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Original Research Article

Synthesis of Silver Nanoparticle from *Saraca Asoca* Leaf Extract & Study of Its Antibacterial and Antioxidant Activity

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ABSTRACT

The field of biotechnology has crossed yet another milestone with development of advanced stream as Nanotechnology. With huge potential of nanotechnology the human race can make immense profit and benefits in his life. One of the most performed uses of nanotechnology comes with the synthesis of silver nanoparticles that is currently a topic of research. These silver nanoparticles or AgNPs have been synthesized from variety of different plant species and have been reduced for detection of antimicrobial activities of various parts of plants. Apart from being eco-friendly, in our present study silver nanoparticles were being generated from the leaves of *Saraca asoca* to evaluate antibacterial effects of plant. The bactericidal effect of biosynthesized nanoparticles was tested against strains of *Bacillus subtilis* and *Proteus vulgaris*. The antibacterial effect was determined using well diffusion method. Both the extract and nanoparticles showed antibacterial activity ranging between various concentrations. Further characterization of silver nanoparticles was done using UV-Vis absorption spectroscopy ranging between 250-800nm. The particle size of the synthesized AgNPs was determined with the help of particle size analyzer. The anti-oxidant activity of the extract was also determined where methods employed included DPPH assay and Reducing Power assay. Lastly, based on the results obtained it was concluded that the plant variety used was effective in bio synthesis of silver nanoparticles and as such can be successively used in various other fields of biotechnology and nanotechnology.

Keywords: *Bacillus subtilis*, DPPH assay, *Proteus Vulgaris*, reducing power assay, Silver nanoparticle and Well diffusion assay

INTRODUCTION

In recent years, Nanotechnology is one such field that has grabbed tremendous attention due to its large capabilities in the field of biotechnology, drug delivery and catalytic industry. [(1,2)] Many metal nanoparticles are being synthesized but silver nanoparticles out of all due to its potential are of particular interest. It has several physical properties like Surface Plasmon Resonance (SPR) due to which the silver particles can be controlled for their size, morphology and various utility. Silver nanoparticles in the colloidal form are superior in performance when it comes against multiple disease causing pathogens. [(3)] Further silver nanoparticles due to their large surface to volume ratio and their crystallographic structure exhibit great deal of anti-microbial properties. Nanoparticles in the range of 1-10 nm have a direct interaction with the bacteria indicating bactericidal property. [(4)] The mechanism of silver action is linked to the interaction

between thiol group compounds of respiratory enzymes of bacterial cells and silver. 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. [(5)] With many approaches in this field and keeping in mind the eco-friendly nature of silver, the nanoparticles are now a day's synthesized using bacteria, yeast, fungi and mainly through plant extract. [(6)]

From ancient time India is known for being rich in herbal medicines that include medicinal plants and its products. Hence, one such plant *Saraca asoca* is being traditionally used due to its pharmaceutical activities [(7)] and nanoparticles are synthesized using it proving more beneficial than using microbes because they contain non-toxic chemicals and also provide us with capping agents. Using plants lea extracts we can synthesize pure silver nanoparticles. [(8)] Presently the leaves from one such pant that is *Saraca asoca* have been used to synthesize silver nanoparticles and for its study. It is one of the most ancient tree found in India and is commonly recognized as ashoka briksh. It belongs to the family of Caesalpiniaceae. [(7,9)] The tree is almost found everywhere including West Bengal, Assam, Odisha, Tamil Nadu, Karnataka, Kerala, Andhra Pradesh, Meghalaya and Maharashtra and is well distributed in the parts of Himalaya and Western Ghats. [(10)] The Ashoka tree is considered to be a scared as it is been revered in traditions followed by Hindu. [(9)] The leaves of Ashoka tree are especially reported to be rich in alkaloids, steroids, flavonoids, tannins, saponins, terpenoids, polyphenolics, glycosides and many carbohydrates. [(2,11-12)] The plant reaches at a height of 7-10 m and is an evergreen deciduous tree that has paripinnate leaves profusely branched. [(7)] The species used in this experiment for determining antibacterial activity were *Bacillus subtilis* and *Proteus vulgaris*.

The following study was undertaken to synthesize silver nanoparticles from leaves of *Saraca asoca* and to study its anti-bacterial activities.

MATERIALS AND METHODS

Collection and identification of plant material

The fresh leaves of *Saraca asoca* were collected from the local park near the P&T Square, Bhopal (M.P.) India. The plant was authenticated by Dr. Zia-Ul-Hassan Head of the Department of Botany at the Safia college of Science Peer Gate, Bhopal, India and the voucher specimen (522/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 *Saraca asoca* were thoroughly washed with tap water and dried in shade and powdered. The extract was obtained by maceration in two solvents; petroleum ether and chloroform. The *Saraca asoca* extract was concentrated by air drying at room temperature. [(13-14)]

Microbial strain

To study the antibacterial property of the leaf extract of *Saraca asoca* the standard strains of *Proteus vulgaris* (0278) and *Bacillus subtilis* (1305) was obtained from Pinnacle Biomedical Research Institute, Bhopal, M.P, India. The strain was maintained on Nutrient Agar Media slants at 4°C temperature.

Phytochemical investigation

Qualitative investigation

The petroleum ether and chloroform extract of leaves of *Saraca asoca* were chemically tested in order to determine the phytochemicals present in it. The standard protocol for the identification of phytochemicals was followed for the phytochemical screening [(15-17)]

Quantitative investigation

Total flavonoids content

The total flavonoid content was estimated by Aluminum Chloride calorimetric assay(we use UV spectrophotometric method for this) where

formation of acid stable complexes with C-4 keto group takes place either at C-3 or C-5 hydroxyl group in addition to $AlCl_3$. Aluminum also forms complexes with ortho-dihydroxyl group with ring of flavonoids that are acid labile. The extract was dissolved in methanol to prepare a required concentration. It was followed by the preparation at five different concentrations by diluting the correct amount of extract with methanol. Then few ml of each concentration was diluted with distilled water followed by addition of $NaNO_2$ solution. Then after few minutes of incubation at room temperature, $AlCl_3$ solution was added to each test tube containing different concentration. It was then allowed to stand for few more minutes where NaOH was further added followed by immediate dilution with distilled water so as to make up a total volume required. The mixture was then again allowed to stand for about few minutes and finally the absorbance was then taken at 510 nm in UV-Visible spectrophotometer (SYSTRONIX 2202). [18]

Total phenolic content

The total phenolic content of the extract was determined by the procedure involving Folin-Ciocalteu reagent. The principle it involves is that it reacts with any reducing agents therefore measuring the total reducing capacity of the given sample. Test sample was prepared and from it four different required concentrations were made. Then little ml of these concentrations was taken in which Folin-Ciocalteu reagent was added, followed by the addition of sodium carbonate solution. With intermittent shaking the mixture was incubated for 30 minutes at room temperature. Then using methanol as blank, absorbance was measured at 765nm using double beam UV- Visible spectrophotometer (SYSTRONIX 2202). [19]

Preparation of leaves for nanoparticles:

25 g of leaves were weighing thoroughly washed in distilled water for 5 min, dried, cut into fine pieces and were boiled in a 500 ml Erlenmeyer flask with 100 ml of sterile distilled water up to 15 min and were filtered.

Synthesis of silver nanoparticle

For the synthesis of silver nanoparticle 1mM of Silver nitrate was added to the plant extract continuously while the sample was stirred on a magnetic stirrer. The sample was then incubated for 24 hours in dark and then after 24 hours and subsequently 48 hours the maximum absorbance of the sample was measured using the UV- Visible Spectrophotometer (SYSTRONIX 2202). Then the silver nanoparticles were purified by centrifugation and synthesized by drying the purified sample for further characterization. [20]

Particle size analyzer

The sample was lyophilized and PSA Analysis was performed. It was then dispersed by the nanoparticle analyzer (HORIBA) to determine the size of the particles.

RESULT

The zeta potential (mean) of the sample was 50.1mV while the electrophoretic mean was -0.000388cm^2 /Vs.

UV-VIS spectrophotometry analysis

For finding the maximum absorbance the sample was observed under UV-VIS Spectrophotometer (SYSTRONIX 2202) and the range of wavelength used was from 250-800nm in order to confirm the reduction of Silver nitrate.

Antibacterial activity

The anti-bacterial activity of extracts and silver nanoparticle was determined by agar well diffusion method. The sterilized media was poured into Petri plates and inoculation was done using spread plate method. The wells were prepared by a cup borer. Different concentrations of extract and silver nanoparticle were used to determine the antimicrobial activity. That is 25 mg/ml to 100 mg/ml and In case of extract four wells were made in concentration ranging from 25 to 100 mg/ml respectively. The plates were incubated for 24 hours at 37°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 ± Standard Deviation. [19]

Antioxidant activity

DPPH assay (2,2-DIPHENYL-1-PICRYLHYDRAZYL)

The radical scavenging activity of *Saraca asoca* with chloroform extract was measured using DPPH assay using method described by. [21] The decrease in the absorption of DPPH solution after the addition of antioxidant was measured at the wavelength of 490nm. The radical scavenging of antioxidants takes place through DPPH by donation of proton. On reduction the color changes from purple to yellow as with free radical inhibition the radical scavenging activity increases. The decrease in coloration indicated the potential of antioxidants. The reaction mixture containing 1 ml of DPPH was prepared with methanol. The various concentrations of the mixture were taken and then incubated at room temperature. Later the absorption of resulting solution was measured at 490 nm using UV Spectrophotometric method.

The % inhibition was calculated using the following equation-

DPPH radical scavenging activity (%) = [(Absorbance of control –Absorbance of sample)/Absorbance of control] × 100

Reducing power assay

Reducing Power assay activity of plant *Saraca asoca* extract was measured using the protocol given by Jain *et al*., 2011. To begin with initially different concentrations of test sample was prepared. The little amount of extract was taken and mixed with phosphate buffer and potassium ferricyanide. The mixture was then allowed to be incubated at about 50ºC for 20 min. The mixture was cooled down and later trichloroacetic acid was added followed by addition of 0.1% ferric chloride. The absorbance was finally measured at 700nm in ELISA reader (Kelvinator) against the blank sample. The increasing value of absorbance of reaction mixture indicated the increased reducing power assay.

The absorbance was reduced that indicated increase in the reducing power activity measured by following equation-

% reducing power = [(Absorbance of control – Absorbance of sample)/Absorbance of control] × 100

Statistical Analysis

The data concerning the anti-bacterial activity is presented in the Mean ± SD form. The State- 32 One Way ANOVA software was used for data analysis followed by the Bonferroni t-test. And the level of significance was considered as $P<0.050$ and $P<0.00$ (n=4).

RESULTS

Phytochemical investigation

Qualitative Analysis

The qualitative phytochemical analysis was performed as per the standard protocols. It revealed the presence of several compounds like tannin, saponins, phenol etc in the extracts as tabulated in Table 1.

TABLE 1: Qualitative Phytochemical Analysis

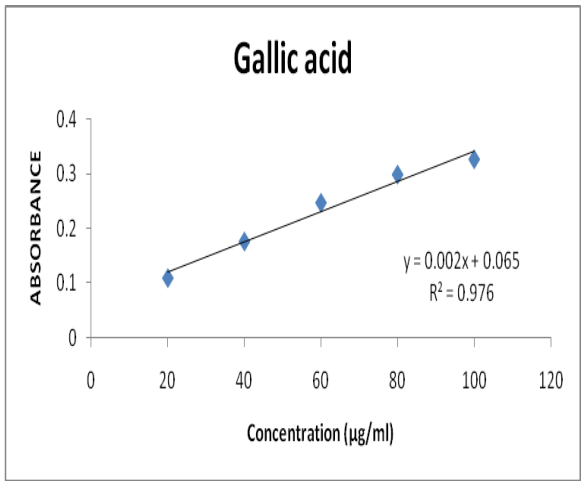
S. No.	Phytoconstituent	Name of Test	Petroleum ether	Chloroform
1.	Carbohydrate	Molish Barfoed	- -	- -
2.	Proteins	Biuret Ninhydrin	- -	- -
3.	Glycosides	Keller-Killiani	-	+
4.	Alkaloids	Mayer's Wagner	+ +	+ +
5.	Saponin	Froth	-	-
6.	Flavonoids	Lead acetate Alkaline reagent	+ +	+ +
7.	Triterpenoid and Steroid	Libermann Burchard's	+	+
8.	Phenol and Tanin	Gelatin Lead acetate	+ +	+ +

Quantitative Phytochemical Investigation

Under quantitative phytochemical analysis the total phenolic content (Table 2 and 3) and total flavonoid content (Table 4 and 5) of the extract were calculated with the help of calibration curve.

TABLE 2: Total Phenolic Content of Gallic Acid (standard)

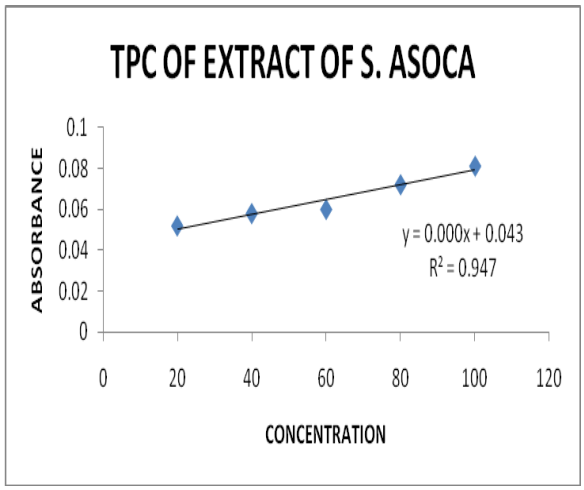
S. No.	Concentration (µg/ml)	Absorbance (nm)
1.	20	0.1098
2.	40	0.1763
3.	60	0.2468
4.	80	0.2981
5.	100	0.3258



Graph 1: Total Phenolic Content of Gallic Acid

Table 3: Total Phenolic Content of Extract of *S. asoca*

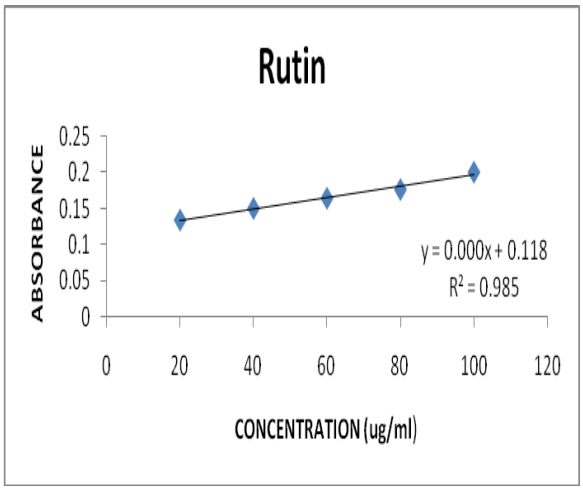
S. No.	Concentration (µg/ml)	Absorbance (nm)
1.	20	0.052
2.	40	0.058
3.	60	0.06
4.	80	0.072
5.	100	0.081



Graph 2: Total Phenolic Content of Extract of *S. asoca*

Table 4: Total Flavonoids Content of Rutin (standard)

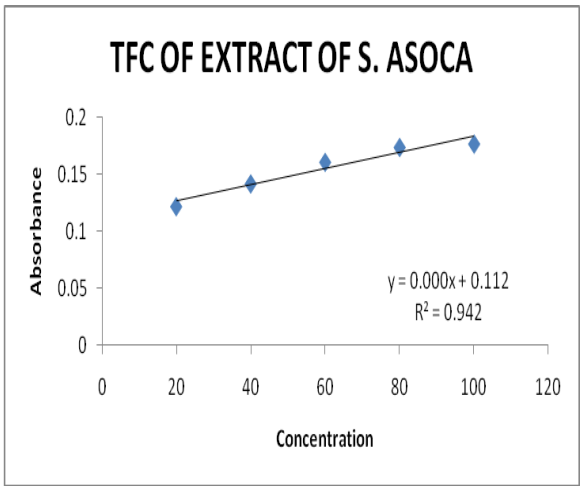
S. No.	Concentration (µg/ml)	Absorbance (nm)
1.	20	0.135
2.	40	0.151
3.	60	0.165
4.	80	0.177
5.	100	0.201



Graph 3: Total Flavonoid Content of Rutin

Table 5: Total Flavonoids Content of Extract of *S. asoca*

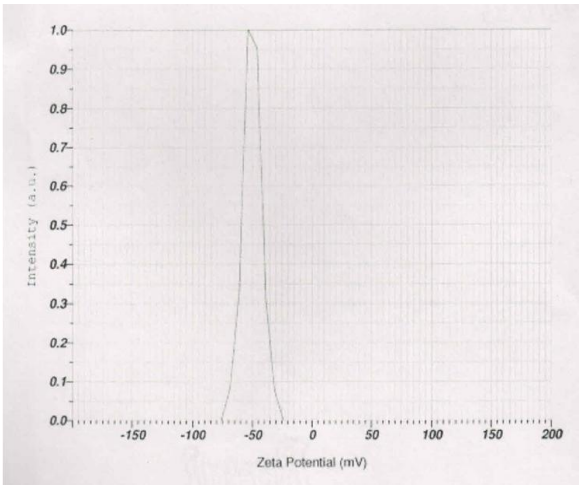
S. No.	Concentration (µg/ml)	Absorbance (nm)
01.	20	0.122
02.	40	0.142
03.	60	0.161
04.	80	0.174
05.	100	0.177



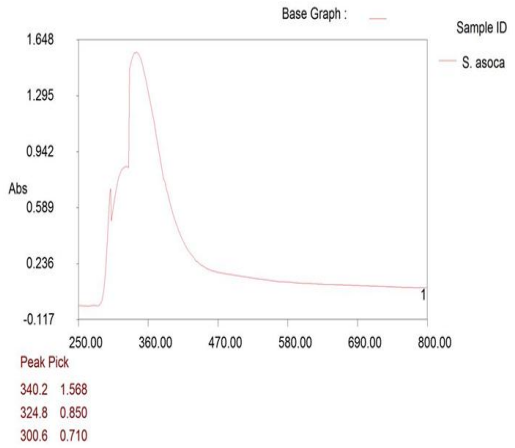
Graph 4: Total Flavonoid Content of *S. asoca*

Antibacterial activity

The antimicrobial activity of silver nanoparticles (Table 6) as well chloroform extract (Table 7) of S. asoca was tested against the bacterial strains of B. subtilis and P. vulgaris using well diffusion assay.



Graph 5: Zeta Potential of Nanoparticles

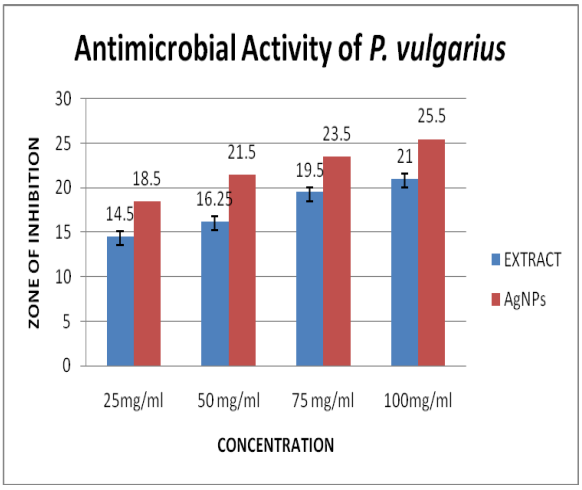


Graph 6: UV-Spectral Analysis of Nanoparticles

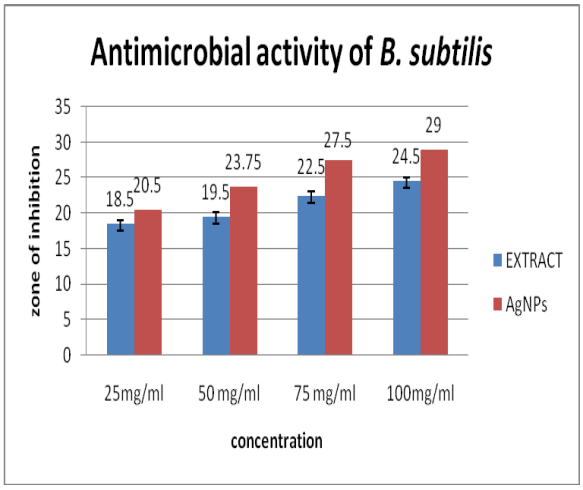
UV- Spectral Analysis:

Table 6: antibacterial activity of *saraca asoca* against *P. vulgaris*

Organism	25mg/ml	50mg/ml	75mg/ml	100mg/ml	Standard (10µg/ml)
EXTRACT	18.5±0.577	21.5±0.577	23.5±0.577	25.5±0.577	30.33±0.577
AgNPs	14.5±0.577	16.25±0.957	19.5±0.577	21.0±0.000	30.33±0.577



Graph 7: Well Diffusion Assay of *P. vulgaris*



Graph 8: Well Diffusion Assay of *B. Subtilis*

Table 7: Antibacterial Activity of *Saraca asoca* against *B. subtilis*

Organism	25mg/ml	50mg/ml	75mg/ml	100mg/ml	Standard (10µg/ml)
Extract	18.50±0.577	19.50±0.577	22.50±0.577	24.50±0.577	30.33±0.577
AgNPs	20.50±0.577	23.75±1.893	27.50±0.500	29.0±0.500	30.33±0.577

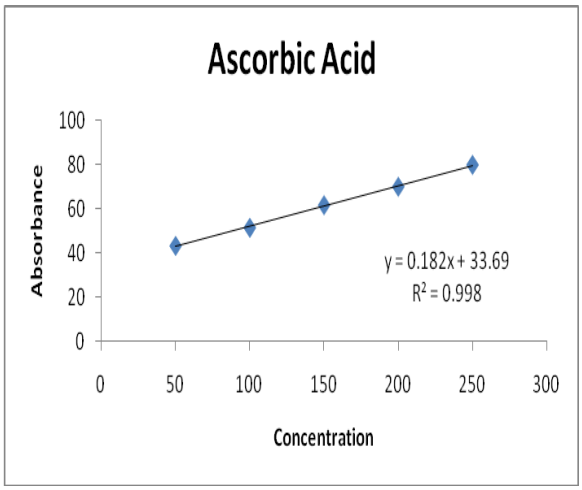
Antioxidant activity

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

The DPPH Assay was performed to know the antioxidant properties of the extract (Table 9).

Table 8: DPPH Assay Standard

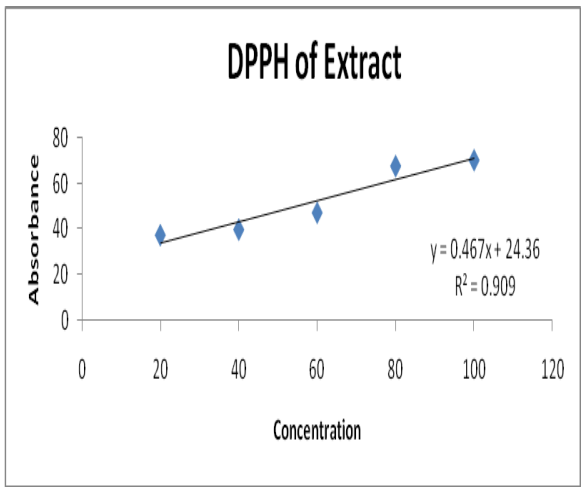
S. No.	Concentration (µg/ml)	Absorbance (nm)
1.	20	0.397
2.	40	0.34
3.	60	0.269
4.	80	0.211
5.	100	0.142



Graph 9: DPPH assay of Ascorbic Acid

Table 9:DPPH assay (2,2-diphenyl-1-picrylhydrazyl) of extract of *S. asoca*

S. No.	Concentration (µg/ml)	Absorbance (nm)
1.	20	0.389
2.	40	0.374
3.	60	0.328
4.	80	0.201
5.	100	0.185



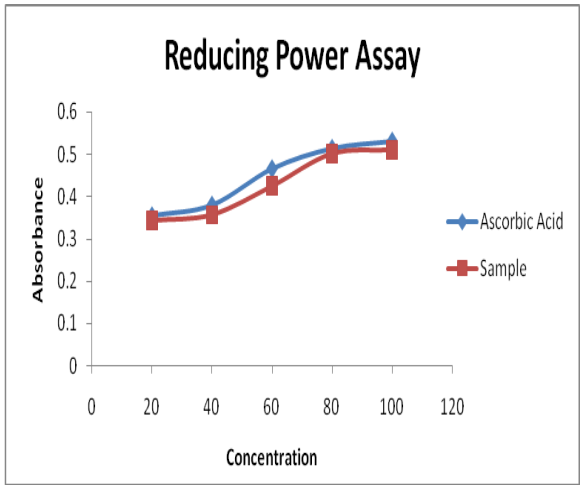
Graph 10: DPPH assay of extract of *S. asoca*

Reducing power assay

The reducing power assay of the extract was demonstrated (Table 10) and was accompanied by decrease in absorbance as the concentration of the plant sample increased.

Table 10: Reducing Power Assay of Extract of *S. asoca*

S. No.	Concentration (µg/ml)	Absorbance (nm)	
		Ascorbic Acid	Sample
1.	20	0.354	0.343
2.	40	0.379	0.357
3.	60	0.465	0.425
4.	80	0.513	0.502
5.	100	0.530	0.511



Graph 11: Reducing Power Assay of *S. asoca* Extract

DISCUSSION

The qualitative phytochemical analysis was performed as per the standard protocols and it was observed that the extracts had several compounds present as stated in Table 1. As per the experimental analysis the extract prepared with petroleum had presence of alkaloids, flavonoids, steroids, triterpenoids, phenol, and tannins. On other hand the chloroform extract had glycosides, alkaloids, tannins, phenol, triterpenoids and steroids present in it.

With the help of quantitative phytochemical analysis the total phenolic content (Table 3) and total flavonoid content of the extract was calculated (Table 5). The total phenolic content of the chloroform extract of S. asoca was calculated with the help of calibration curve and was observed to be 0.947 gallic acid equivalent/gram as shown in Graph 2. Similarly the data acquired from the calibration graph of total flavonoid content of extract, was found to be 0.942Rutin equivalent/gmas stated in Graph 4. Also the Zeta potential of the sample was determined as 50.1mV while the electrophoretic mean was observed to be 0.000388cm 2 /Vs.

The antimicrobial activity of the silver nanoparticles as well of the sample prepared in chloroform extract was tested using the bacterial strains as *B. subtilis* and *Proteus vulgaris* using well diffusion technique. On comparing the results both in tabulating and graphical form it was found that the silver nanoparticles showed greater antimicrobial activity against *B. subtilis* strain while the chloroform sample was most effective against *Proteus vulgaris*. The activity of *Proteus vulgaris* is shown in Graph 7 and that of *B. subtilis* is shown in Graph 8. The antimicrobial activity of AgNPs was found to be greater against *B. subtilis* bacterial strain than that of the plant extract (Table 7). The leaves extract depicted greater antibacterial activity than silver nanoparticles (±14.4mm mean zone of inhibition 0.577 standard deviation) against *P. vulgaris* with 18.5mm mean zone of inhibition ±0.577 standard deviation (Table

6). On the other hand, in case of *B. subtilis* the antibacterial activity of silver nanoparticles was proved to be greater with 20.50mm mean zone of inhibition ±0.577 standard deviation, while the extract showed mean zone of inhibition of 18.50mm, ±0.577 standard deviation (Table 7).

For antioxidant assay the sample was experimented using various concentrations of extract and treating with DPPH scavenging assay and Reducing Power assay. The maximum value of DPPH scavenging for sample was analyzed at 100 µg/ml concentration of the extract (Table 9).

CONCLUSION

Saraca asoca is regarded as the universal plant used in the Ayurvedic medicine. We have here demonstrated the use of *Saraca asoca* for the synthesis of silver nanoparticles. Also since the living organisms have a huge potential for the synthesis of silver nanoparticles they were also used. This plant acts as the versatile plant source for phenolic and flavonoid compounds. The leaves of *Saraca asoca* were used to determine the antimicrobial activities of the plant. With the pharmacological activities this plant carries with it, the use of Saraca asoca should be promoted for control of various diseases.

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