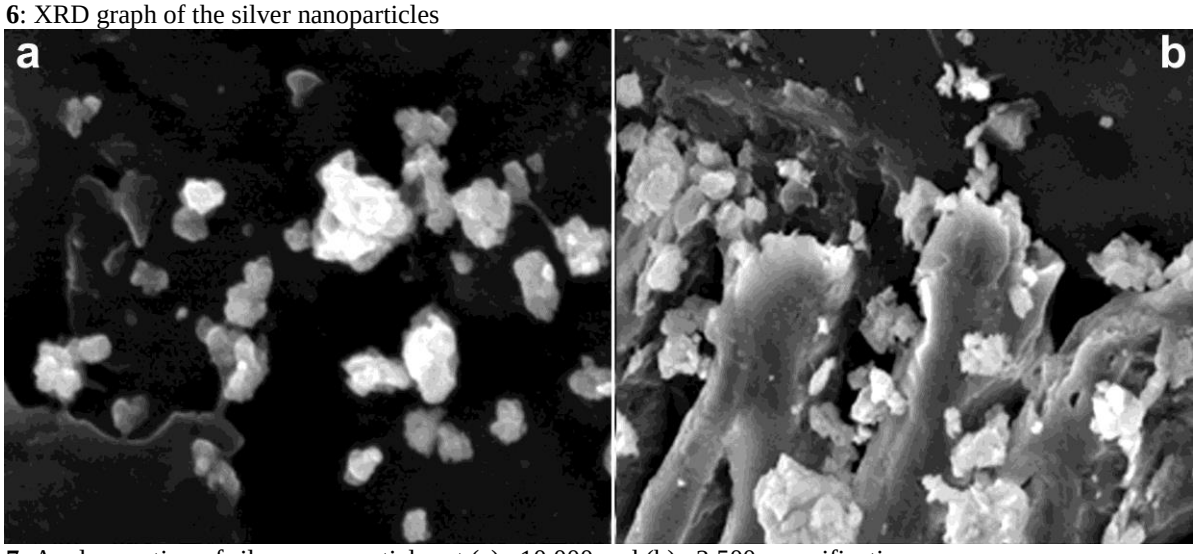

Fig. 7: Agglomeration of silver nanoparticles at (a) x10,000 and (b) x2,500 magnification

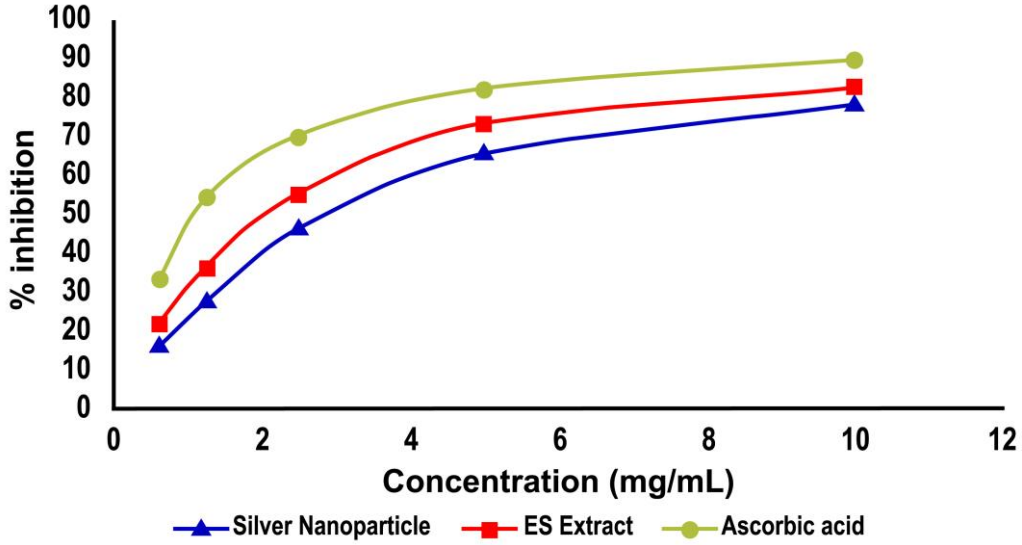

Fig. 8: Percentage inhibition of methanol extract, silver nanoparticle and ascorbic acid on DPPH

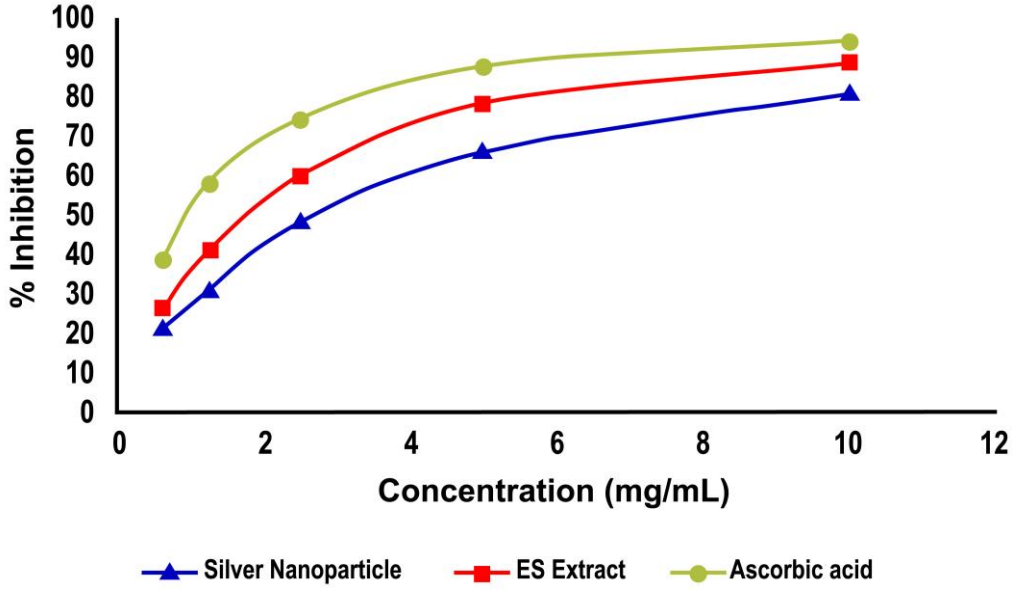

Fig. 9: Percentage inhibition of methanol extract, silver nanoparticles and ascorbic acid on H_2O_2.

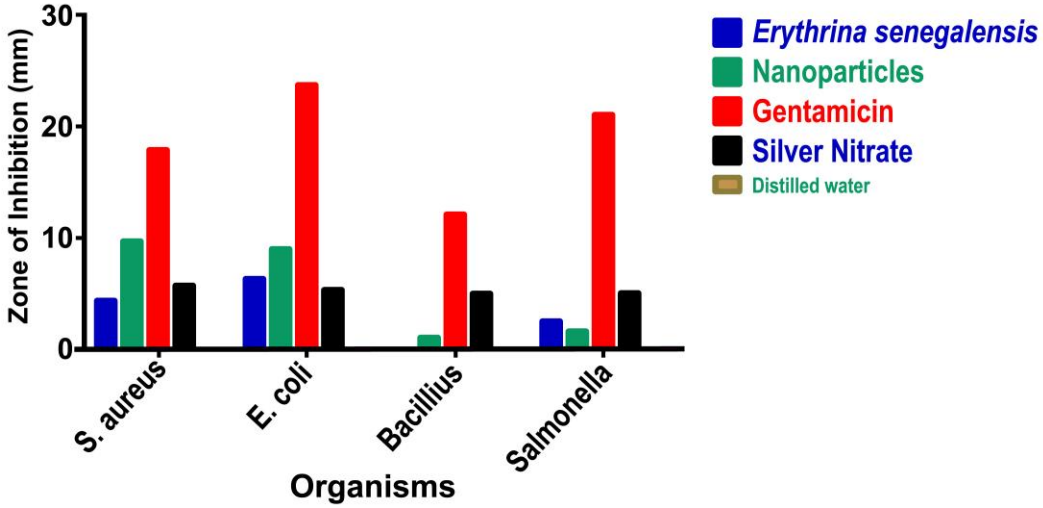

Fig. 10: Zone of Inhibition of samples against the test organisms

Antimicrobial activity of stem bark extract of Erythrina senegalensis

The *Erythrina Senegalensis* stem bark extract and nanoparticles showed comparable antimicrobial activity relative to the positive control (gentamicin) and the zone of inhibition is presented in table 8 and fig. 10. The results showed that nanoparticles demonstrated significant antibacterial activity against all test bacteria, the activity ranged between 1.00-9.67 mm with the highest activity demonstrated against *S. aureus*. The zone of inhibition of growth of all the bacteria used in the study ranged 1.82-6.33 mm for the methanolic extract and 1.00-9.67 mm for the formulated silver nanoparticles.

DISCUSSION

The organoleptic and microscopic evaluations of powder plant samples are preliminarily done with a view to establish some diagnostic characters for the identification of crude vegetable drugs as stated by the WHO (2011). These characters help to distinguish a species from other existing species with well-known features. The bitter taste, characteristic odour and brown colour of the powder bark which are sensory characters may be due to the presence of tannins, flavonoids and volatile substances. The microscopic studies help to authenticate, establish characters of crude plant drugs and are helpful in determining adulteration (Patel *et al*., 2018). The moisture content represents water of crystallization present in the plant sample and when in excess, it leads to the deterioration of the drug via bacterial, fungal growth and undesirable chemical reaction such as enzymatic hydrolysis of the active chemical compounds (WHO, 2011; Sabiha *et al*., 2012). The moisture content obtained in this study was similar to previous studies done by Osuntokun *et al*., (2016) who reported moisture content of 7.5% w/w.

The UV spectroscopy of the extract indicated absorptions in the ultraviolet region. These absorption bands indicted the presence of some chromophores present in the compounds extracted from the plant such as alkaloids, flavonoids, phenols and tannins (Lakshman *et al.,* 2021). On the other hand, the spectrum of the solution of silver nanoparticle showed maximum absorption at 480 nm and this data agrees with previous studies done by Suman *et al*., 2015.

The size, topology and shape of the formulated silver nanoparticles were determined using SEM and it revealed a poly-dispersed spherical shaped nanoparticle (Alhajj *et al*., 2023). XRD analysis revealed a polymorphic and crystalline structure of the AgNPs. The peaks of diffractions were seen at 28º, 32º and 47º relates to the facets 110, 111 and 200 respectively which revealed the crystallinity of the silver nanoparticles (Rajendra and Gregory, 2021). XRD elucidated the molecular structure, crystalline state as well as polymorphism of the formulated AgNPs.

The functional groups present in the methanol extract and the formulated AgNPs were analyzed using FTIR spectrometry. The peaks of the methanol extract were seen at 3301cm^{-1}, 2959cm^{-1}, 1414cm^{-1} and 1027cm^{-1} corresponding to -OH, -C-H, CH_3 and C-O functional groups respectively (table 4; fig. 4). The IR peaks of the formulated AgNPs were seen at 3348cm^{-1}, 2912cm^{-1}, 1582cm^{-1}, 1284cm^{-1} corresponding to -OH, -C-H, NO_2 and C-O functional groups respectively (table 5; fig. 5). The functional groups of the capping ligands that stabilized the AgNPs were studied using FTIR (Nafe *et al.,* 2014).

The percentage inhibitions and IC_{50} values were used to express the free radical scavenging effect of the AgNPs and methanol extract which were then compared with ascorbic acid. From the results, the percentage inhibition increased as the concentration increased. The lower the IC_{50}, the higher the activity. The antioxidant effect displayed by the methanol extract may be due to the presence of phenolic compounds which are more in the extract compared to the nanoparticles. The extract and the formulated nanoparticles had a good antioxidant effect and DPPH scavenging activity when compared with ascorbic acid. From the results of the study, the extract and nanoparticles were also able to scavenge DPPH in a concentration-dependent manner and ascorbic acid being the reference antioxidant had a better scavenging activity than the extract and the silver nanoparticles (Rakmai *et al.,* 2018).

Hydrogen peroxide (H_2O_2) is relatively unreactive however, it can be converted into the highly reactive and toxic hydroxyl radical (OH^-), which can react with nucleotides in the DNA and result in mutagenesis and carcinogenesis. Antioxidants are known to preserve cells from the adverse effects of reactive oxygen species (ROS), which induce stress. Excessive production of these ROS in the body can result in several chronic diseases such as cancer, Parkinson's disease and diabetes. The best way to reduce the amount of oxidative stress in the body is to scavenge these ROS. The ability of a compound to scavenge H_2O_2 is an indicator of its antioxidant activity. The extract and the formulated nanoparticles had a good antioxidant and H_2O_2 scavenging effect when compared with the ascorbic acid. From the results of the study, the extract and nanoparticles were also able to scavenge H_2O_2 in a concentration-dependent manner and ascorbic acid had a better scavenging activity (Rakmai *et al.,* 2018).

The extract and the silver nanoparticles demonstrated significant inhibitory effect against *E. coli* and *S. aureus* ($P<0.05$). However, the extract and the silver nanoparticles did not demonstrate significant antibacterial activity against *Bacillus subtilis* and *Salmonella typhi* ($P>0.05$; table 9). The findings from this study agrees with previous study conducted by Lakshman *et al.,* (2021) who reported that nanoparticles can be used to manage bacterial infections. The antibacterial activity may be attributed to flavonoids, tannins and other compounds which are present in the extract (Haajira *et al*., 2022).

CONCLUSION

Silver nanoparticles of *Erythrina senegalensis* were successfully synthesized in this study. The extract and the nanoparticles demonstrated significant antioxidant and antimicrobial effect against both *E. coli* and *S. aureus* ($P<0.05$). The formulated nanoparticles have potential pharmaceutical applications in the treatment of bacterial infections and diseases caused by free radicals after further studies.

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