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Biosynthesis of Silver and Copper Nanoparticles Using *Cadaba fruticosa* (l.) Druce and its Biological Applications

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Abstract

The current study has been designed to synthesis silver nanoparticles (SNPs) and Copper nanoparticles (CuNPs) using leaf aqueous extracts of *Cadaba fruticosa* an endangered medicinal plant as bioreducing agents. This method authorized the synthesis of SNPs, which was authenticated by Ultraviolet (UV)-visible Spectrophotometry, Fourier-transform infrared analysis, X-ray Diffraction (XRD) studies, Energy Dispersive X-ray, and Scanning Electron Microscopic (SEM) analysis. UV-visible spectra and visual observation exhibited the appearance of greenish brown and dark brown color indicates the synthesis of SNP and CuNP, after the treatment of Ag precursors. Further the, XRD report clearly shows that the synthesized SNPs were crystalline in nature. In addition, SEM analysis confirmed that $AgNO_3$ solution produced SNPs and CuO_2 solution produced CuNPs and their size was about 120–168 nm and 301–538 nm in SNPs and CuNPs, respectively. The leaf SNPs and CuNPs of *C. fruticosa* leaf aqueous extracts considerable antioxidant activities. The results antimicrobial activity exhibited that the bacterial and fungal growth was inhibited by both the samples in a dose-dependent manner.

Keywords: Antioxidant, *Cadaba fruticosa,* copper nanoparticle, silver nanoparticle, spectrophotometry, synthesis

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Introduction

Medicinal and the toxic plants have been recognized and used throughout the human history for pharmaceutical enhancement. In traditional systems of medicines, pharmaceutical intermediates, modern medicines, folk medicines, and chemical entities for synthetic drugs, medicinal plants are the richest bio resources.[1] While comparing synthetic antibiotics with plant based drugs, the plant based drugs are stated to have fewer side effects.[2]

There is a high demand of herbal medicines in both developed and developing countries because of its high source of primary healthcare, having wide biological and medicinal activities, lesser costs, and high safety margins. The struggle produced by the pathogens can be cured by herbal molecules as they exist in a joined form of more than one molecule in the protoplasm of the plant cell which is safe and even less cost.[3,4] Even with the initiation of modern or allopathic medicine, indigenous people derived and used number of important modern drugs from plants.[5]

Plant mediated nanoparticles are the most appropriate method as green, non-polluting, widely available, unharmed with many secondary metabolites. Here as the chemical[6] methods used are too expensive and also involved with the use of toxic and dangerous chemicals that are responsible for various biological risks. The nanoparticles of metal used for the various purposes such as anticancer agents in the cancer treatment, in the field of tissue engineering and also in drug delivery and even they precludes infection, in (burn and traumatic) wound dressings, diabetic ulcers, dental works, biodegradation, scaffold, coating of catheters, and medical devices.[7] To produce various metal nanoparticles, various techniques have been used such as ultraviolet (UV) irradiation, laser ablation, lithography, ultrasonic fields, aerosol technologies, and photochemical reduction.[8] The conservative chemical and physical routes for the synthesis of nanoparticle are harmful to the environment, toxic to human beings because perilous chemicals which we use as reducing and stabilizing agents have biological risks due to their general toxicity and are also economically expensive.[9] Nowadays, research is directed to silver and copper based compounds are the most important materials among many metals such as Gold and Platinum. The metallic copper plays a significant role in modern electronic circuits due to its excellent electrical conductivity and low cost nanoparticles.[10] Hence, copper will gain growing importance as is expected to be an important component in the future nano devices due to its excellent conductivity, biocompatibility, and Surface Enhanced Raman Scattering feature.[11] Metallic copper nanocrystals homogeneously dispersed in silica layers, which have involved great devotion recently and in comparison with bulk copper, are possibly suitable materials for using in pharmacological field and are good alternate for valuable metals such as gold and silver.[12]

Silver nanoparticles (SNP) are substantial product from the field of nanotechnology which expanded boundless interests because of their unique properties such as good conductivity, chemical stability, catalytic, antiviral, and antifungal activities in addition to anti-inflammatory activities which can be incorporated into electronic components and complex fibers.[7] SNP (AgNPs) are most effective against virus, bacteria, and other eukaryotic micro-organism at low concentrations and non-toxic to humans.[13] These AgNPs contribute remarkable applications

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in clinical, pharmaceutical, biological, and immunological fields. Moreover, several salts of silver and their byproducts are commercially manufactured as antimicrobial agents.[14]

In the last decade opportunities for discovering the bactericidal effect of metal nanoparticles are from the development of nanotechnology. These occurs a major threat to public health due to microbial contamination of water, with the occurrence of microorganisms resistant to multiple antimicrobial agents.[15] There is a growing interest in developing new bactericides based on inorganic materials to substitute the traditional organic agents, as organic agents have limited their applications due to their low heat resistance, high decomposability, and short life. Metal nanoparticles with bactericidal activity can be immobilized and coated on to surfaces, which may find application in various fields and their antimicrobial effect has been attributed to their small size and high surface area to volume ratio, which allows them to interrelate closely with microbial membranes.[16]

The Capparaceae or Capparidaceae, commonly known as the caper family contains about 700 species and 33 genera. The largest genera are *Capparis* (about 150 species), *Boscia* (37 species), Maerua (about 100 species), and *Cadaba* (30 species) (https://en.wikipedia.org/wiki/Capparaceae). *Cadaba* is a genus of shrubs in family Capparaceae, with about 30 species.[17] The Indian *Cadaba* or *Cadaba fruticosa* (L.) is a medicinally important, which belongs to Capparidaceae family commonly known as "Capper bush" in English and "vizhuthi" in Tamil. This species is endemic on Indian Subcontinent such as Pakistan, India, Sri Lanka, Bangladesh, and Indo-China (Myanmar). This plant is employed in Indian conventional curative systems. The leaf juice is internally used in the case of diarrhea, general weakness, energetic during dysentery, also to relieve general body pain, antidote against poisoning, and stimulant.[18,19] The roots and leaves are considered anthelmintic, deobstruent, and emmenagogue and are prescribed in the form of a decoction for treating uterine obstructions. The leaves of Indian *Cadaba* are also used as a poultice to promote curative of sores. It has been stated to possess hypoglycemic activity.[20] It is also used as an antiscorbutic, antidote antiallergic, and anti-helminthic herbal drug.[20,21] The leaf extracts also possess antimicrobial activity[22] and are used in traditional medicine to treat gonorrhea and syphilis,[23] antidiabetic activity,[20] and antipyretic activity.[24] In Siddha, *C. fruticosa* fruit and leaf are used to treat swellings, worm infestation, constipation, and eczema. The leaves are used to treat to treat leukoderma[22] and also leave to treat eczema.[20] Moreover, it was report to have the active constituents, that is, Capparisine and α – B –dihydroferulic acid,[25] cadabicine, and cadabicine diaceate.[26]

Biosynthesis of metal nanoparticles, using plant leaf extracts both as capping agent and reluctant is currently under exploitation. It is a cost effective, eco-friendly, and more efficient alternative method for the large scale synthesis of metal nanoparticles. However, only a very little work has been done for the synthesis of silver and copper nanoparticles (CuNPs) using plant leaf constituents (materials). In this study, successfully reported the biosynthesis of silver and CuNPs using *C. fruticosa* leaf aqueous extract and its characterization, antioxidant, and antimicrobial activities.

Materials and Methods

Plant Material and Preparation of Plant Extract

The plant specimens were collected from Government Arts College campus, Coimbatore, Tamil Nadu. The plant leaves of *C. fruticosa* were shade dried at room temperature and powdered 100 g of plant powder was extracted through cold maceration method using double distilled water and kept in a hot air oven for 24 h. The extracts was filtered through Whatman No.1 filter paper to remove all un dissolved matter including cellular materials and other constitutions that are insoluble in the extraction solvent and stored at 4°C used for further experiments.

Biosynthesis of SNP

Aqueous solution of 1 mM Silver nitrate ($AgNO_3$) was prepared and used for the synthesis of SNP. 10 ml of *C. fruticosa* aqueous leaf extract is mixed with 90 ml of $AgNO_3$ for the synthesis of SNP.

Biosynthesis of CuNPs

10 mL of *C. fruticosa* aqueous leaf extract was added to 100 mL of 0.01 M $CuSO_4.5H_2O$ aqueous solution and the mixture was kept at 560°C and 6 h with constant stirring on a magnetic stirrer. The suspension produced was centrifuged 10 min at 3000 rpm and the supernatant liquid was decanted off and the deposit was frequently washed with 10 mL of de-ionized water. The obtained precipitate was dried in an oven at 500°C for 24 h. The synthesized CuNPs were kept for further characterization by UV-Visible spectroscopy.

Characterization of Silver and CuNPs Synthesis

UV-Vis spectrum analysis

Through visual observation change in color of the reaction mixture was noted. Synthesized SNP and CNP was confirmed by sampling the aqueous component of 2 h after reaction and the absorption maxima was scanned by UV-Visible spectrophotometer at 200–800 nm wavelength on UV-Visible Spectrophotometer.

Fourier transform infrared (FT-IR) spectroscopic analysis

FTIR analysis of the aqueous extract of silver and CuNPs synthesized samples was carried out through the potassium bromide (KBr) pellet (FTIR grade) method was recorded using Jasco FT/IR-6300 FTIR spectrometer analyzed with JASCO IRT-7000 Intron Infrared Microscope using transmittance mode functioning at a resolution of 4 cm^{-1}.

X-ray diffraction (XRD) studies

XRD analysis was completed using instrument RICA KU ULTIMA. The crystalline structure of the bio synthesized silver and CuNPs was investigated through XRD technique using X-ray powder diffract meter. The silver and CuNPs dispersion was located on a glass slide and the solution (ethanol) was permitted to evaporate, to get a thin film of silver and CuNPs. This thin film was exposed to XRD operating between 10° and 80° and the scanning rate of 2°/min.

Scanning electron microscopic (SEM) analysis

SEM analysis of SNPs and CuNPs was done using instrument QUANTO 250 SEM. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount

of the sample on the grid, extra solution was removed and using mercury lamp the film on the SEM grid was dried.

Energy dispersive X-ray (EDX) analysis

EDX analysis was carried out for the detection and confirmation of elemental silver and copper. Very small amount of the sample was drop coated onto carbon film and examined for the arrangement of the synthesized nanoparticles.

Bioactive Studies of Silver and CuNPs

Antioxidant activity

DPPH radical scavenging method

The hydrogen donating capacity was evaluated using the stable DPPH method.[27] Briefly a solution of 0.1 mM DPPH was prepared by methanol. The samples 50250 µg/mL concentrations were mixed with 5.0 mL of DPPH solution. Reaction mixture was shaken and incubated at 27°C for 20 min and the absorbance was measured at 517 nm. Results were compared with the activity of ascorbic acid.

Superoxide radical scavenging activity

Superoxide radicals were produced by the modified method of Beauchamp and Fridovich (1971).[28] The assay was constructed on the volume of the sample to inhibit formation by scavenging superoxide radicals made by riboflavin-light-NBT in the system. Each 3 ml reaction mixture contained 20 mg riboflavin, 50 mM sodium phosphate buffer (pH 7.6), 12 mM EDTA, 0.1 mg NBT, and various concentrations (50–250 µg) of sample extracts. Proximately after illumination the absorbance was measured at 590 nm. The entire reaction assembly was surrounded in a box lined with aluminum foil. The reaction mixture served as blank. The percentage inhibition of superoxide anion generation was calculated.

ABTS antioxidant assay

Antioxidant activity was done using an improved ABTS method proposed by Siddhuraju and Manian, (2007).[29] The ABTS radical cation was produced by a reaction of 2.45 mM potassium persulfate and 7 mM ABTS and the mixture was incubated for 1216 h at room temperature in dark, the solution was diluted in ethanol and equilibrated to obtain an absorbance at 734 nm. 1.0 mL of diluted ABTS solution mixed with 10 µL/mL of sample. After 30 min of incubation absorbance was read at 734 nm.

Chelating ability for ferrous ions

The ferrous chelating possible of the extracts was assessed according to the method suggested by Yama Guchi *et al*., 2000.[30] The reaction was initiated with the sequential addition of 250 µg of sample extract, 1.0 mL of 0.2 M Tris–HCl buffer, bipyridyl solution, 0.25 mL of 1 mM FeSO4 solution, 1.0 mL of 2, 2′ 0.4 mL of 10% hydroxylamine hydrochloride, and 2.0 mL of ethanol. The final volume was made up to 5.0 mL with deionized water and the absorbance was determined at 522 nm. EDTA was used to benchmark the chelating abilities, ascorbic acid used as a control.

Antimicrobial activity

Antibacterial activity of *C. fruticosa* SNPs and CuNPs samples was analyzed on three bacterial organisms by well diffusion method. In three bacterial pathogens, two Gram-positive bacteria (*Klensella phemoniae* and *Staphylococcus aureus)* and one Gram-negative bacteria (*Escherichia coli*) were used for this study. Five different concentrations such as 25, 50, 75, and 100 µl were used for this study and which concentrations were prepared by two-fold dilution method by 10 µl in 1ml sterile double distilled water. Nutrient agar medium was used to organism inoculation and after bacterial culture inoculation it was incubated at 37°C at 24 h for bacterial growth.

Results

Synthesis of Silver and CuNPs

The *C. fruticosa* leaf aqueous extract was mixed with aqueous solution of 1 mM AgNO3 solution and 1 mM $CuSO_4$ solution led to the appearance of greenish brown and dark brown color indicates the synthesis of SNP and CuNP.

The complete synthesis of SNP and CuNP was signified by the color change after 24 h incubation at room temperature and there was no significant change afterward [Figure 1].

UV-Visible Spectrophotography

UV–Vis absorption spectrum of the reaction mixture was measured for 24 h at 2 h interval. The first evidence of nanoparticles formation was the color change in the reaction solution that occurred rapidly after mixing the extract with silver nitrate and copper sulfate. SNP show a strong capacity for the absorption of electromagnetic waves in visible light range. The maximum absorption of colloidal *C. fruticosa* leaf SNPs is usually in the range of 430 nm whereas *C. fruticosa* leaf CuNPs range of 510 nm [Figures 2 and 3].

FTIR Spectroscopy

The dual role of the plant extract as a reducing agent and capping agent as well as the presence of some functional groups was confirmed by FTIR analysis of silver and CuNPs. The frequency of the peaks, peak value, and compound names related to the peaks of *C. fruticosa* leaf SNPs is tabulated in Table 1 and depicted in Figure 4. The *C. fruticosa* leaf SNPs shows the six intense peaks at 3306.88, 2915.65, 1634.53, 1244.22, 1194.80, 1081.97, and 647.47 in the region of 4000 cm^{-1}–500 cm^{-1}. The corresponding functional groups are alkynes (3306.88), alkanes (2915.65), 1° amines (1634.53), aliphatic amines (1244.22), alkyl halides (1194.86), aliphatic amines

Table 1: FTIR analysis of *C. fruticosa* leaf aqueous silver nanoparticles

Peak value	*Frequency (cm–^{1})*	*Bond*	*Functional group name*
336.88	3330-3270(s)	-C≡C-H: C-H stretch	alkynes (terminal)
2915.65	3000-2850(m)	C-H stretch	Alkanes
1634.53	1650-1580(m)	N-H bend	1° amines
1244.22	1250-1020(m)	C-N stretch	Aliphatic amines
1194.80	1300-1150(m)	C-H wag (CH_2X)	alkyl halides
1081.97	1250-1020(m)	C-N stretch	Aliphatic amines
647.47	690-515(m)	C-Br stretch	alkyl halides

C. fruticosa: *Cadaba fruticosa*, FTIR: Fourier-transform infrared

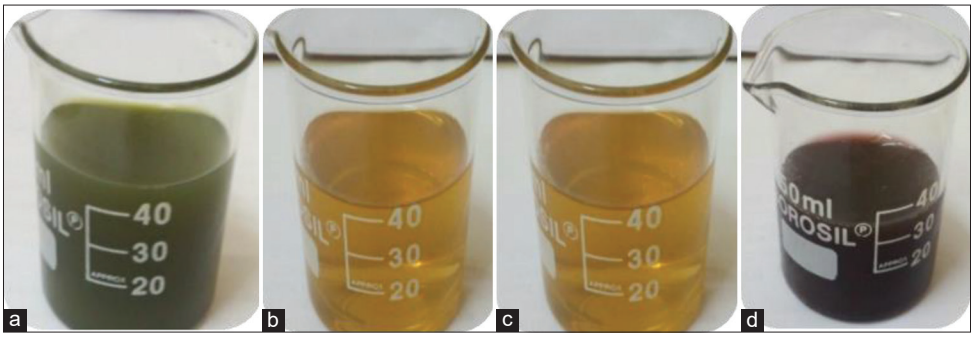

Figure 1: Silver and copper nanoparticle synthesis of *Cadaba fruticosa* leaf aqueous extracts. (a) Color of adding $AgNO_3$ with plant aqueous extract – Initial stage. (b) Color change of adding $AgNO_3$ with plant aqueous extract – After 24 h. (c) Color of adding $CuSO_4$ with plant aqueous extract – Initial stage, (d) color change of adding $CuSO_4$ with plant aqueous extract – After 24 h

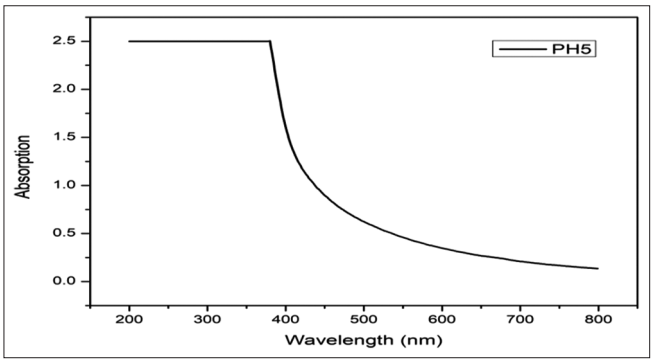

Figure 2: Ultraviolet-visible spectrum analysis of *Cadaba fruticosa* leaf aqueous silver nanoparticles

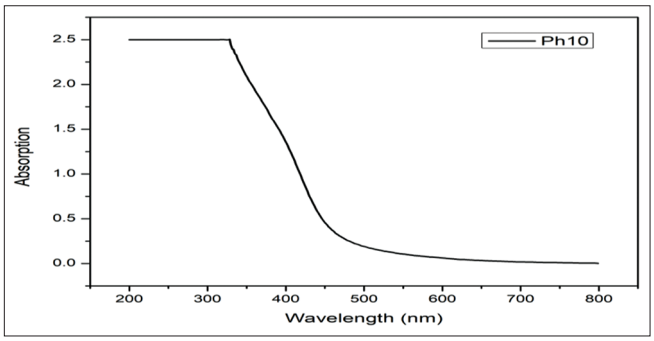

Figure 3: Ultraviolet-visible spectrum analysis of *Cadaba fruticosa* leaf aqueous copper nanoparticles

(1081.97), and alkyl halides (647.47), whereas *C. fruticosa* leaf CuNPs shows the five intense peaks at 3424.32, 2928.66, 1616.46, 1269.40, and 1089.99 in the region of 4000 cm^{-1}–500 cm^{-1}. The corresponding functional groups are alcohols, phenols (3424.32), Alkanes (2928.66), 1° amines (1616.46), alkyl halides (1269.40), and aliphatic amines (1089.99), [Table 2 and Figure 5].

EDX Analysis

The synthesized plant extracts SNPs and CuNPs were analyzed through EDX for the detection and confirmation of element silver and copper. Figures 6 and 7 shows the typical EDX pattern and spectra of silver and CuNPs obtained from *C. fruticosa* leaf aqueous extracts. EDX spectra reveal strong signals in the silver and copper region of 3ke and confirm the formation of nano silver and copper its elemental nature. Due to the excitation of surface plasmon resonance of silver and cooper nanoparticles this signal was formed.

SEM Analysis

The size and shape of the *C. fruticosa* leaf SNPs and CuNPs extracts were ascertained using SEM. The captured SEM image authenticates the presence of globular, elongate, and assymetrical nanoparticles. SEM micrographs showed that the *C. fruticosa* leaf SNPs were spherical in shape and polydispersed and *C. fruticosa* leaf CuNPs synthesized by the reduction of copper sulfide revealed spherical, hexagonal, and cubical NPs. The size of the silver and CuNPs was found to be in the range 120 nm to 168 nm and 301 to 538 nm, respectively [Figure 8].

Table 2: FTIR analysis of *C. fruticosa* leaf aqueous copper nanoparticles

Peak value	*Frequency (cm–1)*	*Bond*	*Functional group name*
3424.32	3500-3200(s,b)	O-H stretch H-bonded	alcohols, phenols
2928.66	3000-2850(m)	C-H stretch	Alkanes
1616.46	1650-1580(m)	N-H bond	1° amines
1269.40	1300-1150(m)	C-H wag (CH_2X)	alkyl halides
1089.99	1250-1020(m)	C-N stretch	Aliphatic amines

C. fruticosa: *Cadaba fruticosa*, FTIR: Fourier-transform infrared

XRD Studies

XRD powder diffraction is the method to analyze the crystalline phase, orientation, and the grain size of silver and CuNPs. The XRD spectrum of *C. fruiticosa* leaf SNPs and CuNPs shows the intense peaks in the whole spectrum of 2θ value ranging between 0 and 80. The three intense diffraction peaks at 23.8°, 33.8°, and 46.6° corresponding to three diffraction factors of silver shows in Figure 9, whereas in CuNPs extract XRD diffraction showed four diffraction peaks at 10.5°, 18.6°, 32.2°, and 46.26° corresponding to four diffraction factors of copper, respectively, [Figure 10].

Antioxidant Activity

DPPH assay

The stable free-radical scavenging activity by the DPPH method is an easy, rapid, and sensitive way to survey the antioxidant

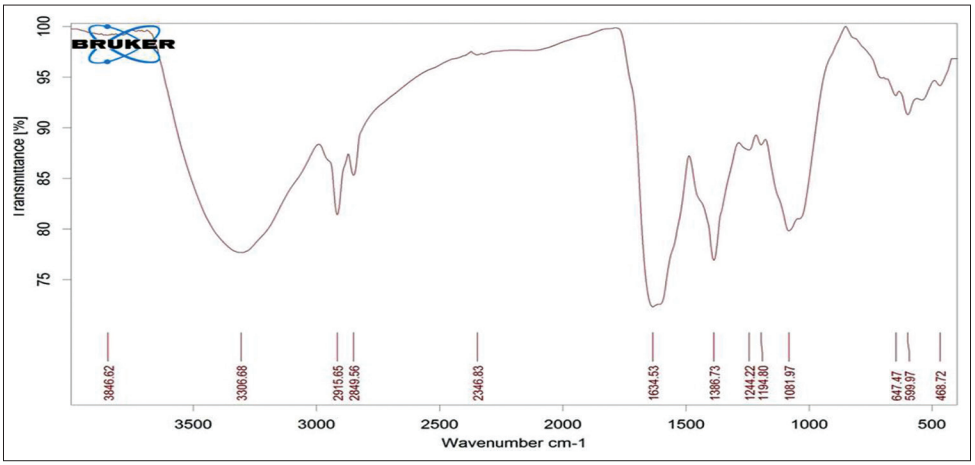

Figure 4: Spectrum obtained from Fourier-transform infrared analysis of *Cadaba fruticosa* silver nanoparticles

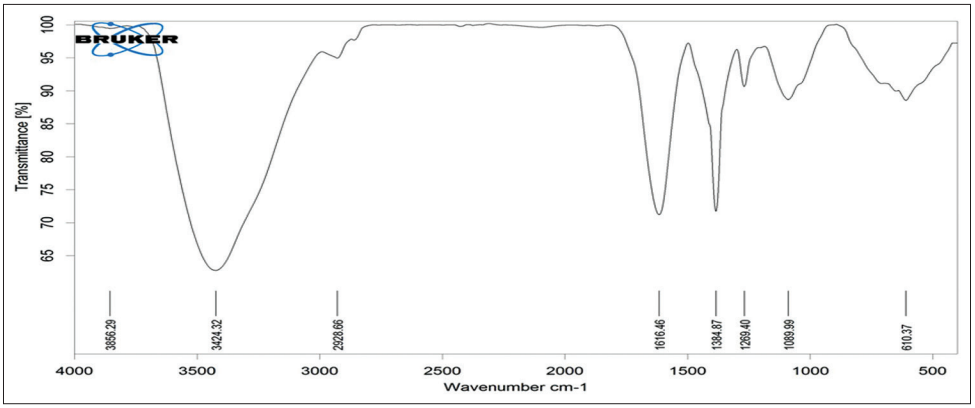

Figure 5: Spectrum obtained from Fourier-transform infrared analysis of *Cadaba fruticosa* copper nanoparticles

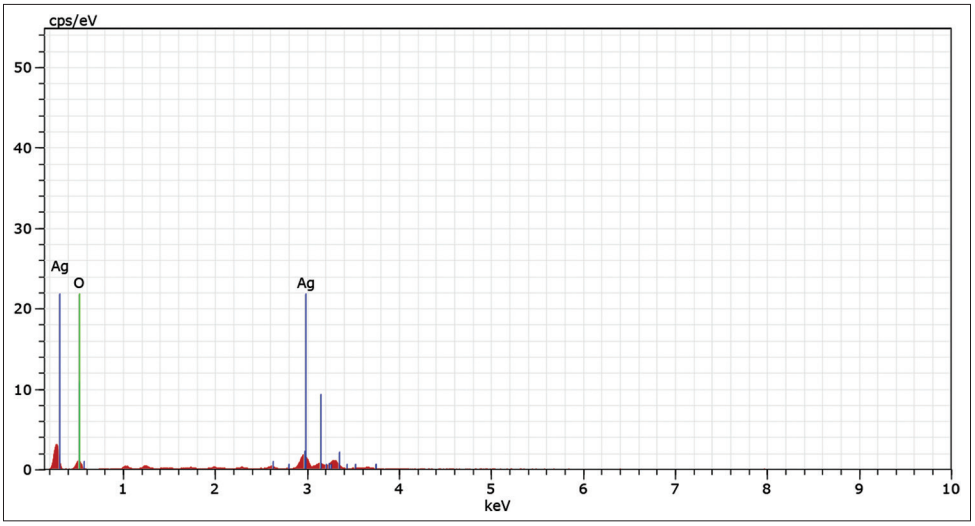

Figure 6: Energy dispersive X-ray spectrum of *Cadaba fruticosa* leaf silver nanoparticles

activity of a specific plant extracts. In this study, percentage inhibition of free radicals was carried out with *C. fruticosa* leaf SNPs and *C. fruticosa* leaf CuNPs extracts. *C. fruticosa* leaf SNPs with 250 µg/ml concentration gives higher percentage 75.32% and *C. fruticosa* leaf CuNPs with 250 µg/ml concentration gives higher percentage 67.08% of free-radical scavenging activity. The free-radical scavenging activity increases with increase in concentration [Table 3]. The percentage inhibition of control ascorbic acid was found to be 81.71% which showed higher activity than the both extracts. The low IC_{50} value observed in SNPs extracts at 85 ±

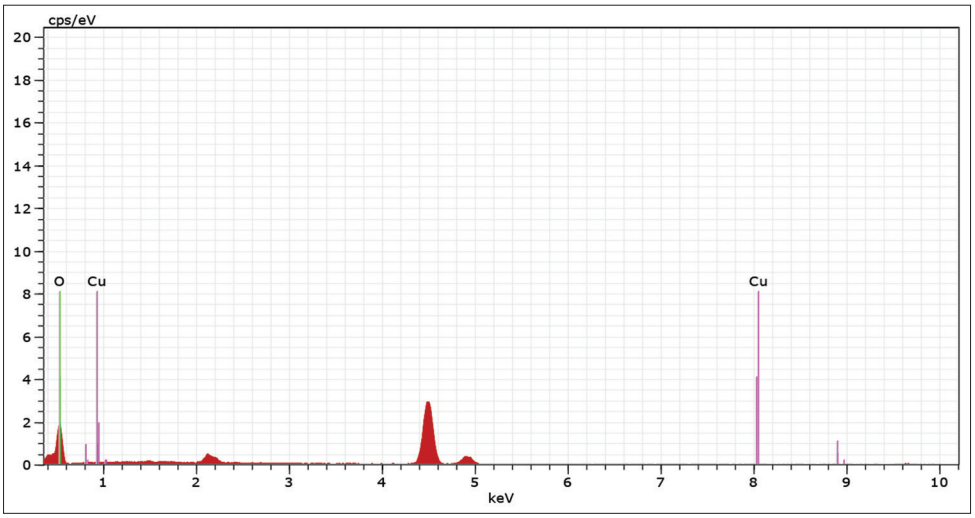

Figure 7: Energy dispersive X-ray spectrum of *Cadaba fruticosa* leaf copper nanoparticles

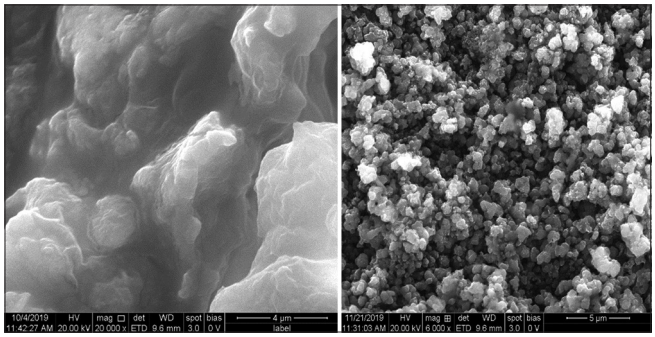

Figure 8: Scanning electron microscopic micrograph of *Cadaba fruticosa* leaf silver nanoparticles and copper nanoparticles

0.08 µg/ml concentrations [Table 4], whereas the CuNPs extract IC_{50} value was 185 ± 1.21 µg/ml against control (60 ± 1.2 µg/ml).

Superoxide scavenging activity

The scavenging ability of superoxide anion radical's percentage was examined at five different concentrations (50, 100, 150, 200, and 250 µg/ml) of *C. fruticosa* leaf SNPs and *C. fruticosa* leaf CuNPs extracts presented in Table 5. Highest scavenging ability of superoxide anions was observed in *C. fruticosa* leaf SNPs and *C. fruticosa* leaf CuNPs with 55.32% and 51.08% at 250 µg/ml concentration. The lowest IC_{50} value observed with *C. fruticosa* leaf CuNPs extracts showed at 190 ± 0.99 µg/ml concentrations [Table 4], whereas the IC_{50} value of SNPs was observed in 220 ± 0.17 µg/ml concentration.

ABTS assay

The decolorization of the sample through ABTS assay absorbance was measured at 734 nm. Among the nanoparticles SNPs had the highest scavenging activity with 58.32% and CuNPs 57.08% at 250 µg/ml concentration [Table 6]. Ascorbic acid was the strongest ABTS radical scavenging with 74.71 % and low IC_{50} value was observed in CuNPs extract at 190 ± 1.12 µg/ml concentrations against the standard ascorbic acid 50 ± 0.1 µg/ml concentrations [Table 4].

Table 3: DPPH free radical scavenging activity of *C. fruticosa* SNPs and CuNPs

Concentration µg/ml	*DPPH radical scavenging activity of C. fruticosa SNPs and CuNPs*		
	SNPs	*CuNPs*	*Ascorbic acid*
50	49.21±0.21	38.27±0.09	50.09±0.56
100	51.56±0.32	40.32±0.18	54.12±0.98
150	62.43±1.23	46.35±0.87	67.08±0.34
200	65.98±1.78	51.27±1.11	70.52±0.08
250	75.32±0.98	67.08±1.21	81.71±0.06

C. fruticosa: *Cadaba fruticosa*, CuNPs: Copper nanoparticles, SNPs: Silver nanoparticles

Table 4: IC_{50} value of antioxidant activity of *C. fruticosa* SNPs and CuNPs

Sample	*Antioxidant methods (IC_{50} value±SEM µg/ml)*			
	DPPH	*Super oxide*	*ABTS*	*Chelating ferros ions*
C. fruticosa leaf SNPs	85±0.08	220±1.09	225±0.17	160±1.09
C. fruticosa leaf CuNPs	185±1.21	190±0.99	190±1.12	210±1.12
Ascorbic acid	60±1.17	50± 0.1	85±0.02	130±0.18

C. fruticosa: *Cadaba fruticosa*, CuNPs: Copper nanoparticles, SNPs: Silver nanoparticles

Table 5: Superoxide scavenging activity of *C. fruticosa* SNPs and CuNPs

Concentration µg/ml	*Superoxide scavenging activity of C. fruticosa SNPs and CuNPs*		
	SNPs	*CuNPs*	*Ascorbic acid*
50	28.09±0.09	11.23±0.24	42.09±0.11
100	35.56±0.24	28.32±1.23	52.59±0.12
150	42.43±0.12	36.35±0.98	69.08±0.34
200	45.98±1.13	41.27±0.45	70.52±0.09
250	55.32±1.12	51.08±1.78	76.71±0.09

C. fruticosa: *Cadaba fruticosa*, CuNPs: Copper nanoparticles, SNPs: Silver nanoparticles

Chelating ability for ferrous ions activity

The metal chelating ability of both extracts was measured by the formation of ferrous ion- ferrozine complex. Iron binding capacity in terms of percentage of inhibition of the SNPs and CuNPs

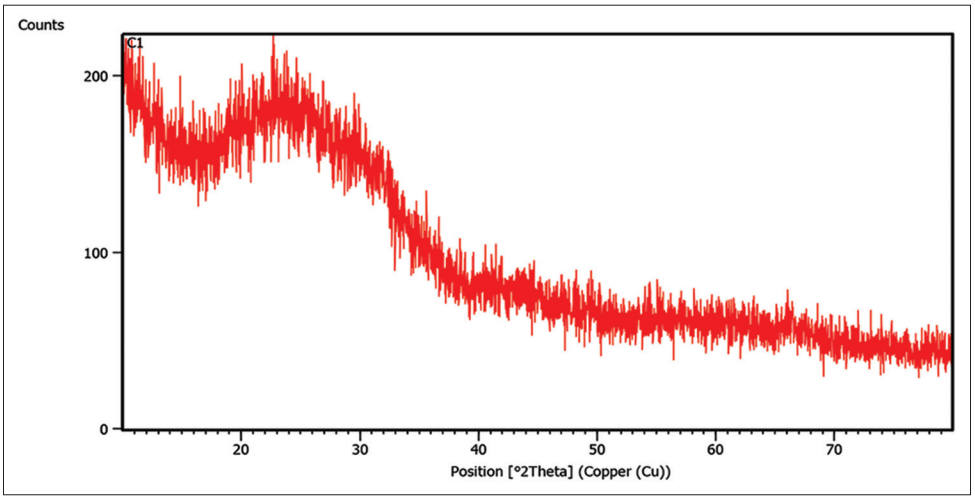

Figure 9: X-ray diffraction pattern of *Cadaba fruticosa* leaf silver nanoparticles

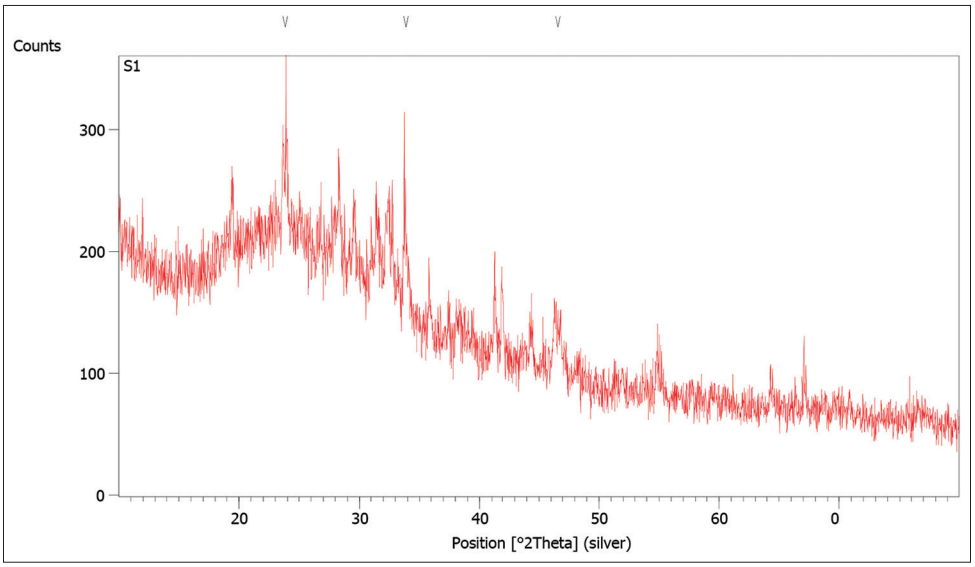

Figure 10: X-ray diffraction pattern of *Cadaba fruticosa* leaf copper nanoparticles

Table 6: ABTS activity of *C. fruticosa* SNPs and CuNPs

Concentration µg/ml	*ABTS activity of C. fruticosa SNPs and CuNPs*		
	SNPs	*CuNPs*	*Ascorbic acid*
50	32.21±0.31	20.27±1.82	42.09±0.11
100	35.56±0.98	30.32±0.61	54.12±1.32
150	38.43±1.48	46.35±1.26	57.08±1.32
200	45.98±1.42	51.27±1.14	65.52±1.34
250	58.32±0.31	57.08±1.65	74.71± 1.2

C. fruticosa: *Cadaba fruticosa*, CuNPs: Copper nanoparticles, SNPs: Silver nanoparticles

leaf extracts of *C. fruticosa*. The chelating ability of the both silver and CuNPs was analyzed at five different concentration 50, 100, 150, 200, and 250 µg/ml and ascorbic acid was taken as standard. The mean percentage inhibition value of SNPs and CuNPs of *C. fruticosa* was 22.21, 36.21, 48.43, 61.98, 75.32 and 20.27, 32.32, 39.35, 39.35, 49.27, and 59.08 percentages, respectively. The standard ascorbic acid values were 35.09, 41.12, 55.08, 68.52, and 81.71 percentages with increasing the concentration of nanoparticles. The IC_{50} values of SNPs and CuNPs were 160 ± 1.09 µg/ml and 210 ± 1.12 µg/ml against the control ascorbic acid 130 ± 0.18 µg/ml concentrations [Tables 4 and 7].

Antimicrobial Activity

Antimicrobial studies

Antimicrobial activity of *C. fruticosa* SNPs and CuNPs leaf aqueous extracts of different concentrations (25, 50, 75, and 100 µl) was assayed against two species of Gram-positive bacteria (*K. pneumoniae S. aureus*) and one Gram-negative bacteria (*E. coli*) by well diffusion method. Observation results of the microbial activity are presented in Tables 8 and 9 and Figures 11 and 12. The *C. fruticosa* SNPs showed best antibacterial activity at the concentration of 100 µl against *K. pneumoniae* (3.2 ± 0.15), *S. aureus* (2 ± 0.34), and *E. coli* (3.6 ± 0.45). The second best inhibition was observed at 75 µl concentration against *K. pneumoniae* (1.5 ± 0.33), *S. aureus* (1.6 ± 0.17), and *E. coli* (1.3 ± 0.07), respectively, whereas CuNPs of

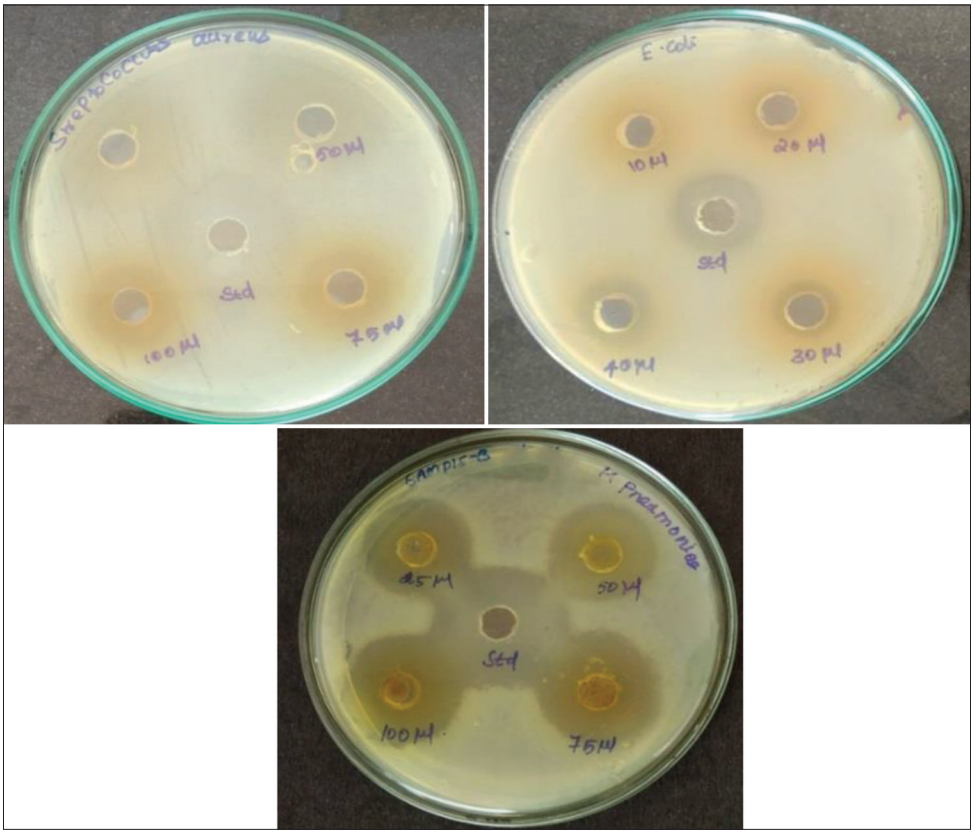

Figure 11: Antimicrobial inhibition zone of *Cadaba fruticosa* leaf aqueous extract silver nanoparticles

Table 7: Chelating ability for ferrous ions activity of *C. fruticosa* SNPs and CuNPs

Concentration µg/ml	*Chelating ability for ferrous ions activity of C. fruticosa SNPs and CuNPs*		
	SNPs	*CuNPs*	*Ascorbic acid*
50	22.21±1.25	20.27±0.87	35.09±2.10
100	36.56±1.09	32.32±1.65	41.12±1.23
150	48.43±0.99	39.35±0.19	55.08±1.72
200	61.98±1.01	49.27±0.87	68.52±0.21
250	75.32±1.98	59.08±1.76	81.71±0.09

C. fruticosa: Cadaba fruticosa, CuNPs: Copper nanoparticles, SNPs: Silver nanoparticles

Table 8: Antimicrobial activity of C. *fruticosa* leaf aqueous extract SNPs

Bacteria name	*Zone if inhibition(mm)*				
	25 µl	*50 µl*	*75 µl*	*100 µl*	*Standard*
Klebsiella pneumoniae	0.8±0.65	0.9±0.11	1.5±0.33	3.2±0.15	2.8±0.11
Streptococcus aureus	0.8±0.23	1.2±0.93	1.6±0.17	2.0±0.34	1.6±0.15
Escherichia coli	0.8±0.32	1.0±0.89	1.3±0.07	3.6±0.45	1.5±0.10

C. fruticosa: *Cadaba fruticosa*, SNPs: Silver nanoparticles

C. fruticosa leaf aqueous extracts showed best antibacterial activity at the concentration of 100 µl against *K. pneumoniae* (2.6 ± 0.22), *S. aureus* (1.2 ± 0.32), and *E. coli* (0.8 ± 0.13). The second best inhibition was observed at 75 µl concentration against *K. pneumoniae* (1.3 ± 0.11), *S. aureus* (1.0 ± 0.12), and *E. coli* (0.6 ± 0.78), respectively.

Table 9: Antimicrobial activity of C. *fruticosa* leaf aqueous extract CuNPs

Bacteria Name	*Zone if inhibition(mm)*				
	25 µl	*50 µl*	*75 µl*	*100 µl*	*Standard*
Klebsiella pneumoniae	0.8±0.12	1.0±0.21	1.3±0.11	2.6±0.22	1.5±0.01
Streptococcus aureus	0.2±0.34	0.8±0.99	1.0 ±0.12	1.2±0.32	1.6±0.09
Escherichia coli	0.2 ±0.67	0.4 ±0.45	0.6±0.78	0.8±0.13	1.0±0.21

C. fruticosa: *Cadaba fruticosa*, CuNPs: Copper nanoparticles

Discussion

The phytochemicals includes lipids, proteins, polyphenols, carboxylic acids, Saponins, polysaccharides, and enzymes present in plants can serve as metal reducing agents and as capping agents to provide strong and healthy coating on the metal nanoparticles leads to the change in color.[31] *C. fruticosa* plant extract was mixed with $AgNO_3$ and $CuSO_4$, the color of the solution was changed from greenish brown to dark brown and light yellow to dark brown due to the reduction of Ag^+ ions into SNP and reduction of Cu+ ions into CuNPs.

The changes in solution color confirmed the synthesis of SNPs. This indicates that the leaves of *C. fruiticosa* plants have ability to synthesis of SNPs and CuNPs successfully. The SNP and CuNP formation was fast for leaf extract and keep stable in its color for more time at room temperature. The silver and CuNPs formation was portrayed by change in the color from greenish brown to dark brown color and light yellowish to dark brown through

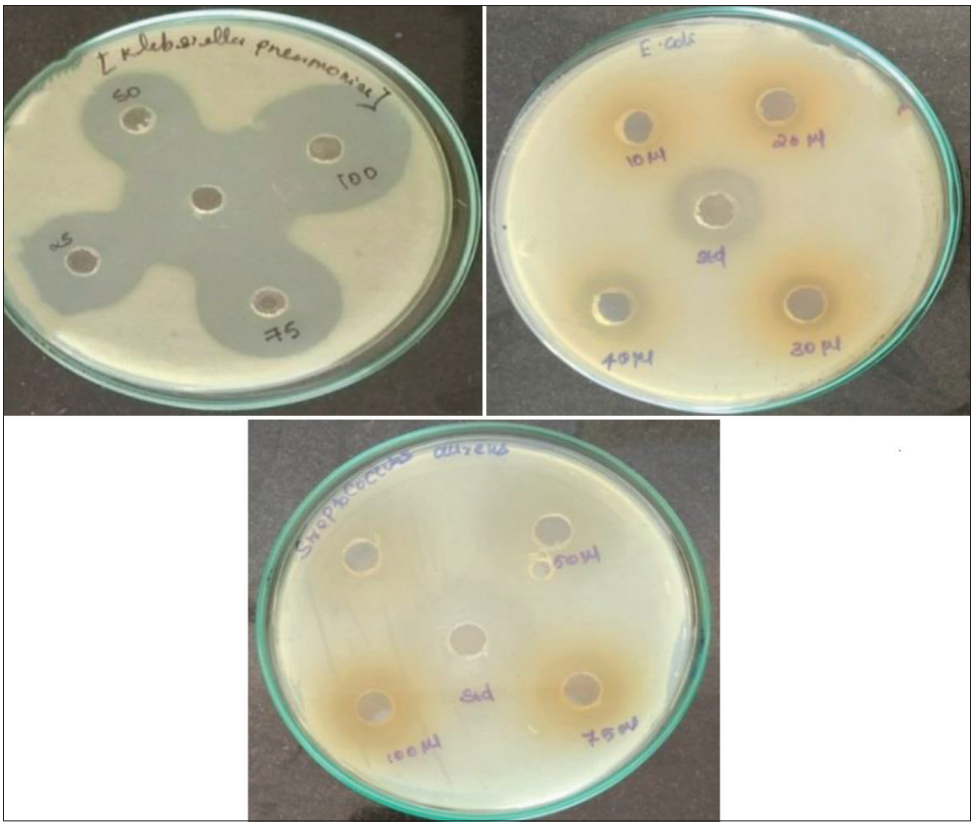

Figure 12: Antimicrobial inhibition zone of *Cadaba fruticosa* leaf aqueous extract of copper nanoparticles

visual observation and its indicates the reduction of copper and silver ion into their corresponding nanoparticles.[32,33] The present results are in consonance with many findings.[34,35] The appearance of yellowish brown color during this process could be due to the excitation of surface plasma vibrations in SNPs. Initial confirmation of Silver and CuNPs synthesis was visual observation by color change. Moreover surface plasmon resonance phenomenon impart a suitable indication to point out the formation of copper and SNP by change in color in the reaction mixture.[32]

In the present study, FTIR spectrum was investigated to determine the possible functional groups in biomolecules behind for capping and effective stabilization of SNPs and CuNPs synthesized by *C. fruticosa* leaf aqueous extracts. Different peaks deployed at 3306.88, 2915.65, 1634.53, 1244.22, 1194.80, 1081.97, and 647.47 cm^{-1} for SNPs and 3424.32, 2928.66, 1616.46, 1269.40, and 1089.99 cm^{-1} for CuNPs in FTIR spectrum of *C. fruticosa* leaf extract. The peaks at 3424.32 cm^{-1} are due to O-H stretching vibrations.[36] 2915.65 and 2928.66 bands emerged due to uneven C-H stretching vibrations of –CH2 and CH3. The peak 1634.53 and 1616.46 are a unique feature of N-H bending vibrations in amide of protein as capping agent.[37] The peaks noticed at 1081.97 and 1089.99 can be designated to C-N stretching vibrations aliphatic amine.[38] Our results implied that the biomolecules possibly carry out the formation of synthesis and stabilization of SNPs and CuNPs.[39] The results showed that the hydroxyl group present in the biomolecules has strong ability to interact with nanoparticles. Therefore, it may be understood that the bioreduction capping and stabilizing of nanoparticles occurred due to the presence of proteins, polysaccharides, amide, and long chain fatty acids in biomolecules.

Formation of silver and CuNPs and its morphological dimensions where elucidated with the help of SEM. The spherical, ellipsoidal, and some irregular shapes of silver nanoparticles and spherical hexagonal and cubical of CuNPs were observed from SEM images. The size of the silver and CuNPs was found to be in the range 120–168 nm and 301–538 nm, respectively. The SEM results were confirmed by earlier reports.[40]

The complete inorganic composition of the synthesized silver and CuNPs is analyzed through EDX analysis. EDX study confirmed the presence of silver and copper. In the present study of the EDX give a clear indication and intense signal of silver atom in the region of 3keV and cond confirmed the formation of nano silver, which in the region for absorption of SNP.[41] Similar findings were also obtained in SNPs of *Tephrosia villosa,*[40] whereas the EDX analysis of CuNPs possesses metallic copper with some impurities such as K, Na, Mg, O, and Ca. Other than CuNPs these compounds the complex of plant extracts.

The synthesized silver and CuNPs from *C. fruticosa* leaf aqueous extract were conformed through XRD analysis. The SNPs have three broad diffraction peaks observed 2ɵ range at 0–80 is 23.8, 33.8, and 46.6 ° can be indexed to the (100) (111) and (200) orientation, respectively. This is the confirmation that the synthesized SNPs are tranquil of pure crystalline silver. In addition, some unusual peaks were also seen in the diffract gram. Crystallization of the biogenic phases take place on the crystallization of the SNP may be the reason for this.[39,42] The present results are in accordance with many earlier finding.[40,43,44]

Furthermore, the synthesized CuNPs showed the major intense peaks at 10.5, 18.6, 32.2, and 46.26 ° diffracted from (100), (120), (111), and (200) places, respectively. Apart from this, some

other peaks were also observed at 2θ and point out the presence of oxide shell around the nanoparticles. This is agreement with the earlier findings.[45,46]

Antioxidants are compounds which inhibit or arrest the oxidation and in general extent the life of the oxidizable matter. Oxidative stress is always linked with majority of the diseases or disorders due to free radicals. Free radicals are species with high reactively, very short half-life and may cause damage to macro molecules such as proteins, nucleic acids, lipid, and enzymes. In addition, the antioxidants may suppress cancer cells by inhibiting oncogenes expression or by affecting cyclo oxygenease-2-enzyme.[47] Secondary metabolites produce by the medicinal plants act as small molecular weight antioxidants. Based on the structure and environment they have variable mechanism of action.

The leaf SNPs and CuNPs of *C. fruticosa* leaf aqueous extracts considerably scavenged the DPPH radicals. The DPPH method is a valid, simple, rapid, accurate, less expensive, and widely used to measure the potential of the rational compounds such as extract of plant to act as free radicals scavengers or hydrogen donors.[48,49]

Antioxidants scavenge the DPPH by donating of protein and reduced the DPPH provided a significant decrease in the DPPH radical concentration due to the scavenging ability of the extract. In the present study, the scavenging activity of leaf aqueous SNPs and CuNPs of *C. fruticosa* was increased based on the increase in the concentration of the extract. Similar findings were also reported SNPs of *T. villiosa* and CuNPs of *Eclipta prostrata*.[40,50] The IC_{50} value of SNPs of *C. fruticosa* was less where compared to the IC_{50} value of CuNPs. Similar results were also observed in *Iresine herbstii* where the SNPs exhibited more inhibition with more scavengers activity of DPPH. The ABTS decolorization occurs due to the reduction of the radical action measured as percentage inhibition of absorbance at 734 nm. Through the incubating ABTS chromo phone through the reaction ABTS was generated.[51]

The presence of specific bioactive compounds in the aqueous extract SNPs and CuNPs of *C. fruticosa* may be inhibit the potassium persulfate activity and leads to reduce the producing of ABTS. The relative antioxidant ability to scavenge the radical $ABTS^{+}$ has been compared with standard ascorbic acid. In this study, ABTS scavenging activity of leaf aqueous extract SNPs and CuNPs *C. fruticosa* was in dose-dependent manner. The highest percentage inhibition was observed at 250 ug/ml concentration in both the nanoparticles. The IC_{50} value of CuNPs of *C. fruticosa* was lesser when compared to IC_{50} value of CuNPs. This is in accordance with the findings of Nilima and Hande, (2011).[52]

Both silver and CuNPs synthesized from *C. fruticosa* leaf aqueous extracts exhibited good scavenging ability against super oxide anions. The scavenging ability of superoxide anions was found to increase in a dose-dependent manner. However, the AgNPs showed more inhibition with more scavenging ability when compared to CuNPs leaf extract. This is agreement with the SNPs obtained from *Desmostachya bipinnata* extract.[53] Furthermore, IC_{50} value of CuNPs showed better superoxide scavenging capacity (190 ± 0.09) than SNP (220 ± 1.09) obtained from *C. fruticosa* leaf extracts. Iron is required for oxygen transport, respiration, and for activating many enzymes. Hence, it is very essential for life never the less, it is an reactive metal and oxidative damages occur in protein, lipids, and other cellular parts could be catalyzed by iron.[54]

Silver and CuNPs obtained from leaf extract of *C. fruticosa* metal cheating ability were as certain by the formation of ferrous ion-ferozine complex. Ferrozine interacted with ferrous ions forming a red colored complex.[30] Duh *et al*., 1999,[55] reported that the chelating agents which forms bond with a metal are potent as secondary antioxidants because they reduce the redox potential there by stabilizing the oxidized form of the metal ion. In the present study, the chelating ability of SNPs and CuNPs of *C. fruticosa* leaf extract occurred in a concentration dependent manner. This study is in accordance with the earlier studies on *Hypochaeris radicata*[56] and *D. bipinnata*.[53] Further, the IC_{50} values of leaf extract SNP of *C. fruticosa* are lower (160 ± 1.09) than CuNPs (210 ± 1.12 ug/ml). However, both the samples are higher IC_{50} values than standard ascorbic acid (130 ± 0.18).

Nowadays, many of the researchers have been separated active principles from medicinal plants and these are playing a significant role in covering health needs. These medicinal plants may be used to produce a new drug for microorganism.[57] The SNPs and CuNPs synthesized from plant extracts and compounds present in the nanoparticles are of new interest as antimicrobial and antiseptic agents. Phytochemicals present in the plant extract along with metal particles play a main roll within medicinal properties of nanoparticles activity. Antimicrobial activity of synthesized SNPs and CuNPs showed moderate antimicrobial activities against human pathogens. The plant extract along with metal particles are significantly active against microorganism used in the bioassay. This result in agreement with the studies on *C. fruticosa*[24] and *C. trifoliata* leaf extract.[58]

Conclusion

The leaf aqueous extract of *C. fruticosa* was used to synthesize silver and CuNPs. The synthesis was simple, ecofriendly, economical, and rapid without using disastrous chemicals. This study recommends the use of plant materials instead of chemical substances as reducing agent. The synthesized SNPs and CuNPs exhibited a potent scavenging and antimicrobial activities.

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