

Synthesis of Silver Nanoparticle from *Spathodea Campanulata* Leaf Extract and Study of Its Antimicrobial and Antioxidant Activity

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ABSTRACT

The developments and progress in artificial intelligence and molecular technology have spawned a new form of technology; Nanotechnology. Nanotechnology could give the human race eternal life. Eco friendly methods of green mediated synthesis of nanoparticle are the current research in the appendage of nanotechnology. Silver nanoparticle (AgNPs) have been synthesized from myriad of plant species and used to determine the antimicrobial activity of various micro-organisms. In the present study silver nanoparticle were synthesized from leaf extract of *Spathodea campanulata*. The formation of the silver nanoparticle was monitored using UV-Vis absorption spectroscopy in the range from 250-800 nm. The particle size and range was further confirmed using particle size analyzer. The antibacterial activity of extract and nanoparticle was determined using well diffusion method. The bacterial species taken into consideration were gram positive- *Bacillus cereus* and *Actinomyces* and both extract and silver nanoparticle showed antibacterial activity at concentration ranging between 25, 50, 75, 100 mg/ml. The extract also showed anti-oxidant activity, the method employed were DPPH assay and reducing power assay. Based on the result obtained, it can be concluded that the plant resources can efficiently used in the production of silver nanoparticle and it could be utilized in various fields such as biomedical, nanotechnology, microbiology etc.

Keywords: Silver nanoparticle, well diffusion assay, DPPH assay, reducing power assay

INTRODUCTION

Nanotechnology is one of the very frontiers of science today. It involves attuning of materials at atomic level to accomplish unique properties. It refers to the ability to control the composition of molecules and atoms within the range of 100nm. [1] It is currently employed as a tool to explore various fields of medical sciences like imaging, [2] targeted drug delivery, [3] gene delivery systems [4] and artificial implants. [5] Research and product developments in the area of nanotechnology have steadily increased especially due to

new, beneficial properties of nonmaterial. There has been rapid technological advances and commercialization yet there has been debate on associated regulatory and legal aspects. [6] Silver has been known to have disinfecting effect for a long period of time. Various salts of silver and their derivatives are commercially employed as antimicrobial agents. [7] Silver nanoparticles (AgNPs) have been aptly investigated for their antibacterial property. [8-11] The mechanism of silver action is linked to the interaction between thiol group compounds of respiratory enzymes of bacterial cells and

silver. Silver has large surface area as compared to other elements, therefore has better contact with micro-organisms and show significant antimicrobial property. AgNPs attach to cell membrane of bacteria containing proteins and form low molecular weight region, thus bactericidal property of AgNPs depend on their stability in growth medium. [12]

Medicinal plants have been used for ailment of several microbial and non-microbial originated diseases. During the past decade, the therapeutic use of herbal medicine is gaining considerable momentum in the world. The use of herbal medicine due to toxicity and side effects of allopathic medicines, has led to sudden increase in the number of herbal drug manufacturers. Herbal medicines as the major remedy in traditional system of medicine have been used in medical practices since antiquity. [13] The practice continues today because of its biomedical benefits and has made a great contribution towards maintaining human health. The use of plant derived natural compounds used as alternative sources of medicine continues to play major roles in the general wellness of people all over the world. The curative properties of medicinal plants are due to the presence of various complex chemical substances of different composition which occur as secondary metabolites. [14] Antioxidants are abundantly found in a number of trees. In the present study the plant material selected were leaves of *Spathodea campanulata*. *Spathodea* is a medium sized plant belonging to family Bignoniaceae and is native to tropical Africa. It is found in African countries like Ghana, Nigeria. [15] The plant is commonly known as Rudrapalash, African tulip and bears orange scarlet bell shaped flowers. [16] The plant has reported anti-malarial, [17] anticonvulsant, [18] analgesic, anti-inflammatory, [19] wound healing. [20,21] The species used in this experiment for determining antibacterial activity were *Bacillus cereus*, *Actinomyces pseudofradera*.

This study was undertaken to synthesize nanoparticles from leaf extract of *Spathodea campanulata* and determine its antibacterial activity.

MATERIALS AND METHODS

COLLECTION AND IDENTIFICATION OF PLANT MATERIAL

The plant selected for the study was *Spathodea campanulata* and its fresh and healthy leaves were collected from the local park near the P & T Square, Bhopal. The plant was identified by Dr. Zia-Ul-Hassan from the Department of Botany at the Safia college of Science Peer Gate, Bhopal, India and the voucher specimen (329/Botany/Safia/15) has been deposited at the Herbarium of the Safia college of Science Peer Gate, Bhopal.

PREPARATION OF PLANT EXTRACT

The leaves of *Spathodea campanulata* were thoroughly washed and let to dry in air. The leaf extract was prepared by using two different extract processes- Maceration and Decoction. The petroleum ether extract and chloroform extract of the specimen were obtained through Maceration technique by using petroleum ether and chloroform as solvents respectively. To obtain the concentrated extract, the marc is pressed, and the liquid retained is then purified by filtration after standing. The aqueous extract of the specimen was obtained through Decoction by boiling the leaves of *S. campanulata* in distilled water. To concentrate the extract, continuous boiling is done. The concentrated extract is then filtered. [22]

PHYTOCHEMICAL ANALYSIS

• QUALITATIVE

PHYTOCHEMICAL ANALYSIS

The petroleum ether and chloroform extract of leaves of *Spathodea campanulata* were chemically tested in order to determine the Phytochemical present in it. The standard protocol for the identification of

phytochemicals was followed for the phytochemical screening. [23-25]

- **QUANTITATIVE PHYTOCHEMICAL ANALYSIS**

- **Total Phenolic Content:**

The total phenolic content of the chloroform extract of the leaves of *Spathodea campanulata* was determined by employing the Folin-Ciocalteu reagent. Initially, the test sample was allowed to incubate for 30 minutes at room temperature. Then absorbance was measured at 765nm by using methanol as blank. The double beam UV-Visible spectrophotometer (SYSTRONIX 2202) was used to measure the absorbance. [26]

- **Total Flavonoids Content:**

The total flavonoids content of chloroform extract of leaves of *Spathodea campanulata* was determined by employing aluminium chloride calorimetric assay. Once the sample was ready, it was allowed to stand for 15 minutes. Later the absorbance was measured at 510 nm against the blank by using the UV-Visible spectrophotometer (SYSTRONIX 2202). [27]

PREPARATION OF NANOPARTICLE

In 1mM aqueous solution of silver nitrate (AgNO_3) 10 ml of plant extract was added drop wise. The sample was incubated in dark. The sample was measured for its maximum absorbance using UV-Visible spectrophotometer (SYSTRONIX 2202) once after 24 hours of incubation then subsequently after 48 hours. [28]

PARTICLE SIZE ANALYSER

The PSA analysis was carried out for the sample which is lyophilized and dispersed by nano-particle analyzer (HORIBA) for the determination of size.

UV-VIS SPECTROPHOTOMETRY ANALYSIS

The samples were observed under UV-Vis spectrophotometer (SYSTRONIX 2202) for its maximum absorbance and wavelength to confirm the reduction of Silver nitrate.

MICROBIAL STRAIN

The standard strain of *Bacillus cereus* (MTCC 1305), *Actinomyces pseudofradrea*

(MTCC 7802), were obtained from Pinnacle Biomedical Research Institute (M.P.), India.

ANTIBACTERIAL ACTIVITY

The anti-bacterial activity of extract and silver nanoparticle was evaluated using agar well diffusion method. The media was poured into petri-plates and inoculation was done using spread plate method. The wells were created using a cup borer and extract were poured into it in concentration from 25 mg/ml to 100 mg/ml. The plates were incubated for 48 hours at 28°C. Antibacterial activity was determined by measuring the diameter of the zone of inhibition surrounding bacterial growth. The experiments were repeated three times and the mean values were presented with \pm Standard Deviation (SD). [29]

ANTIOXIDANT ASSAY

- **DPPH assay (2,2-diphenyl-1-picrylhydrazyl)**

The radical scavenging activity of chloroform extract of *S. campanulata* was determined by using DPPH assay. [30] DPPH (1, 1-Diphenyl-2-picryl-hydrazyl) is a stable free radical with red colour. On scavenging, these free radicals turn to yellow. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability. This common principle is utilised in this assay. The stock solution of 1mg/ml of extract was prepared with methanol. A 0.1 mM solution of DPPH in methanol was prepared. Different concentrations ranging from 20 to 100 $\mu\text{g/ml}$ of extract was made with chloroform. Then to 2ml of test sample 1ml of DPPH solution was added and vortexed. It was kept for incubation in dark for 30 minutes. The absorbance was measured at 490 nm in ELISA Reader (Kelvinator) and % inhibition.

- **Reducing Power assay**

The reducing power of the extract was determined as per the protocol. [31] Initially 0.5 ml of the extract was mixed with 0.5 mL of phosphate buffer (pH 6.6, 0.2M) and 0.5 mL of potassium ferricyanide (0.5 ml, 1%W/V). The mixture was then incubated

at 50°C for 20 min. Once the mixture cooled down, 1.5 mL of trichloroacetic acid (10% W/V) was added. Then 0.5 ml of 0.1% ferric chloride was added and the absorbance of the reaction mixtures was measured at 700 nm in ELISA reader (Kelvinator) against blank sample. Increased absorbance of the reaction mixture indicated increased reducing power.

Biostatistical analysis

All data are presented in mean± SD. Data was analyzed by state-32 One Way Anova followed by Bonferroni t-test. P<0.001 and P<0.050 was considered as level of significance (n=4).

RESULT AND DISCUSSION

Qualitative Analysis

The qualitative phytochemical analysis, performed as per the standard protocols, revealed the presence of several compounds in the extracts as tabulated in Table 1. The analysis revealed that the petroleum extract had glycosides, alkaloids, saponins, flavonoid, triterpenoid, steroid, phenol and tannin whereas the chloroform extract comprised of carbohydrates, glycosides, alkaloids, saponins, flavonoid, triterpenoid, steroid, phenol and tannin.

Table 1: QUALITATIVE ANALYSIS OF PLANT EXTRACTS

S. No.	Phytoconstituent	Name of test	Petroleum ether extract	Chloroform extract
1	Carbohydrate	• Molish • Barfoed	- -	+ +
2	Proteins	• Biuret • Ninhydrin	- -	- -
3	Glycosides	• Keller-Killiani	+	+
4	Alkaloids	• Mayer's • Wagner	+ +	+ +
5	Saponin	• Froth	+	+
6	Flavonoid	• Lead acetate • Alkaline reagent	+ +	+ +
7	Triterpenoid and steroid	• LibermannBurchard's	+	+
8	Phenol and Tanin	• Ferric chloride • Lead acetate	+ +	+ +

QUANTITATIVE PHYTOCHEMICAL ANALYSIS

Under quantitative phytochemical analysis, the total phenolic content (Table 2.1 and 2.2) and total flavonoid content (Table 3.1 and 3.2) of the extract were calculated.

Total phenolic content

The total phenolic content of the chloroform extract of *S. campanulata* was calculated with the help of calibration curve and was found to be 0.912 gallic acid equivalent/gm (Fig.1 b). Gallic acid was used as the standard.

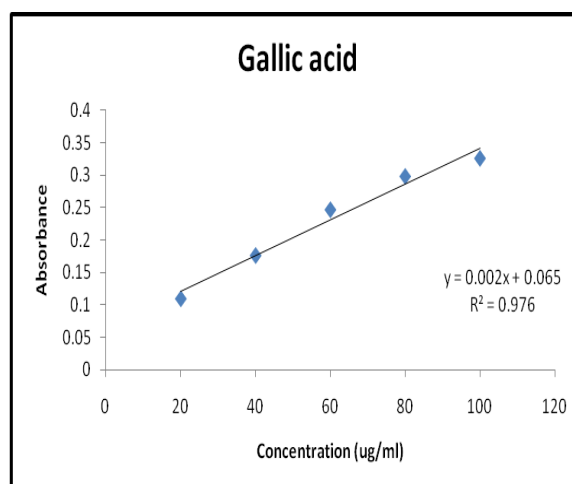


FIG.1 a: STANDARD CALIBRATION CURVE OF GALLIC ACID

Table 2.1: TOTAL PHENOLIC CONTENT OF STANDARD (GALLIC ACID)

S.No.	Concentration	Absorbance
1.	20	0.1098
2.	40	0.1763
3.	60	0.2468
4.	80	0.2981
5.	100	0.3258

Table 2.2: TOTAL PHENOLIC CONTENT OF PLANT EXTRACT

S.No.	Concentration	Absorbance
1.	20	0.425
2.	40	0.453
3.	60	0.484
4.	80	0.490
5.	100	0.501

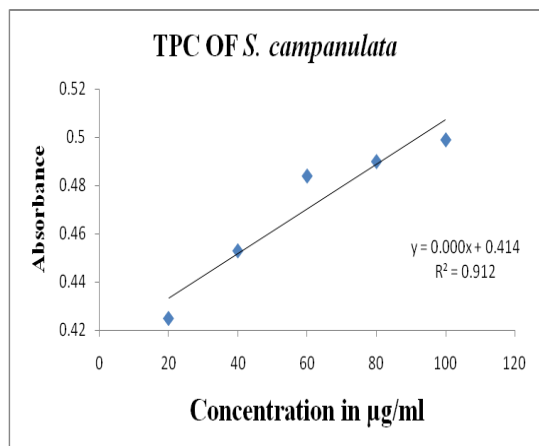


FIG. 1 b: TOTAL PHENOL CONTENT OF LEAF EXTRACT OF *S. campanulata*

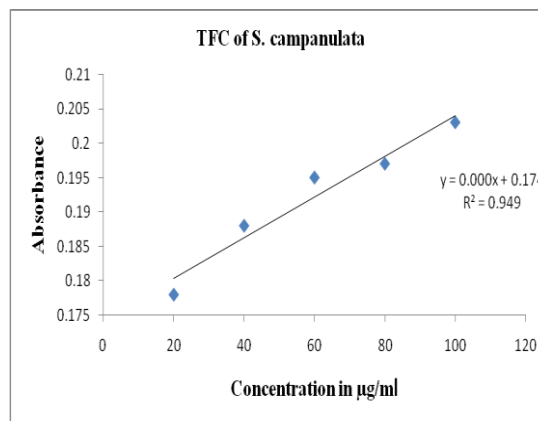


FIG 2 b: TOTAL FLAVONOID CONTENT OF LEAF EXTRACT OF *S. campanulata*

Total flavonoid content

The total flavonoid content of the chloroform extract of *S. campanulata*, as calculated from the calibration graph, was found to be 0.949 Rutin equivalent/gm (Fig 2 b). Rutin was used as the standard.

Table 3.1: TOTAL FLAVONOID CONTENT OF STANDARD (RUTIN)

S.No.	Concentration	Absorbance
1.	20	0.135
2.	40	0.151
3.	60	0.165
4.	80	0.177
5.	100	0.201

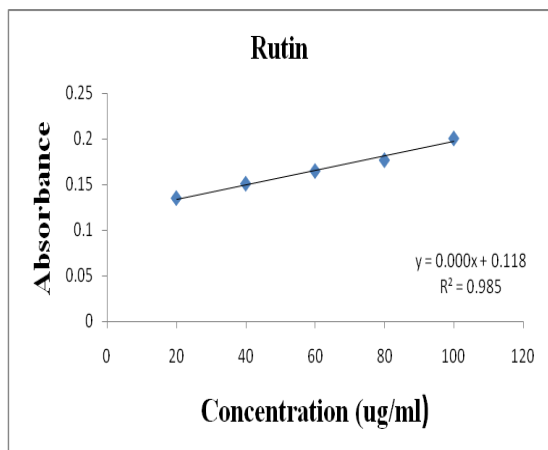


FIG. 2 a: STANDARD CALIBRATION CURVE OF RUTIN

Table 3.2: TOTAL FLAVONOID CONTENT OF EXTRACT

S.No.	Wavelength (nm)	Absorbance
1.	20	0.178
2.	40	0.188
3.	60	0.195
4.	80	0.197
5.	100	0.203

• UV-SPECTROPHOTOMETER ANALYSIS

The zeta potential (mean) of the sample was 33.6mV while the electrophoretic mean was $0.000261\text{cm}^2/\text{Vs}$

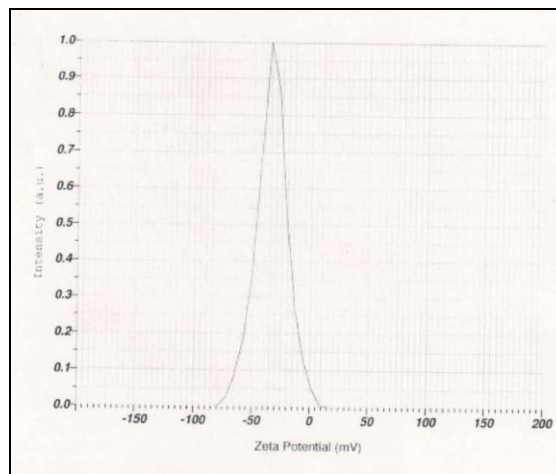


FIG. 3 a: Particle Size Analyzer image of synthesized silver nanoparticles

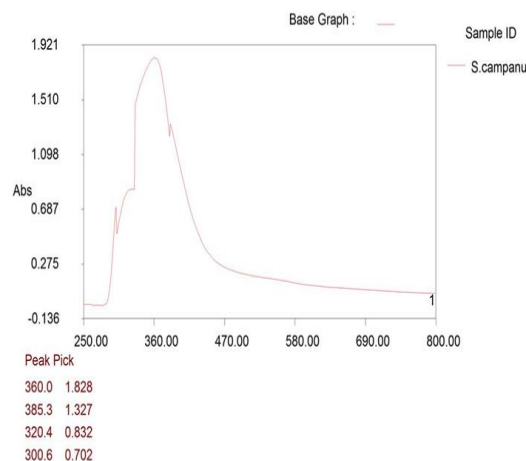


FIG. 3 b: UV-Vis Spectrum Of Silver Nanoparticles Obtained

• ANTIMICROBIAL ACTIVITY

The antimicrobial activity of silver nanoparticles (Table 4.1) and chloroform

extract (Table 4.2) of *S. campanulata* was tested against the bacterial strains of *B.*

cerius and *Actinomyces pseudofradea* using well-diffusion assay.

Table 4.1: ANTIMICROBIAL ACTIVITY OF AgNPs of *Spathodea campanulata* AGAINST *B. cereus* AND *Actinomyces*.

Organism	25mg/ml	50mg/ml	75mg/ml	100mg/ml	Standard (10µg/ml)
<i>B. cereus</i>	14.75±0.957	15.75±0.500	19.25±0.500	20.50±0.577	30.33±0.577
<i>Actinomyces</i>	16.50±1.000	17.50±0.577	19.00±0.816	21.50±0.577	

Table 4.2: ANTIMICROBIAL ACTIVITY OF EXTRACT OF *S.campanulata* AGAINST *B. cereus* AND *Actinomyces*.

Organism	25mg/ml	50mg/ml	75mg/ml	100mg/ml	Standard (10µg/ml)
<i>B. cereus</i>	7.50±0.577	8.25±0.500	9.75±0.500	11.75±0.500	30.33±0.577
<i>Actinomyces</i>	9.50±0.577	11.75±0.500	13.50±0.816	15.50±0.577	

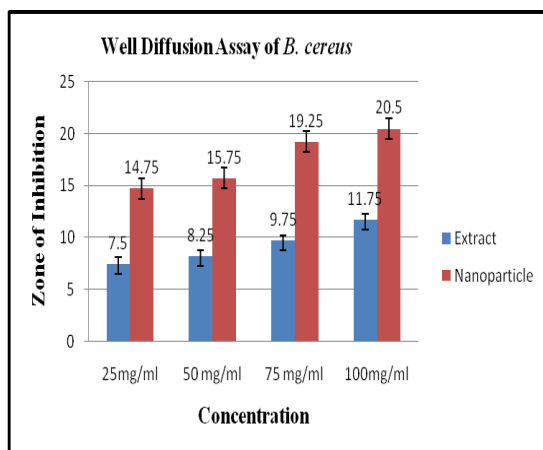


Fig. 4 a: well diffusion assay of *B.cereus* for leaf extract of *S.campanulata* and silver nanoparticles.

The activity of *B.cereus* is shown in Fig.4a and that of *Actinomyces pseudofradea* is shown in Fig.4b. On comparing the efficiencies, it was found that both the AgNPs as well as the chloroform extract were most effective against *Actinomyces pseudofradea* exhibiting an inhibition zone of 21.50±0.577 mm at a concentration of 100mg/ml. The

antimicrobial activity of AgNPs was found to be greater against *B.cerius* (20.50±0.577mm at 100mg/ml) than that of plant extract (11.75±0.500mm at 100mg/ml).

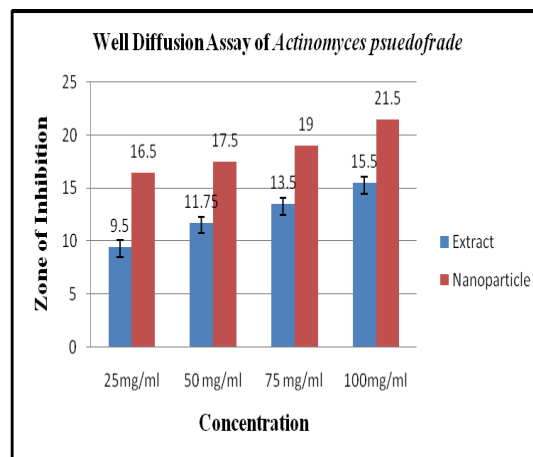


FIG. 4 b: well diffusion assay of *Actinomyces* for leaf extract of *S.campanulata* and silver nanoparticles.

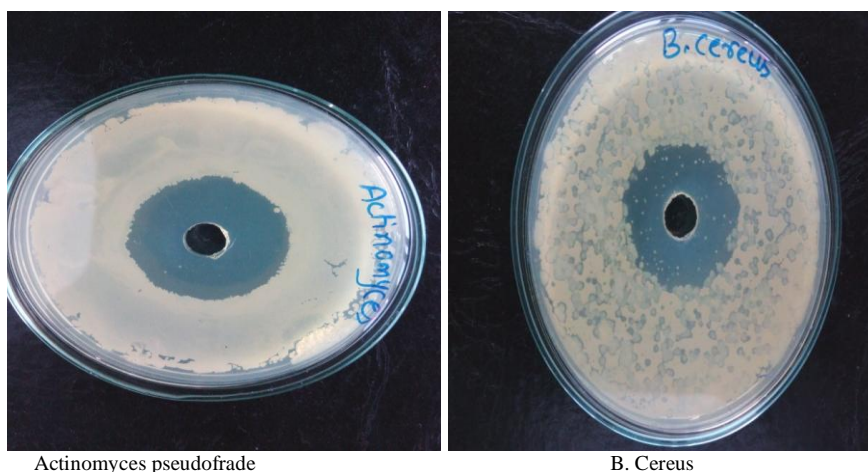


FIG. 4 c: Anti-bacterial analysis

ANTIOXIDANT ASSAY

• DPPH Assay (2,2-DIPHENYL-1-PICRYLHYDRAZYL)

The DPPH Assay was performed to know about the antioxidant properties of the extract (Table 5.2).

TABLE 5.1: DPPH ASSAY (2,2-DIPHENYL-1-PICRYLHYDRAZYL) STANDARD

Concentration	Control	Absorbance
20	0.699	0.397
40	0.699	0.34
60	0.699	0.269
80	0.699	0.211
100	0.699	0.142

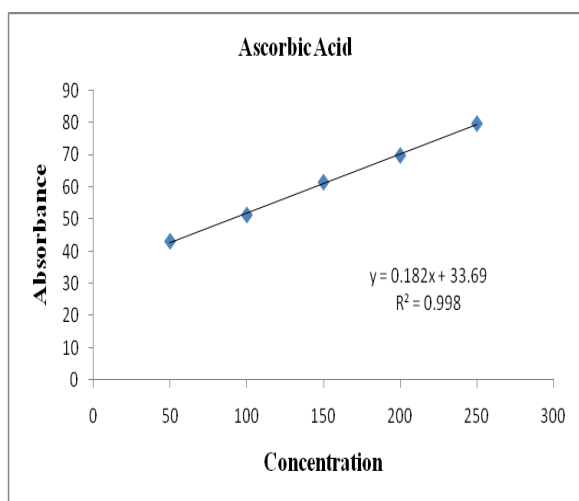


Fig.5 a: DPPH ASSAY OF ASCORBIC ACID

Table 5.2: DPPH ASSAY OF *S.campanulata* LEAF EXTRACT

Concentration	Control	Test absorbance
20	0.621	0.595
40	0.621	0.471
60	0.621	0.403
80	0.621	0.365
100	0.621	0.338

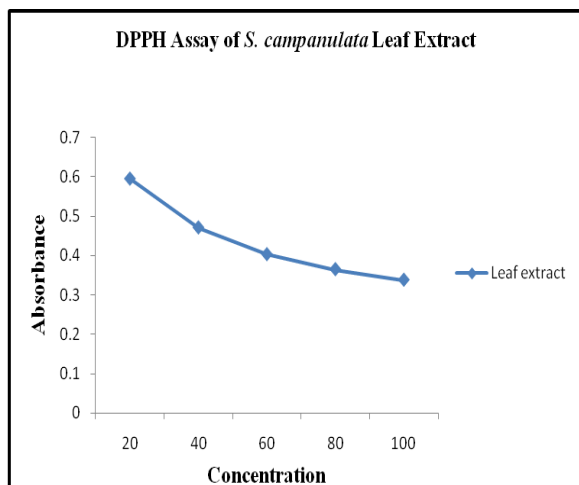


FIG. 5 b: DPPH ASSAY OF CHLOROFORM EXTRACT OF LEAVES OF *S.Campanulata*

• Reducing power assay

The reducing power assay was accompanied by decrease in absorbance as the concentration of the plant sample increased. The extract showed a decrease of absorbance from 0.501 to 0.295 as concentration increased from 20µg/ml to 100µg/ml (Table 5.3).

TABLE 5.3: REDUCING POWER ASSAY OF ASCORBIC ACID AND SAMPLE

Concentration	Absorbance of Ascorbic acid	Absorbance of Sample
20	0.354	0.501
40	0.379	0.422
60	0.465	0.411
80	0.513	0.341
100	0.53	0.295

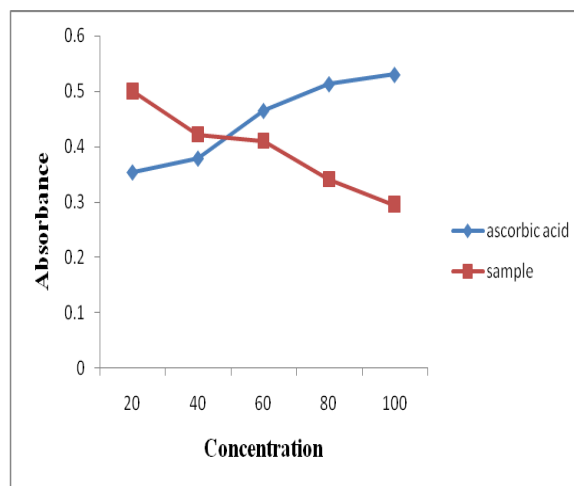


FIG. 5 c: REDUCING POWER ASSAY OF ASCORBIC ACID AND SAMPLE (CHLOROFORM EXTRACT OF LEAVES OF *S.Campanulata*).

DISCUSSION

This paper demonstrates the experiment which was performed to study the antibacterial and antioxidant properties of the leaf extract of the plant *S. campanulata* and the silver nanoparticles derived from it. The qualitative phytochemical analysis revealed the phytoconstituents in the leaf extract whereas TPC and TFC determined the phenolic and flavonoid content respectively. UV-VIS spectrophotometry analysis was performed to confirm the formation of silver nanoparticles.

The antimicrobial tests, conducted against *B.cereus* and *A.pseudofradera*, revealed the antimicrobial properties of

plant extract as well as the silver nanoparticles extracted from it. From the results it can be concluded that the nanoparticles have higher antimicrobial activity against *B.cereus* as compared to the leaf extract. Not much difference was observed in activity against *A.pseudofradera*. This suggests that this plant has the potential to be used as an antibacterial agent. Further studies have to be done to determine the potency of this plant against other microbial species.

The DPPH scavenging and reducing power assay helped to reveal the antioxidant activities of the plant. As the results suggest, the plant exhibits antioxidant properties which can be exploited for further benefits to mankind.

This study indicates that *S. campanulata* has a wide range of properties which can be exploited for human benefits. Nanotechnology, the newly evolved technique, can be used to assist and enhance these properties. Further work has to be done in this aspect to discover more astonishing properties of this plant.

CONCLUSION

This study revealed the phytochemical constituents of the extract of the plant *Spathodea campanulata*. Through this study it can be concluded that this plant and the silver nanoparticles extracted from it have the potential to be used for their antimicrobial and antioxidant properties.

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