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Research Article

Synthesis and Evaluation of Antibacterial Potency of Silver Nanoparticles of Extracts of *Ziziphus mauritina* and *Coriandrum sativum*

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Abstract: The major advantage of using plant extracts for silver nanoparticle synthesis is that they are easily available, safe, and nontoxic in most cases, have a broad variety of metabolites that can aid in the reduction of silver ions, and are quicker than microbes in the synthesis. The main mechanism considered for the process is plant-assisted reduction due to phytochemicals. In present we had selected two plant - *Ziziphus mauritina* commonly known as Indian jujube (Ber) and *Coriandrum sativum* commonly known as coriander for the phytochemical screening, nanoparticle synthesis and antibacterial activity against *Kliebsella pneumonia, Clostridium per fringes, Citrobacter freundii* and *Staphylococcus aureus* bacteria. All the bacteria selected in this study are highly and drug resistant. Potency of these plant extract against selected bacterial culture increases with formulation of nanoparticles .When compared with free extract in terms of zone of inhibition exhibited by bacterial culture. Zeta potential analysis of nanoparticles confirms that all the nanoparticles are of size loss them 100 nm. Stability of nanoparticles up to 15 days was observed which confirms the method of preparation of nanoparticle is stable and successful. Research finding confirms the format and stable nature of nanoparticles, which make them, potential and significant in drug delivery agent.

Keywords: Silver nanoparticles, *Ziziphus mauritina, Coriandrum sativum*, anti-bacterial, potency.

INTRODUCTION

For nanoparticles preparation three major types of reactions involves - physical, chemical and biological. Silver nanoparticles have attracted much attention because their properties for utilizing in different application inclusive pharmaceutics, agriculture, water detoxification, Air filtration, textile industries and as an activated in oxidization reaction [1]. Nanoparticles can be synthesized by non-biological and biological methods. Among non-biological methods, they can be synthesized by physical and chemical methods. But main disadvantages of using these methods are that they are expensive and cause release of hazardous chemicals. Due to this, many researchers move towards the biological method of synthesis. In this, many bacteria, fungi, plant extracts, yeasts and biological particles have been exploited for nanoparticle synthesis. Silver nanoparticles can be synthesized extracellulary or intracellularly by these biological agents [2].

The main phytochemicals involved are terpenoids, flavones, ketones, aldehydes, amides, and carboxylic acids. Flavones, organic acids, and quinones are water-soluble phytochemicals that are responsible for the immediate reduction of the ions. Studies have revealed that xerophytes contain emodin, an anthraquinone that undergoes tautomerization, leading to the formation of the silver nanoparticles.

The antimicrobial effects of leaf extract of these three medicinal plants and their respective biologically synthesized Ag NPs was evaluated by disc diffusion method. Comparative studies were also performed to analyze the toxicity of these biologically synthesized Ag NPs on three legume plants of the coriandrum (*Coriandrum sativa*), and ber (*Ziziphus mauritiana*) as they are native to the Indian Subcontinent and widely consumed.

MATERIAL AND METHOD

Maintenance of bacterial culture

Pure culture of *Keliebsell pneumonia, Staphococus aures, Closridium perfiuges* and *citrobactear freundii* were obtained from Department of Biotechnology of Barkatullah university from slants and pure culture were maintained though out the dissertation work through sub culturing, fresh slants were also prepared for storage of pure culture.

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Well Diffusion Method

For In vitro antibacterial screening agar plates were made, then the bacteria culture are poured and with the help of a spreader inoculum were spreaded on petriplates. After that small holes were punctured on the agar plates. In those wells extracts (both ethanol and aqueous) of different dilution series ranging from 10µl-100µl were poured with the help of a micropipette and these plates were kept in the incubator for 24 hours at 37℃ . After incubation the growth of the bacteria is noted. The area near the well shows no growth that is called the zone of inhibition which means that the phytoconstituents present in the extract have the potency to inhibit the growth of the bacterial strains. The zone of inhibition is calculated with the formula πr^2 (these experiments were also done in triplicate). All the test were conducted is triplicates, Cappuccino and Sherman [3].

Plant Collection and Extraction

Ziziphus maritain leaves were collected from various regions of campus of Barkatullah university Bhopal. Each sample was tagged and placed in separate zip lock polythene bags, brought back to the lab and processes collection. These plant samples were washed in running tap water for 15 minutes. The *Coriander sativum* plant procured from the local market.

The collected plants were washed with tap water and kept in room temperature for dry. After the plant dries, the leaves and stems of the plant are made by cutting the plant or cutting it into small pieces or with the help of, scissors or knives. The plant extracts were obtained by putting the air dried and crushed leaves in a soxillate extraction machine. The extraction was made using alcohol and distilled water.

Soxhlet Extraction

In conventional soxhlet, the sample in this case plant leaves of Indian jujube and leaves and leaf of coriander and giloy was washed, dried and crushed then placed in a thimble-holder and during operation is gradually filled with condensed fresh solvent (Ethanol and water) from a distillation flask. When the liquid reaches an overflow level, a siphon aspirates the whole contents of the thimble-holder and unloads it back into the distillation flask, carrying the extracted analytes in the bulk liquid. This operation is repeated until complete extraction is achieved after six rounds of soxhlet cycle. After the completion extraction process the collected extract was dried at 50°C to achiev to achieve the desired consistency required for future use, extracted material was then stored in air tight container [4].

Yield of extract

After extraction, each cycle was analysed for % yield of the extract is calculated in percentage by the formula % Yield =wt. of the extract/ wt. of the plant material× 100

Phytochemical analysis

The commonly known phytochemicals from *Coriander sativum, Ziziphus mauritina* are cardiac glycosides, terpenoids, steroids, saponin, tannin, flavonoids and alkaloids. Extracts were subjected to various chemical tests in order to determine the secondary plant constitutuents present by employing standard procedures as follows. (All the tests weredone in triplicates) [4].

Total ash

Presence of ash in any drug (natural) is a limited factor which can interfere with the pharmacological properties of extract. During validation and formulation ash value must be in lower rang, lower the value higher the potency of the extract. To determine total ash about 2 of the air-dried extract was placed in a crucible, extract was then spread in an even layer and ignited and gradually the temperature was increased to 450°C until it is white, indicating the absence of carbon. The content was cooled in a desiccator and weighed. Ash value can be calculated by using formul: - Ash value = Initial Weight – final Weight × 100/ Intial weight Uprieti *et al*. [5].

Water soluble ash

The total ash obtained from 2g extract was boiled with 25 ml of distilled water for 5 minutes. The insoluble matter was collected on an ash less filter-paper, washed with hot water and ignited to constant at low temperature. The weight of the insoluble matter was subtracted from the weight at total ash, represent the water soluble ash. The percentage of water soluble ash was calculated with reference to the air dried drug Uprieti *et al*., [5].

Test for cardiac glycosides

Test for Cardiac Glycosides 0.5 ml of each extract was treated with 0.2 ml glacial acetic acid then 1 drop of 3.5%ferric chloride ($FeCl_3$) was added to the solution. This was layered with 1 ml of concentrated H_2SO_4. A reddish brown ring was occurred at the interface indicates the presence of cardiac glycosides.

Test for terpenoids

0.5 ml of plant extract was added to the test tube then 2ml of chloroform was mixed to the solution. 3 ml of concentrated $_1$was added carefully from the wall of the test tube, to form a lower layer. Occurrence of reddish-brown colour at the interface indicated the presence of terpenoids.

Test for steroids

Test for Steroid 0.5ml of extract was dissolved in 3ml of chloroform. The solution was filtered, 2ml of concentrated H_2SO_4wasadded to the filtrate to form a lower layer. A reddish-brown colour ring at the interface was indicated the presence of steroid.

Test for aponin

Test for Saponin 0.5ml of extract was taken in the test tube, and then 5ml of distilled water was added to it. The solution was vigorously shaken and stable persistent froth was observed for the presence of saponin.

Test for tannin

Test for Tannin 0.5ml of extract and 5ml of distilled water was taken in test tube then it was boiled then filtered. Few drops of concentrated H_2SO_4 and 1% $FeCl_3$ were added to the filtrate. Deep green, brownish green or blue black coloration was indicated the presence of tannin.

Test for flavonoid

Test for Flavonoid 0.5ml of extract and 5ml distilled water was added to test tube then it was filtered. 5ml of diluted ammonia solution was added to the filtrate then concentrated $H_2SO_4$was added. A yellow coloration indicated the presence of flavonoid. The yellow color disappeared on standing.

Test for alkaloids

Test for Alkaloid 0.5 ml dried extract was taken and 3ml of methanol was added to it. Then 300µl of acetic acid (10% of methanol) was added to the solution ammonium hydroxide was added drop wise. Appearance of precipitate indicated the presence of alkaloid.

Biosynthesis of nanoparticles and antibacterial activity

Aqueous solution of silver nitrate (1mM) was prepared and mixed with fresh plant extract of *Coriander sativum* and *Ziziphus mauritina* at a ratio of 8:2. This solution was placed on a shaker with magnetic stirrer in the room temperature at 27 ± 2°C for 24 h. All stages of the experiment were implemented in three replicates. Silver nanoparticles synthesized by plant extract were tested for antibacterial activity agar well diffusion method against pathogenic bacteria.

Well diffusion assay

In this method 20 µl bacterial cultures were seeded on the surface of Nutrient Agar Media plates by spread plate technique. Media was punched with a sterile cork borer to make open wells (4 mm) in all plates. Different colloidal solutions of silver nanoparticles (2µl, 4 µl, 6 µl and 8 µl) synthesized above were poured in separate wells. Then these plates were incubated at 37°C for 24 hrs. After 24 hours incubation antibacterial activities of different nanoparticles sample were recorded in terms of diameter of inhibition zone measured in mm by using scale.

Characterization of silver nanoparticles

The brown colloidal solution containing silver nanoparticles obtained in above experiments is further subjected to following characterization techniques for determination of their physical, chemical properties viz., size, stability etc.

UV-Visible spectroscopic analysis

Change in colour of the mycelium free water extract incubated with 1 mm silver nitrate solution visually observed over a period of time indicates the bio reduction of silver ions to silver nanoparticles. The colloidal brown solution was monitored by absorption measurements carried out on UV–Visible Spectrophotometer (UV 1800 spectrophotometer Schimadzu) at a resolution of 1 nm between 300 to 600 nm (which is a characteristic wavelength absorption range for silver nanoparticles) wavelength range for confirming the synthesis of silver nanoparticles in the solution [6]. For absorption measurements, different brown colloidal solutions were poured in cuvette and placed in sample holder where wavelength of specific range is passed through it and absorption values are displayed in the form a spectra. Maximum absorption at a particular wavelength was depicted as a peak .Two plant *Ziziphus mauritiana* and *coriandraum staivum* through soxhlation aqueous and ethenolic extracts were obtained.

RESULTS AND DISCUSSION

Yield of Extract

The table 1 shows the %yield of the both ethanolic as well as aqueous extract of all the three plants .The yield of extract of leaf of *zizipus mauritiana* was found to be more when ethanol used as a solvent (15.9 ± 0.77) as compared to when water was used as a solvent(5.1± 1.3).

Total Ash Value

Ash value of different extract was calculated and results of ash value are given in table 2.The total ash value of *Zizipus mauritiana* was calculated 0.72±0.1%, 1.083±0.01% for ethanolic and aqueous leaf extract respectively. For water soluble ash the values are 0.24±0.06% , 0.25±0.02% for ethanol and aqueous extract respectively whereas acid insoluble ash are 0.19±0.01%, 0.2±0.01% respectively for *Coriander sativum* it was 0.36±0.02gm, 0.19±0.01gm for the ethanol and aqueous leaf extract respectively. The water soluble ash values are 0.15±0.05gm, 0.12±0.02gmfor ethologic and aqueous extract respectively whereas acid insoluble ash are 0.14±0.06%, 0.05±0.01 gm for ethanolic and aqueous extract respectively

Phytochemical screening of extracts

Results are shown in table 3, The present study revealed that the ethanol extract of leaf of *Zizipus mauritiana* has tannims (cathecolic), the aqueous extract of leaf of reducing sugar, saponin, glycoside. However, the aqueous extract of leaf of *Zizipus mauritiana* contained reducing sugar and saponins(table

3). Both the extracts showed the presence of rich variety of secondary metabolites. Glycosides are present in ethanolic extract while absent in aqueous. ethanolic extract of plant of *Coriandrum sativum* contains, tannins (cathecolic), and saponins, However, the aqueous extract contains only saponins.

Biosynthesis of silver nanoparticles

Aqueous solution of silver nitrate (1mM) was prepared and mixed with fresh plant extract of *Coriander sativum, Ziziphus mauritina* and *Tinospora cordifolia*at a ratio of 8:2. This solution was placed on a shaker with magnetic stirrer in the room temperature at 27 ± 2°C for 24 h. All stages of the experiment were implemented in three replicates.

Screening of antibacterial activity

The *in vitro* antibacterial activities of ethanol extract of *Z. mauritiana* is given in the table4. In one of the previous study (Deshpande et al, 2020) free plant extract fails to inhibit the growth with of *Kliebsella pneumonia* but, nanoparticles preparation is effective against *Kliebsella pneumonia* population. It is evident from the results that the nanoparticles inhibit two population of *Clostridium perfringes* 0.5cm^2, 1.54cm^2, 1.13cm^2, 4.52cm^2 and *Staphlococcus aureus* 2.01cm^2, 2.54cm^2, 1.54cm^2, 3.80cm^2.More effective compared to free extract while nanoparticles inhibit population of *Citrobacter freundii* while free plant extract fails inhibit do the population and area is 0.50cm^2, 0.78cm^2, 1.13cm^2, 1.54cm^2.

The table 5 shows the yield% of ethanolic and aqueous extract of plant (*coriandrum sativum)* using aqueous and ethanolic solvent. The yield of extract of the plant of *Coriandrum sativum* found to be more when water was used as a solvent (18.0±5.0%) as compared to when ethanol was as a Solvent (7.09±4.0%). *Kliebsella pneumonia* was not affected by nanoparticle of *Ziziphus mauritiana* even at higher concentration. Organism like *Citrobacter freundii* and *Clostridium perfringes* shows no inhibition when exposed to free extract of *Coriandrum sativum* plant while when treated with nanoparticles 8µl *Kliebsella pneumonia*, *Staphylococcus aureus Citrobacter freundii* and *Clostridium perfringes* zone of inhibition was observed as 1.3cm2 , 0.7 cm^2, 0.9cm^2, 1.1cm^2. In one of the our previous study free plant extract do not inhibit the growth of *Clostridium perfringes* even nanoparticles preparation also stop *Clostridium perfringes* 1.13cm^2, 1.54cm^2, 2.01cm^2, 2.54cm^2 area population. The zone of inhibition of *Kliebsella pneumonia*3.8cm^2,3.8cm^2, 4.52cm^2 ,5.31cm^2 area. It is evident from the results that the nanoparticles inhibit two population of and *Staphylococcus aureus*0.78,cm^2 0.78cm^2,1.13cm^2, 1.54cm^{2}more effective compared to free extract While nanoparticles inhibit population of *Clostridium perfringes* while free plant extract not inhibit do the population and area is 1.54cm^2, 0.78cm^2, 5.31cm^2, 4.52cm^2 area.

Nanoparticle prepration

The fresh extract of *Coriandrum sativum* and *Ziziphus mauritiana*, was yellow in color, but after addition of $AgNO_3$ solution, 1N $AgNO_3$ solution was prepared by mixing 10ml D/W in 0.1g $AgNO_3$, the solution for nanoparticles prepared by mixing 16ml of D/W with 3ml of $AgNO_3$ and 1ml plant extract was added. With continuous stirring for 24hours at room temperature, gradually the color of the solution changes to red. The intensity of the color increased, which confirmed Ag ion reduction and the formation of Ag NPs. Silver nanoparticle surface plasmon excitation causes colour change in the solution, which is the primary and notable evidence for the formation of Ag NPs.

UV–Vis analysis

UV–Vis absorption spectrum of Ag NPs is shown in Fig. Broad bell-shaped spectrum curve was obtained from UV–Vis analysis. Various metabolites from plant extract introduced to solution make the Plasmon band broad because they may be read in this spectrophotometric range, too. Surface plasmon resonance (SPR) of silver occurs at 300nm. To 600 nm. This peak increased with time up to 360 min. According to Mie theory, spherical nanoparticles show only a single SPR band. The number of peaks increases by increasing diversity of particles shapes. Then, it can be concluded that biosynthesized Ag NPs are unanimously spherical in nature. In present study nanoparticles of all the extract are giving λ mean of 440- 500 nm. Nanoparticles gives λ mean at around 460-500 nm. Result confirms that at nanoparticles formed is present research are table and intact which is a good.

Stability of nanoparticles

The activity was determined by spectrophotometer analysis. All the nanoparticles formed give lambda mean at 380-400 nm up to 15 days of prepration which conferm the integrity and stablility of nanoparticles formed.

From both the result we can conclude that method which was adopted by to form nanoparticles is best suited for plant extract and stability up to 15 days is a good sign and holds future properties for plant extract nanoparticles for application use.

Table-1: Result showing % yield of extract

S NO.	PLANT NAME	SOLVENT USED FOR EXTRACTION	YIELD OF EXTRACT (gm)
1.	*Coriandrum sativum*	ETHENOL	15.9± 0.77
2.	*Coriandrum sativum*	AQUEOUS	5.1±1.3
3.	*Ziziphus mauritiana*	ETHANOL	7.09± 4.0
4.	*Ziziphus mauritiana*	AQUEOUS	18.0±5.0

Table-2: Total cash value

S. NO.	PLANT NAME	SOLVENT USED FOR EXTRACTION	TOTAL ASH VALUE (gm)	WATER SOLUBLE ASH (gm)	ACID SOLUBLE ASH(gm)
1	*Coriandrum sativum*	ETHANOL	0.75±0.1	0.35± 0.06	0.20±0.01
2	*Coriandrum sativum*	AQUEOUS	1.095±0.01	0.28± 0.02	0.3±0.01
3	*Ziziphus mauritiana*	ETHANOL	0.36±0.02	0.15±0.05	0.21±0.01
4	*Ziziphus mauritiana*	AQUEOUS	O.19±0.01	0.12± 0.02	0.06±0.01

Table-3: Result of phytochemical analysis of ethanolic and aqueous extracts of *zizipus mauritiana*

S.NO.	PLANT NAME	PHYTOCHEMICAL COMPOUND	ETHANOLIC EXTRACT	AQUEOUS EXTRACT
1	***Zizipus mauritiana***	TANNINS(cathecolic)	+	-
2		VOLATILE OIL	-	-
3		REDUCING SUGAR	+	+
4		SAPONIN	+	+
5		GLYCOSIDE	+	-
6		ALKALOIDS	-	-
7	***Coriandrum sativum***	TANNINS(cathecolic)	+	-
8		VOLATILE OIL	-	-
9		REDUCIN SUGAR	-	-
10		SAPONINS	+	+
11		GLYCOSIDE	-	-
12		ALKALOID	-	-

+ =present; -=absent

Table-4: Zone of inhibition of nanoparticle of ethanolic extract of *Ziziphs mauritiana* leaf.

S. No	Dose of plant extract and nanoparticles in µl	Area of zone of inhibition in cm^2			
		Kliebsella Pneumonia	***Staphlococcus Aureus***	***Citrobacter Freundii***	***Clostridium Perfringes***
1.	2	**	0.8	0.4	0.5
2	4	**	0.9	0.5	0.6
3	6	**	0.7	0.6	0.7
4	8	**	1.1	0.7	1.2

**No zone of inhibition was observed.
##results are the men value of triplicate

Table-8: Zone of inhibition of nanoparticles of ethanolic extract of Coriandrum sativum leafs

S. NO	DOSE OF EXTRACT IN (µl)	Area of zone of inhibition in (cm^2)			
		Kliebsella pneumonia	***Staphylococcus aureus***	***Citrobacter freundii***	***Clostridium perfringes***
1	2	1.1	0.5	0.6	0.7
2	4	1.1	0.5	0.7	0.5
3	6	1.2	0.6	0.8	1.2
4	8	1.3	0.7	0.9	1.1

** No zone of inhibition was observed.
Results are the mean value of triplicate.

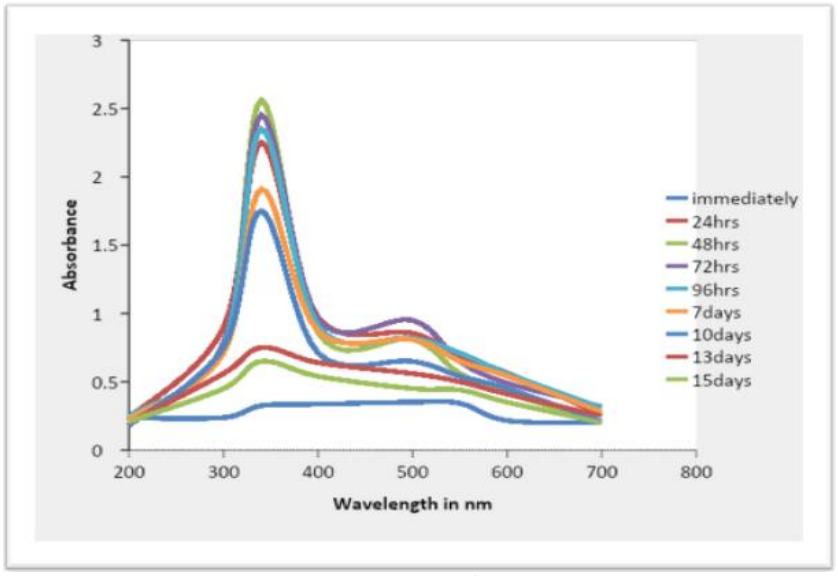

Fig-1: Graph showing the activity of nanoparticles (15 days)

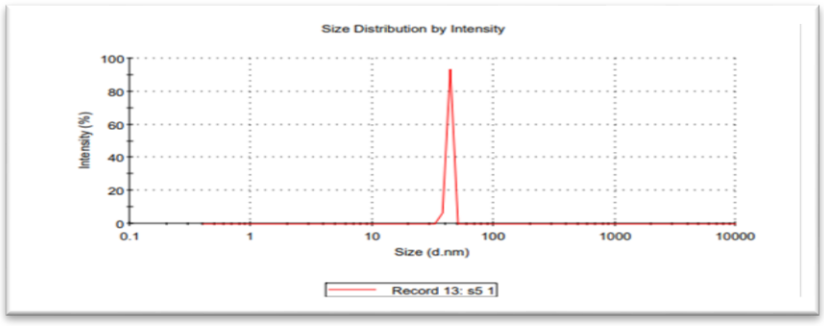

Fig-2: Zeta potential analysis

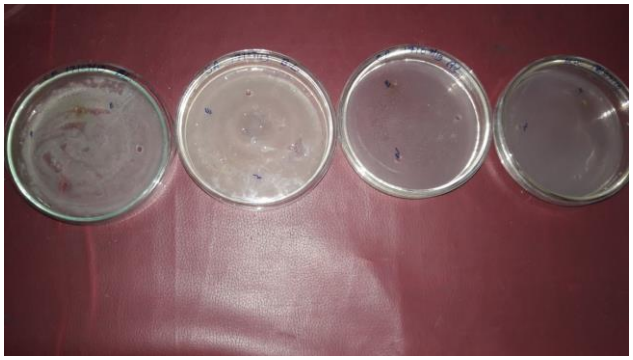

Fig-3: Agar plates showing susceptibility of Clostridium perfringes, Citrobacter freungii, kliebsella pneumonia and staphylococcus aureus through zone of inhibition against ethanolic extract of Ziziphus mauritiana

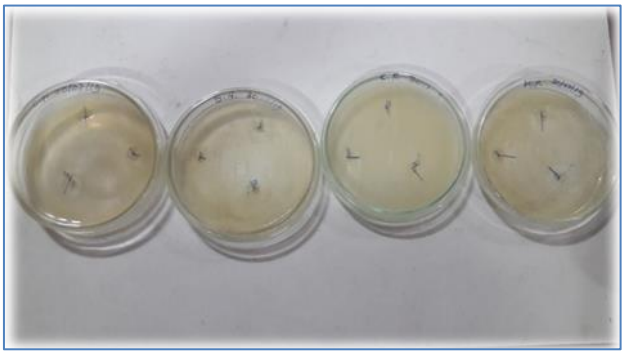

Fig-4: Agar plates showing susceptibility of Clostridium perfringes, Citrobacter freungii, kliebsella pneumonia and staphylococcus aureus through zone of inhibition against ethanolic extract ofCoriandrum sativum

Fig-5: (A): Plant extract with $AgNO_3$ (B) after 24h in magnetic stirrer

CONCLUSION

Bactericidal activity assessment of the biosynthesized Ag NPs showed their inhibitory function against both Gram-positive and Gram-negative bacteria. Zeta potential analysis of nanoparticles confirms that all the nanoparticles are of size loss them 100 nm. Stability of nanoparticles up to 15 days was observed which confirms the method of preparation of nanoparticle is stable and successful. Organism like *Citrobacter freundii* shows no inhibition when exposed to free extract but, zone of inhibition was recorded when exposed to Nanoparticle $0.7cm^2$.

FUTURE RECOMMENDATION

In place of crude extract purified extract like (tannins, saponins, flavonoid, glycosides etc.) can lead to a potent drug discovery. These plants can be further exploited as potent alternative to current drug therapy against bacterial infection. Nanoparticle of purified plant extract can be exploited in future which will definitely leads to positive outcomes.

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