Available online on 15.06.2019 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Research Article

Preparation, Characterization and Evaluation of Silver Nanoparticles of *Thunbergia Grandiflora* and Its Antimicrobial Activity

Priyanka Pal*, Naveen Gupta, Shailesh Jain

Patel College of Pharmacy, Ratibad, Bhabdhada Road Bhopal (MP) 462044, India

ABSTRACT

Nanoparticles are gaining interest in biomedical applications due to its importance such as anti-bacterial, anti-fungal and anti-cancer agents. A conventional method for the synthesis of metal nanoparticles involves toxic reagents which produce harmful by-products and are hazardous to the environment. To overcome these limitations, green synthesis of nanoparticles was established. Eco-friendly methods using plant extracts are gaining popularity due to the abundance of raw materials and the production of non-toxic by-products threatening to the environment. Moreover, the nanoparticles synthesized from the plant extract are cost-effective. In addition, nanoparticles produced by green synthesis methods produce synergetic effect where both the nanoparticles as well as the natural bioactive constituents of the plant influence the biocidal properties. The present investigation evaluates phytochemical screening, antimicrobial, antioxidant activities and green synthesis, characterization of silver nanoparticles and its antimicrobial activity. Three dissimilar solvents viz., petroleum ether, ethyl acetate and methanol were used to prepare crude extracts of *T. grandiflora* leaves. Antioxidant activity was examined by means of DPPH and reducing power assay method. AgNPs were synthesized by using 1mM $AgNO_3$ solution mixed with leaf aqueous extract of *T. grandiflora*. The characterization of the prepared AgNPs was done by UV-Vis spectrometry and FTIR spectroscopy. Antimicrobial activity was studied by agar well diffusion method. The phytochemical screening results unveiled the bearing of different phytochemicals viz., flavonoids, alkaloids, saponins, carbohydrates, terpenoids, steroids, tannins and free anthraquinones particularly with relatively high abundance in methanol extract. The total phenolics content of leaves of methanolic extract was (0.058mg/gm), followed by flavonoids (1.080mg/gm). Likewise methanol extract too exhibited effective free radical scavenging and antimicrobial activities were concentration dependent. The characterization results of the prepared AgNPs displayed that the silver nanoparticles are formed and stabilized by plant phyto-constituents and also exhibited virtuous antimicrobial property. Green synthesis process is a pivotal area in nanotechnology and usage of natural resources is the best choice for the making of NPs as a sustainable, eco-friendly, inexpensive and free of chemical contaminant method. These AgNPs have several potential biological and medical applications.

Keywords: *Thunbergia grandiflora,* Phytochemical screening, Antioxidant activity, Silver nanoparticles, FTIR spectroscopy, Antimicrobial activity.

Article Info: Received 25 April 2019; Review Completed 27 May 2019; Accepted 31 May 2019; Available online 15 June 2019

Cite this article as:

Pal P, Gupta N, Jain S, Preparation, Characterization and Evaluation of Silver Nanoparticles of *Thunbergia Grandiflora* and Its Antimicrobial Activity, Journal of Drug Delivery and Therapeutics. 2019; 9(3-s):229-235

http://dx.doi.org/10.22270/jddt.v9i3-s.2830

***Address for Correspondence:**

Miss Priyanka Pal, Patel College of Pharmacy, Ratibad, Bhabdhada Road Bhopal (MP) 462044, India

INTRODUCTION

There is an imperative need to explore new warfare schemes to combat against multi drug resistant bacteria and also to subdue the problems of chemical drug consumption in order to control the microbial infections. These drugs from plants are less harmful; side effects are minimal and also cost effective[1]. Plants and their parts such as roots, stems, barks, leaves, flowers, fruits, seeds and exudates form an important major ingredient of drugs used in traditional herbal medicinal systems. The therapeutic competence of the drugs used in these systems really depends on the use of proper and authentic raw materials[2]. The screening of medicinal plant extracts and plant products for antimicrobial and antioxidant properties show that many of such plants are primary sources of antibiotics[3]. Native groups have used curing plants as their personal phytomedical remedies[4]. To control human diseases antioxidant effects play an important role. Reactive oxygen species (ROS) related to lipid peroxidation is responsible for most of the pathogenesis[5]. Antioxidants provide confrontation against the oxidative stress by scavenging free radicals. Antioxidant activity is one of the most significant properties of plant extracts, because scientists have looked for sources of natural antioxidants to be introduced in many cosmetic, pharmaceutical and food formulations. The synthesis of nanomaterials is currently

one of the most active areas in nanoscience. Special attention has been dedicated to nanomaterials that help improve the human quality life. A remarkable example is the silver nanoparticles (AgNPs) which are known by their inhibitory and bactericidal effects. AgNPs can be produced with various sizes and shapes depending on the fabrication method which can be physical, chemical, biological and hybrid. The chemical methods use toxic chemicals, which are not friendly to environment making them unsuitable for biomedical applications. Specifically, the widely used chemical reduction methods[6] usually employ toxic and perilous chemicals that are responsible for various biological risks. On the other hand, physical methods are expensive and incompatible with sizeable production of nanoparticles. Therefore, to avoid toxic and hazardous chemicals, the green synthesis methods have been developed, attracting significant interest because they are environment friendly, rapid, facile and energy-efficient[7]. Green synthesis using huge biological molecules derived from plant extracts[8]could facilitate size and morphology control of metal nanoparticles due to the presence of an innumerable quantity of biomolecules possessing bioreduction and biostabilization ability[9]. Specifically, many plants have been used for silver nanoparticles synthesis[10], such as stem bark of *Callicarpa maingayi*, *Terminalia cuneata*, *Illicium verum* (star anise) and pod extract of *Acacia nilotica*[11]. *T. grandiflora* Roxb. exRottler (Acanthaceae) is an evergreen vine. It is a large climbing and twining plant, native to China, India, Nepal, Indochina and Burma and many tropical countries of Africa[12-15]. Generally; it is known as black clock vine and blue trumpet vine. Other common names include Bengal clockvine, Bengal trumpet, blue skyflower, blue thunbergia, clock vine, skyflower and skyvine[14]. The shape and texture of the leaf as well as being oval and narrowing to pointed tip makes the plant different from other *thunbergia* species. The trumpet-shaped flowers have a short broad tube, blue to mauve on the outside, pale yellow inside, which expands to five rounded, pale lavender-blue petals, one larger than the others. The seed is flat, up to 1 cm long and covered with brown scales, the pods containing the seeds are ejected several metres upon ripening[13]. The plant is cultivated as house plant in temperate regions and has gained the Royal Horticultural Society's Award of Garden Merit[15,17]. The species is used for the treatment of blood dysentery, cataract, diabetes, gout, hydrocele, hysteria, malaria, marasmus, post eclampsia, pre-eclampsia, rheumatism, spermatorrhoea[18], stomach complaints[19], ophthalmia, conjunctivitis[20], elephantiasis, and urinary bladder stone[21]. Phytochemical studies of *T. grandiflora* confirmed several active compounds including iridoid glycosides, isounedoside, and grandifloric acid[22]. The plant has also been reported to possess important pharmacological effects including antibacterial[23], anti-inflammatory and anti-arthritic effect[24]. Due to enormous pharmacological and ethnobotanical benefits of *T. grandiflora*, the present study aimed to assess the qualitative/quantitative nature of the phytochemical constituents and to evaluate the antimicrobial and antioxidant activities of *T. grandiflora* leaf extracts. Green synthesis of plant leaf mediated silver nanoparticles; its characterization and antimicrobial activity were also attempted as there is no earlier report on the green synthesis of *T. grandiflora* leaf AgNPs.

MATERIALS AND METHODS

Plant material

The leaves of *T. grandiflora* were collected from local area of Bhopal (M.P.) in the month of February, 2018.The sample was identified by senior Botanist Dr. Zia-Ul-Hassan, Professor and head of department of Botany, Safia College of Arts and Science, peer gate Bhopal. A herbarium of plants was submitted to the specimen library of Safia College of Arts and Science, peer gate Bhopal and The specimen voucher no. of *T. grandiflora* is 119/Bot/Saf/41.

Chemical reagents

Silver nitrate ($AgNO_3$) is purchased from Sigma-Aldrich Chemicals for this study. Dimethyl sulphoxide (DMSO) was purchased from Merck, India. The pH buffer tablets were purchased from Himedia. Nutrient Agar, Nutrient Broth, Agar Agar, Muller Hinton Agar (MHA) and Sabouraud Dextrose Agar Media were purchased from Himedia Laboratories, Mumbai, India. The aqueous solutions were prepared with triple distilled water. All the chemicals used in this study were of analytical grade.

Extraction of plant material

Cold maceration

Leaves of *T. grandiflora* were collected, washed and rinsed properly. They were dried in shade and powdered mechanically. About 500gm of the leaves powder was macerated with pet. ether, ethyl acetate and methanol and stored for 72 hours in ice cold condition for the extraction of phytochemicals. At the end of the third day extract was filtered using whatmann No. 1 filter paper to remove all un-extractable matter, including cellular materials and other constitutions that are insoluble in the extraction solvent. The entire extract was concentrated to dryness using rotary flash evaporator under reduced pressure and stored in an air tight container free from any contamination until it was used. Finally the percentage yields were calculated of the dried extracts[25]. For extraction of aqueous extract, 250g of powdered plant material was soaked in 450mL of water and kept at room temperature for 48 hours with periodical shaking. The solvent extract was filtered through muslin cloth and collected in a beaker and then filtrate was place on water bath for solvent evaporation. Finally browinesh black, solid crude aqueous leaf extract of plant obtained.

Qualitative phytochemical analysis of plant extract

The *T. grandiflora* leaves extract obtained was subjected to the preliminary phytochemical analysis following standard methods by Khandelwal and Kokate[26,27]. The extract was screened to identify the presence or absence of various active principles like phenolic compounds, carbohydrates, flavonoids, glycosides, saponins, alkaloids, fats or fixed oils, protein and amino acid and tannins.

Quantification of secondary metabolites

Quantitative analysis is an important tool for the determination of quantity of phytoconstituents present in plant extracts. For this TPC and TFC are determined. Extracts obtained from leaves of *T. grandiflora* plant material of subjected to estimate the presence of TPC and TFC by standard procedure.

Total phenolic content estimation

The amount of total phenolic in extracts was determined with the Folin Ciocalteu reagent. Concentration of (20-100 µg/ml) of gallic acid was prepared in methanol. Concentration of 100 µg/ml of plant extract were also prepared in methanol and 0.5ml of each sample were introduced in to test and mixed with 2 ml of a 10 fold dilute folin Ciocalteu reagent and 4 ml of 7.5% sodium carbonate. The tubes were covered with para film and it was then incubated at room temperature for 30 min with intermittent shaking and the absorbance were taken at 765 nm against using methanol as blank. Total phenolic content was

calculated by the standard regression curve of gallic acid and the results were expressed as gallic acid equivalent (mg/g) [28].

Total flavonoid content estimation

Different concentration of rutin (20 to 100µg/ml) was prepared in methanol. Test sample of near about same polarity (100µg/ml) were prepared. An aliquot 0.5ml of diluted sample was mixed with 2 ml of distilled water and subsequently with 0.15 ml of a 5% $NaNO_2$ solution. After 6 min, 0.15 ml of a 10% $AlCl_3$ solution was added and allowed to stand for 5min, and then 2 ml of 4% NaOH solution was added to the mixture. The final volume was adjusted to 5ml with distilled water and allowed to stand for another 15 min. Absorbance was determined at 510 nm against water as blank. Total flavonoid content was calculated by the Standard regression curve of Rutin/ Quercetin[29].

Antioxidant activity

DPPH radical scavenging activity

For DPPH assay, the method of Gulçin *et al.,* 2006[30] was adopted. A solution of 0.1mM DPPH (4mg/100ml) in methanol was prepared and 1 ml of this solution was mixed with 1 ml of different concentrations of the different extracts. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. Ascorbic acid was used as reference standard while methanol was used as control. Reduction of the stable DPPH radical was used as a marker of antioxidant capacity of *T. grandiflora* extracts. The change in colour was measured at 517 nm wavelength using methanolic solution as a reference solution. This was related to the absorbance of the control without the plant extracts. The percentage inhibition of free radical DPPH was calculated from the following equation: % inhibition = [(absorbance of control - absorbance of sample)/absorbance of control] × 100%. All the tests were carried out in triplicates. Though the activity is expressed as 50% inhibitory concentration (IC50), IC50 was calculated based on the percentage of DPPH radicals scavenged. The lower the IC50 value, the higher is the antioxidant activity.

Reducing power assay

A spectrophotometric method was used for the measurement of reducing power. For this 0.5 ml of each of the extracts was mixed with 0.5ml phosphate buffer (0.2 M, pH 6.6) and 0.5 ml of 1% potassium ferricyanide (10 mg/ml). The reaction mixture was incubated at 50ºC for 20 min separately and then rapidly cooled, mixed with 1.5 ml of 10% trichloroacetic acid and centrifuged at 6500 rpm for 10 min. An aliquot (0.5ml) of the supernatant was diluted with distilled water (0.5ml) and then ferric chloride (0.5ml, 0.1%) was added and allowed to stand for 10 min. the absorbance was read spectrophotometrically at 700 nm. Ascorbic acid (AA) was used as standard for construction of calibration curve[31].

$$\text{Reducing Power (\%)} = (As / Ac) \times 100$$

Here, Ac is the absorbance of control (AA) and as is the absorbance of samples (extracts) or standards.

Green synthesis of silver nanoparticles

100 ml of 1mM aqueous solution of $AgNO_3$ solution was taken in Erlenmeyer flask and 15 ml of aqueous stem extract was added drop wise into it for bio reduction process at room temperature. The reaction mixture was allowed to stir at 200 rpm using magnetic stirrer till the solution was turned from yellow to dark brown indicating the formation of AgNPs. The reduced solution was centrifuged at 5000 rpm for 30 min to get clear supernatant. The supernatant was discarded and the particles obtained were centrifuged with water repeatedly to get pure nanoparticles.

Characterization of silver nanoparticles

UV-spectrophotometer analysis

The synthesized AgNPs formulation F3 (solution of 1 mg/ml in distilled water as a dispersive medium) were monitored using UV-Vis spectrophotometer (Systronics double beam spectrophotometer 2202, India) between the range of 200nmand800nm. Distilled water was used as blank for UV-vis Spectrophotometer analysis.

Fourier transforms infra red spectroscopy (FTIR)

The infrared spectra for the plant extract and synthesized AgNPs were attained for the identification of functional groups in a (Perkin Elmer Spectrum 2, Germany) spectrophotometer IR affinity-1 by employing KBr pellet technique and registering amplitude waves ranging from 400 to 4000 cm−1.

Antibacterial activity study

Antibacterial study of different extract was carried out using agar well diffusion assay against selected bacterial strains. Prepared AgNPs were diluted to the concentration of 50, 100, 200 and 250 mg/ml utilizing DMSO as the solvent. Nutrient agar media was prepared. Microbial strains grown in nutrient media were used. Microbial suspension of density 10^8 CFU/ml were used for inoculation on the Nutrient agar media. Four wells of 6 mm diameter and 5 mm depth were prepared on the solid agar in each plate using a sterile borer. Different concentrations of extracts, positive control (ofloxacin 10 µg/ml) and negative control (DMSO) were dispensed (50 µl). The plates were allowed to stand for 1 h at room temperature for diffusion of the extract and incubated at 37°C for 24 h. After 24 h, the zones of inhibition were measured using a digital Verniercaliper. All experiments were conducted in triplicate and the mean values of the diameter of inhibition zones ± standard deviations were calculated.

Antifungal activity

Antifungal study of prepared AgNPs was carried out using well diffusion assay. AgNPs were diluted to the concentration of 50, 100, 200 and 250 mg/ml utilizing DMSO as the solvent. Sabouraud dextrose agar media was prepared. Fungal strains (*A. niger* and *C. krusei*) grown in Sabouraud broth were used. Microbial suspension of density 10^8 CFU/ml were used for inoculation on the Sabouraud dextrose agar media. Four wells of 6 mm diameter and 5 mm depth were prepared on the solid agar in each plate using a sterile borer. Different concentrations of extracts, positive control (Amphotericin B 10 µg/ml) and negative control (DMSO) were dispensed (50 µl). The plates were allowed to stand for 1h at room temperature for diffusion of the extract and incubated at 28°C for 48 h. After 48 h, the zones of inhibition were measured using a digital Verniercaliper. All experiments were conducted in triplicate and the mean values of the diameter of inhibition zones ± standard deviations were calculated.

RESULTS AND DISCUSSIONS

The crude extracts so obtained after each of the successive maceration extraction process were concentrated on water bath by evaporation the solvents completely to obtain the actual yield of extraction. The percentage yield of extraction is very important in phytochemical extraction in order to evaluate the standard extraction efficiency for a particular

plant, different parts of same plant or different solvents used. The yield of extracts obtained from the leaves of the plants using petroleum ether, ethyl acetate and methanol as solvents are depicted in the Table 1. The results of qualitative phytochemical analysis of the crude powder of leaves of *T. grandiflora* are shown in Table 2. Methanolic extracts of leaves sample of *T. grandiflora* showed the presence of alkaloids, flavonoids, phenols, tannins and glycosides but in Pet ether and ethyl acetate extracts all phytoconstituents was absents, only carbohydrates, alkaloids presents.

Table 1 Results of percentage yield of leaves extracts

Plant Name	Percentage yield (%)		
	Pet. Ether	Ethyl acetate	Methanol
T. grandiflora	4.6	5.2	6.9

Table 2 Phytochemical evaluation of *T. grandiflora* leaves

Tests	Pet ether	Ethyl acetate	Methanol
Carbohydrates			
Molish	+ ve	- ve	+ ve
Fehlings	+ ve	- ve	+ ve
Benedit's	+ ve	- ve	+ ve
Protien & amino acids			
Biurets	- ve	- ve	+ ve
Ninhydrin	- ve	- ve	+ ve
Glycosides			
Borntrager	- ve	+ ve	+ ve
Killer killani	- ve	+ ve	+ ve
Alkaloids			
Mayers	+ ve	- ve	+ ve
Hagers	+ ve	- ve	+ ve
Wagners	- ve	- ve	+ ve
Saponins			
Froth	- ve	- ve	+ ve
Flavonoids			
Lead acetate	- ve	- ve	+ ve
Alkaline reagent test	- ve	+ ve	+ ve
Treterpenoids & Steroids			
Salwoski	+ ve	- ve	+ ve
Libberman Burchard	+ ve	+ ve	+ ve
Tannin & Phenolics			
Ferric chloride	- ve	- ve	+ ve
Lead acetate	- ve	- ve	+ ve
Gelatin	+ ve	- ve	+ve

Quantitative phytochemical assay was performed by calculating total phenolic content (TPC) and total flavonoid content (TFC). The TPC was calculated with respect to gallic acid (standard) and TFC was then calculated with respect to rutin taken as standard. The TPC and TFC in methanolic extract were found to be 0.058mg/gm and 1.080 mg/gm respectively table 3& fig 1, 2.

Table 3 Total phenolic and flavonoid content of extracts

Test	Methanolic extract
TPC	0.058 mg/gm equivalent to Gallic acid
TFC	1.080 mg/gm equivalent to Rutin

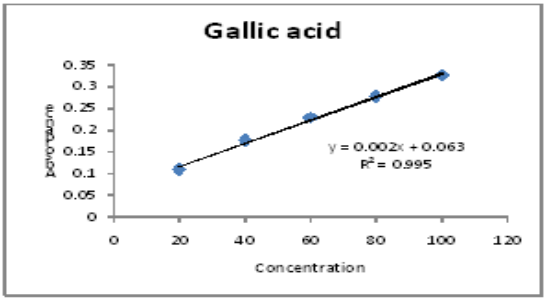

Fig. 1 Graph of estimation of total phenolic content

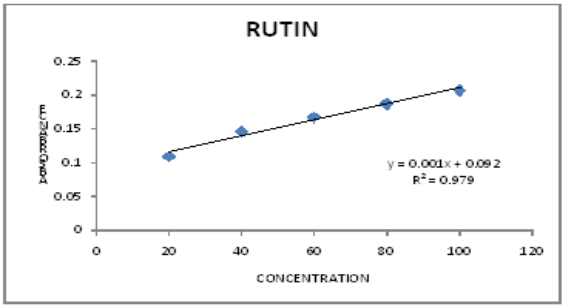

Fig. 2 Graph of estimation of total flavonoids content

Antioxidant activity of the samples was calculated through DPPH assay and reducing power assay. % inhibition was calculated as an indicative of antioxidant potency. The higher the % inhibition the better the activity. Ascorbic acid was taken as standard in both the tests and the values were comparable with concentration ranging from 20µg/ml to 100µg/ml. A dose dependent activity with respect to concentration was observed. % inhibition was higher in the ascorbic acid while the values were lesser in methanolic extract table 4. The reducing ability of the compound usually depends on the reductants, which have been exhibited antioxidative capacity by breaking the free radical chain, donating a hydrogen atom. Reducing power assay was

calculated in extracts and the values indicated a superior activity table 5& fig 3

Table 4 DPPH assay of ascorbic acid and methanolic extract

S. N.	Conc. (µg/ml)	Ascorbic acid (% Inhibition)	Methanolic Extract (% Inhibition)
1.	20	52.74123	47.80702
2.	40	56.35965	56.4693
3.	60	61.51316	65.57018
4.	80	68.9693	68.85965
5.	100	71.71053	74.01316
IC 50 Value		11.54	21.29

Table 5 Result of reducing power assay

S. N.	Conc. (µg/ml)	Ascorbic acid	Methanolic Extract
1.	20	0.987	0.533
2.	40	1.032	0.712
3.	60	1.145	0.716
4.	80	1.159	0.762
5.	100	1.196	0.800

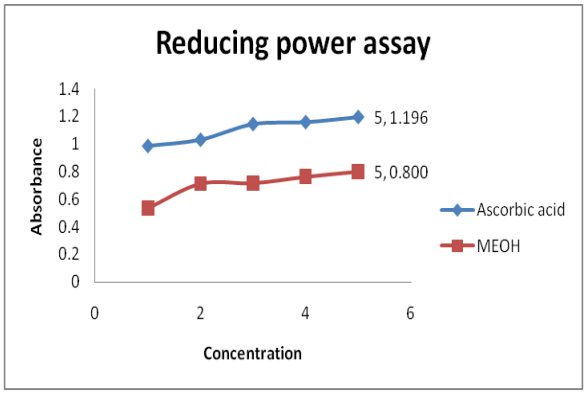

Fig 3 Reducing power assay

Synthesis of the AgNPs in aqueous solution was checked by recording the absorption spectrum at a wavelength range of 200-700 nm. The UV-Vis absorption spectrum of AgNPs Fig. 4 obtained showed the absorption maxima ranged from 408-442 nm which gives the confirmation for the AgNPs synthesis. FTIR spectra of *T. grandiflora* leaf extract and synthesized AgNPs are shown in Fig 5& 6.

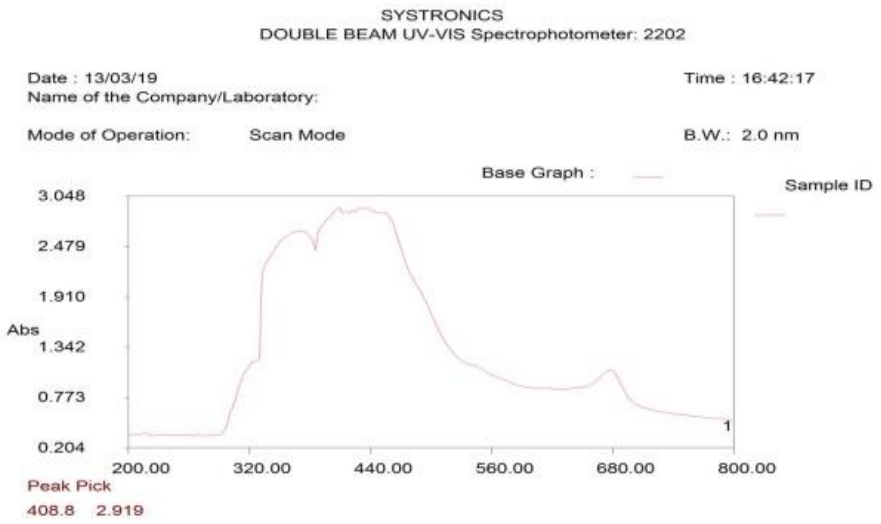

Fig. 4 UV-Vis spectra of *T. grandiflora* leaf extract AgNPs

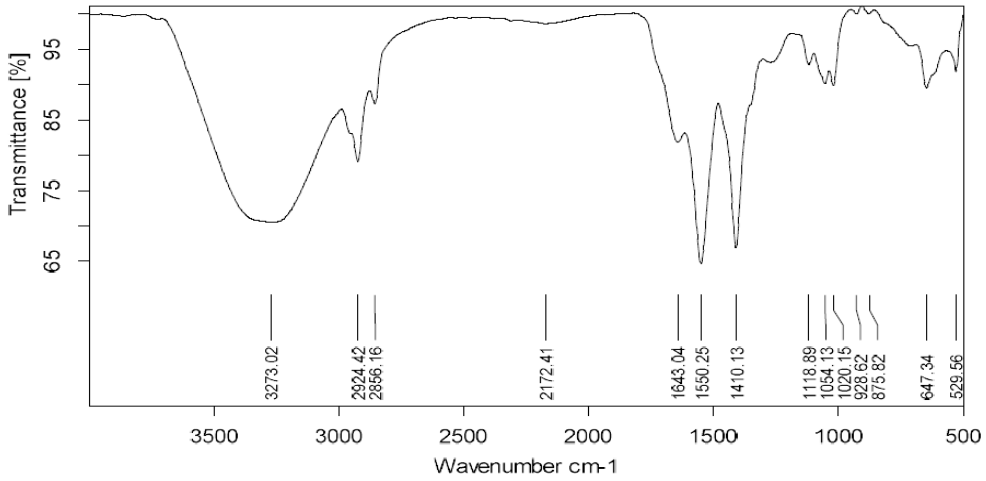

Fig. 5 FTIR of stem extract of *T. grandiflora*

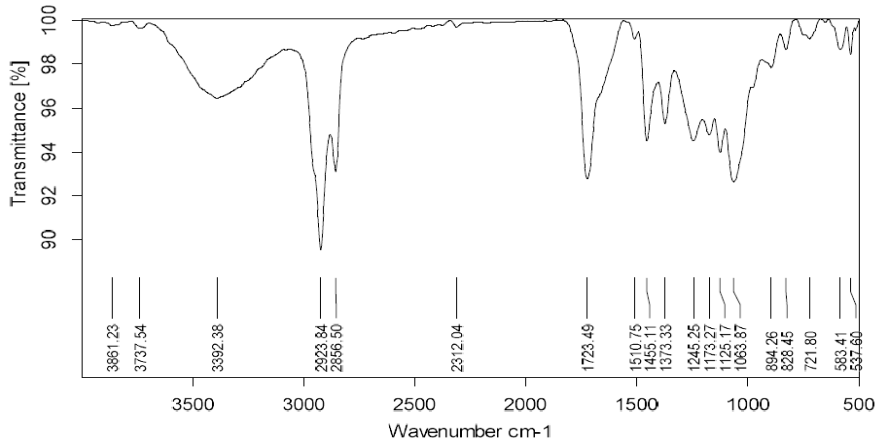

Fig. 6 FTIR of synthesized AgNPs

T. grandiflora plant is also possessed well antimicrobial activity. To study the antibacterial property of plant extract, $AgNO_3$, AgNPs (15ml), gram positive and gram negative bacteria were used and standard antibiotic ofloxacin were used in this study. Zone of inhibition against bacterial growth produced by AgNPs was compared to standard antibiotic ofloxacin. From the table, it is concluded that synthesized AgNPs exhibit inhibition zone nearly close to standard antibiotic values Table 6.

Table 6 Inhibition zone of extract, $AgNO_3$, AgNPs and antibiotic against four bacteria

Bioactive agent	Zone of inhibition (Diameter, cm)			
	Actinomyces	***Streptococcus mutans***	***Proteous vulgaris***	***Pseudomonas aeruginosa***
Extract	2.2	1.5	2.1	2.2
$AgNO_3$	2.3	1.4	1.5	1.5
AgNPs	2.8	2.1	1.6	1.8
Ofloxacin	3.1	2.9	2.8	2.8

The data revealed that significant reduction in growth of test fungus *A. niger* and *C. Krusei* was observed with the extract, $AgNO_3$, synthesized AgNPs and standard Amphotericin B shown in Table 7. Studies revealed that AgNPs may kill fungal spores by destructing the membrane integrity or may interact with phosphorus and sulphur containing compounds and their interaction may cause damage to DNA and protein which lead to cell death. In the present study reduction in colony diameter on prepared plates is an indication of antifungal activity of AgNPs. *C. krusei* was inhibited to higher extent comparative to *A. niger*.

Table 7 Inhibition zone of extract, $AgNO_3$, AgNPs and antibiotic against fungi

Bioactive agent	Zone of inhibition (diameter, cm)	
	Aspergillus niger	***Candida krusei***
Extract	2.4	2.8
$AgNO_3$	2.3	2.6
AgNPs	2.6	2.8
Amphotericin B	2.6	3.0

CONCLUSION

The present investigation involved the screening and evaluation of *T. grandiflora* leaf crude extracts for the phytochemicals, antimicrobial activity and antioxidant activity. Green synthesis of AgNPs using aqueous leaf extract, characterization and antimicrobial activity of synthesized nanoparticles were also carried out in the study. Outcome of all the experiments carried out suggests the existence of most of the phytochemicals in the leaves and are having some important biological activities. Further work is needed to isolate, purify and identify the exact active principle which is the cause for the biological activities. The Green synthesis is a simple, low cost and ecofriendly approach without any huge inputs in terms of energy. This is the first report of green synthesis of silver nanoparticles for this plant. Being exhibiting greater antimicrobial activity, phytochemical based nanoparticles may stand as a potential remedy in developing drugs against antibiotic resistant bacteria.

REFERENCES

1. Harishchandra MR, Rajan PR, Singh SRP. Study of krimighna effect of nimba (*Azadiracta indica* A.Juss.) Patra as rakshoghna dhoopan by culture and sensitivity method W.S.R. to pyogenic bacteria. Int Res J Pharma 2012; 3:142-146.
2. Imaga N OA et al. Antisickling property of *Carica papaya* leaf extract. African J Biochem Res 2009; 3(4):102-106.
3. Suresh K, Deepa P, Harisaranraj R, Vaira AV. Antimicrobial and phytochemical investigation of the leaves of *Carica papaya L, Cynodon dactylon (L.) Pers, Euphorbia hirta L, Melia azedarach L* and *Psidium guajava L*, Ethnobotanical Leaflets 2008; 12:1184-1191.

4. Zunjar MD, Trivedi BM, Daniel M. Pharmacognostic, physicochemical and phytochemical studies on *Carica papaya* Linn leaves. Pharmacog J 2011; 3(20):5-10.
5. Mrudula G, Mallikarjuna P, Arshad MD. Anti-stress and antioxidant effects of *Prunella vulgaris* leaves, Pharmacologyonline, 2020; 2:952-962.
6. Bhui DK, Misra A. Synthesis of worm like silver nanoparticles in methyl cellulose polymeric matrix and its catalytic activity, Carbohydrate Polymers 2012; 89(3):830-835.
7. Mohapatra B, Kuriakose S, Mohapatra S. Rapid green synthesis of silver nanoparticles and nanorods using *Piper nigrum* extract, J Alloys Comp 2015; 637: 119-126.
8. Ahmed S, Ahmad M, Swami BL, Ikram S. A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: a green expertise, J Adv Res 2016; 7(1):17-28.
9. Narayanan KB, Park HH. Antifungal activity of silver nanoparticles synthesized using turnip leaf extract (*Brassica rapa* L.) against wood rotting pathogens, Eur J Plant Pathol 2014; 140(2): 185-192.
10. Logeswari P, Silambarasan S, Abraham J. Synthesis of silver nanoparticles using plants extract and analysis of their antimicrobial property, J Saudi Chem Soc 2015; 19(3):311-317.
11. Velez E, Campillo G, Morales G, Hincapie C, Osorio J, Arnache O. Silver nanoparticles obtained by aqueous or ethanolic *Aloe vera* extracts: an assessment of the antibacterial activity and mercury removal capability. J Nanomaterials 2018: Article ID 7215210.
12. *Thunbergia grandiflora*. Australian Plant Name Index (APNI), IBIS database. Centre for Plant Biodiversity Research, Australian Government, Canberra; 2013.
13. *Thunbergia grandiflora*. Weed Identification. Australian Weeds Committee; 2013.
14. Taxon: *Thunbergia grandiflora* Roxb." Germplasm Resources Information Network (GRIN). United States Department of Agriculture, Agricultural Research Service, Beltsville Area; 2013.
15. Motaleb MA, Hossain MK, Alam MK, Abdullah-Al Mamun MM, Sultana M. Commonly used medicinal herbs and shrubs by traditional herbal practitioners. Glimpses from Thanchi Upazila of Bandarban. Dhaka: International Union for Conservation of Nature and Natural Resources; 2013.
16. RHS Plant Selector-*Thunbergia grandiflora*. Retrieved 27 June 2013.
17. RHS A-Z encyclopedia of garden plants. United Kingdom: Dorling Kindersley. 2008; 1136.
18. Uddin SN. Traditional uses of ethnomedicinal plants of the Chittagong hill tracts. Dhaka: Bangladesh National Herbarium. 2006; 992
19. Ahmed ZU, Begum Z NT, Hassan MA, Khondker M, Kabir SMH, Ahmed M, Haque E U. Encyclopedia of flora and fauna of Bangladesh, angiosperms: *Dicotyledons* (Acanthaceae-Asteraceae). Dhaka: Asiatic Society of Bangladesh 2008; 6:74-75.
20. Bor NL, Raizada MB. Some beautiful Indian climbers and shrubs. Bombay Natural History Society. Bombay: Oxford University Press 1954; 110-112.
21. Ahmed ZU, Hassan MA, Begum ZNT, Khondker M, Kabir SMH, Ahmed M et al. Encyclopedia of flora and fauna of Bangladesh, angiosperms: *Dicotyledons* (Fabaceae-Lythraceae). Dhaka: Asiatic Society of Bangladesh 2009; 8:40-41.
22. Ismail LD, El-Azizi MM, Khalifa TI, Stermitz FR. Iridoid glycosides from *Thunbergia grandiflora*. Phytochemistry 1996; 42:122-1225.
23. Jeeva S, Johnson M, Aparna JS, Irudayaraj V. Preliminary phytochemical and antibacterial studies on flowers of selected medicinal plants. Int J Med Aromat Plants 2011; 1:107-114.
24. Alam MN, Biozid MS, Islam MR, Rahman MM, Chowdhury AI, Uddin MM. In-vitro comparative study of anti-inflammatory and anti-arthritic effects of the methanol extract of *Cissus pentagona* Roxb and *Thunbergia grandiflora* Roxb leaf. The Pharma Innovation 2015; 4:39-42.
25. Sangeetha J, Vijayalakshmi K. Determination of bioactive components of ethyl acetate fraction of Punica granatum rind extract. Int J Pharm Sci Drug Res 2011; 3(2):116-122.
26. Khandelwal KR. Practical pharmacognosy technique and experiments. 23rd Ed. Nirali Prakashan; 2005.
27. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy; 23 ed., Nirali prakashan: 2006; 493-497.
28. Ainsworth EA, Gillespie KM. Estimation of total phenolic content and other oxidation substrates in plant tissue using Folin-Ciocalteu reagent. Nature Protocol 2007; 2(4):875-877
29. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem 1999; 64:555-559.
30. Gulçin I, Elias R, Gepdiremen A, Boyer L. Antioxidant activity of lignans from fringe tree (*Chionanthus virginicus* L.). Eur Food Res Technol 2006; 223:759-767.
31. Jain R Jain SK. Total phenolic contents and antioxidant activities of some selected anticancer medicinal plants from Chhattisgarh State, India. Pharmacologyonline, 2011; 2:755-762.