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ECO-FRIENDLY APPROACH FOR THE GREEN SYNTHESIS OF SILVER NANOPARTICLES USING FLOWER EXTRACTS OF *SPHAGNETICOLA TRILOBATA* AND STUDY OF ANTIBACTERIAL ACTIVITY

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ABSTRACT

In the present study, the Silver nanoparticles (AgNPs) were synthesized through green route using flower extracts of Sphagneticola trilobta. The aqueous silver ions were reduced into AgNPs as mixed with the Sphagneticola trilobta flower extracts. Synthesized AgNPs were characterized by UV–visible spectroscopy, Fourier transform infra-red spectroscopy (FTIR), X-ray diffraction (XRD) and Scanning electron microscopy (SEM) analysis. The phytochemical analysis of the plant Sphagneticola trilobta flower extracts reveals the presence of flavonoids, alkaloids, cardiac glycosides and saponins. The synthesised silver nanoparticles (AgNPs) have shown good antibacterial activity against E-coli, Klebsiella aerogenes, Staphylococus aureus and Pseudomonas aerogenes.

KEY WORDS

***F**lower extracts, silver nanoparticles (AgNPs), antibacterial activity UV-Vis, FT-IR, XRD and SEM.*

I. INTRODUCTION

Nanotechnology is the creation, control and utilization of materials at the nanometer measure scale (1 to 100 nm). At this size scale, there are significant differences in many material properties that are typically not found in similar materials at bigger scales. In spite of the fact that nanoscale materials can be created utilizing an assortment of customary physical and concoction forms, it is currently conceivable to naturally blend materials through condition amicable green science based methods. As of late, the union amongst nanotechnology and science has made the new field of nano biotechnology that consolidates the utilization of natural elements, for example, actinomycetes green growth, microscopic organisms, parasites, infections, yeasts, and plants in various biochemical and biophysical forms. The organic blend by means of nano biotechnology procedures have a critical potential to help nanoparticles generation without the utilization of, toxic and expensive chemicals usually utilized as a part of customary physical and compound procedures. Nanoparticles are of great interest due to their novel physicochemical, magnetic, and optoelectronic properties that are governed by their size, shape, and size distribution [1–6]. In recent years, noble metal nanoparticles have been the subject of focused research due to their unique mechanical and chemical properties that are significantly different from those of bulk materials [7]. Silver nanoparticles have many important applications such as these can be used as an antimicrobial agent, used in textiles, in home water purification systems, medical devices, cosmetics, electronics, and household appliances [8]. Other than their antimicrobial properties the silver nanoparticles exhibit strong optical features making the nanoparticles suitable for biological sensing and imaging [9]. Since the Silver nanoparticles possess high conductivity, these are used in conductive inks, adhesives and pastes for a range of electronic devices [10].

Until date it has been reported by the researchers about the synthesis of AgNPs by green methods using extracts of fruits, vegetables, microorganisms, plant tissues etc. Nobel metals such as gold, silver, copper exhibits different applications those are used in cancer detection, catalysis, drug delivery and antibacterial activity. Some of these works, which used plant substances, are synthesis of AgNPs by using leaf extracts of *Pterocarpus santalinus* [11], *Cardiospermum halicacabum* L. [12], *Ocimum tenuiflorum* and *Catharanthus roseus* [13], *Cynodon dactylon* [14], *Ficus microcarpa* [15], *Ficus microcarpa* [16], *Hibiscus rosa sinensis* flower extracts [17], *Excoecaria agallocha* [18], *Ipomoea pescaprae* [19] and *Olive* [20].

Sphagneticola trilobata is belongs to Asteraceae family (Fig.1) and found in the West Indies, Hawaii, south Florida, Central America, West Africa, China and India. It is also called as African Marigold, grown especially at low elevations. For the present study the flowers of *Sphagneticola trilobata* were selected as it has many medicinal values such as to treat backache, muscle cramps, rheumatism, stubborn wounds, sores and swellings, and arthritic painful joints and also demonstrated its antimicrobial properties against Gram positive and Gram-negative bacteria [21].

Hence in the present work, we investigated the synthesis of stable silver nanoparticles with the bioreduction method using aqueous flower extracts of *Sphagneticola trilobata* and evaluated their antibacterial activity against drug resistant bacterial strains.

Fig.1 Sphagneticola *trilobata.*

II. EXPERIMENTAL

Collection and preparation of flower extracts:

Sphagneticola trilobata flowers were collected from the campus garden of Shridevi Institute of Engineering and Technology, Sira Road, Tumakuru, Karnataka, India. The flowers of *Sphagneticola trilobata* were washed thoroughly with tap water to remove the dust and dirt particles and then washed with double distilled water. 20 g of chopped flowers were added to 100 ml double distilled water and stirred at 60°c for 30 min on heating mantle. After boiling, the mixture was cooled for 20 min and filtered through Whatman filter paper number-1. The collected flower extracts (pale yellow color) was used as reducing and capping agents in AgNPs synthesis.

Phytochemical analysis.

The flower extracts of *Sphagneticola trilobata* were assessed for the qualitative determination of phyto constituents i.e. flavonoids, saponins, phenols, tannins, alkaloids and cardiac glycosides by applaying standard procedures.

Synthesis of Silver Nanoparticles using flower extracts: 10 ml of *Sphagneticola trilobata* flower extracts were added to the 90 ml of $AgNO_3$ solution at ambient temperature and stirred continuously for 10 min using magnetic stirrer. Slow reduction takes place and kept for 24 hours to obtain the color change for bio-reduction process.

III. CHARACTERIZATION

UV-Vis spectroscopy: The sample was analysed by UV-Vis spectrophotometry (model Shimadzu UV) for its maximum absorbance v/s wavelength to confirm the formation of AgNPs.

Fourier Transform Infra-Red spectroscopy (FT-IR) analysis:

The FTIR measurement sample was recorded in the range of 400-4000cm^{-1} using Nicolet Avatar model. It gives information on the rotations and vibrations modes were identified and purposed to determine the distinct functional groups present.

X-Ray diffraction analysis: The reduced AgNPs powder was coated on a glass substrate and the X-ray diffraction measurement were carried out by using a powder X-ray (PAN analytical BV model) instrument operating at a voltage of 40kV and current of 30mA. The output was recorded in the form of a graph with 2θ on x-axis and then intensity on y-axis. The crystallite

average size of particle was calculated by using the Debye-Scherrer formula.

$D=k\lambda/\beta\cos\theta$, where λ is wavelength, D is particle diameter size, β is the full width half maximum, k is a constant (value 0.9) and θ is Braggs diffraction angle.

Scanning Electron Microscopy (SEM) of silver nanoparticles:

Particle size and its morphological distribution were assessed with Scanning Electron Microscopy (SEM).

Antimicrobial activity of silver nanoparticles:

The antibacterial activity of AgNPs produced by *Sphagneticola trilobata* flower extracts were evaluated by the disc diffusion method. *Psedomonus aerogenes, Staphylococus aureus, Klebsiella aerogenes and E-coli* bacterial strains were collected from department of microbiology, Shridevi Institute of Medical Sciences and Research Hospital, Tumakuru, Karnataka, India. These bacterial strains were developed in nutrient broth (NB) media for 24 h at 37°c and 1 ml of each broth culture was spreaded over the nutrient agar media. 5 mm sterilized filter paper discs were dipped in synthesized Silver nanoparticles suspension (10µg/ml), double distilled water (negative control), Taxim (1µg/ml) as standard and flower extract was placed over the agar plates and incubated for 24 h at ambient temperature.

IV. RESULTS AND DISCUSSION

Phytochemical analysis

The results of phytochemical analysis of *Sphagneticola trilobata* are presented in table (1) and flavonoids, saponins, alkaloids and cardiac glycosides are present.

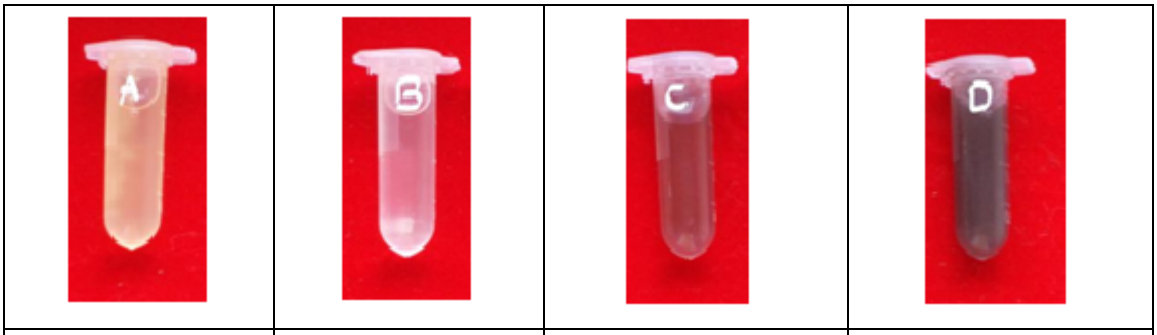
Table.1 Phytochemical analysis of *Sphagneticola trilobata* (flower)

S.No.	Phytochemicals	Flower extracts
1	Flavonoids	+++
2	Alkaloids	+++
3	Phenols	---
4	Tannins	---
5	Cardiac glycosides	+++
6	Saponins	+++

+++: Confirms, ---: Absent.

Synthesis of Silver Nanoparticles using flower extracts:

10 ml of *Sphagneticola trilobata* flower extracts were added to the 90 ml of $AgNO_3$ solution at ambient temperature and stirred continuously for 10 min using magnetic stirrer. After 24 h pale yellow color changed to dark brown color which indicates the formation of AgNPs (Fig.2) The AgNPs obtained from the solution was purified by repeated centrifugation at 8,000 rpm for 15 min using Remi Cooling Centrifuge C-24. The AgNPs obtained were dried and stored for further analysis.



A- Flower extract	**B**- 5mM $AgNO_3$ solution	**C**- Flower extract + $AgNO_3$ after 1 hour	**D**- Flower extract + $AgNO_3$ after 24 hours

Fig.2. Formation of AgNPs.

UV-Vis-spectroscopy analysis:

UV-vis spectra of AgNPs synthesized by *Sphagneticola trilobata flower* extracts was observed at 426nm which is a broadening peak with an increase in absorbance due to increase in number of AgNPs formed as a result

of reducing of Ag^+ ions present in the aqueous $AgNO_3$ solution Fig. (3).

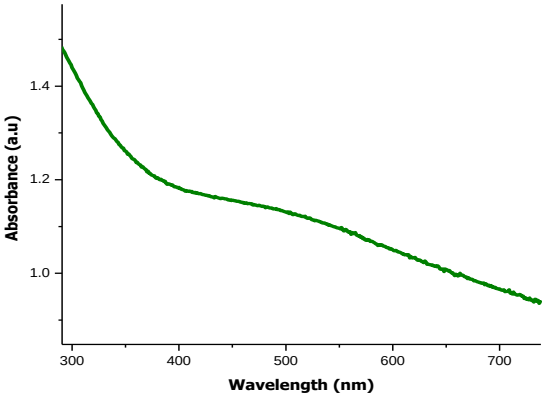
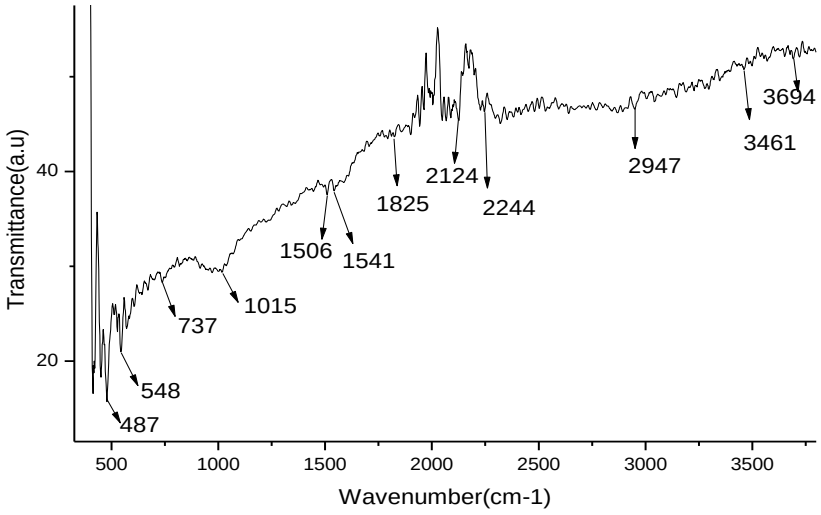


Fig 3: UV-vis spectrum of AgNPs synthesized by *Sphagneticola trilobata* flower extracts.

FT-IR analysis.

FT-IR spectrum was performed to identified and assigned to determine the different functional groups present in the AgNPs synthesized by *Sphagneticola trilobata flowers* extract (Figure.4.(a)).The IR bands were observed at 3694, 3461, 2947, 2244, 2124, 1825, 1541, 1506, 1015, 737, 548 and 487cm^{-1}(fig.4.(a)). The strong bands which appeared at 3694cm^{-1} Amide N-H stretch and 3461cm^{-1} Amine N-H, the bands at 2947 cm^{-1} Alkyl C-H, 2244cm^{-1} Nitrile CN, 2124 cm^{-1} Alkyne C≡C, 1825 cm^{-1} Carbonyl C=O, 1541 cm^{-1} Nitro N-O, 1506 cm^{-1} Aromatic C=C, 1015 cm^{-1} Alkyl halide C-F, 737 cm^{-1} Alkyl halide C-Cl, 548 cm^{-1}Alkyl halide C-Br and the low band at 487 cm^{-1} corresponds to Alkyl Halide C-I.



Figures: 4(a) - IR spectra of silver nanoparticles synthesized using *Sphagneticola trilobata* flower extract.

FT-IR spectrum was performed to identified and assigned to determine the different functional groups present in the *Sphagneticola trilobata flowers* extract (Figure.4. (b)). The strong band were observed at 3304 cm^{-1} Alcohol O-H stretch, the bands at 2156 cm^{-1} Alkyne C≡C, 1630cm^{-1} Alkenyl C=C, 1056 cm^{-1} Alcohol C-O, 565cm^{-1} Alkyl halide C-Br and low band at 487 cm^{-1} Alkyl halide C-I.

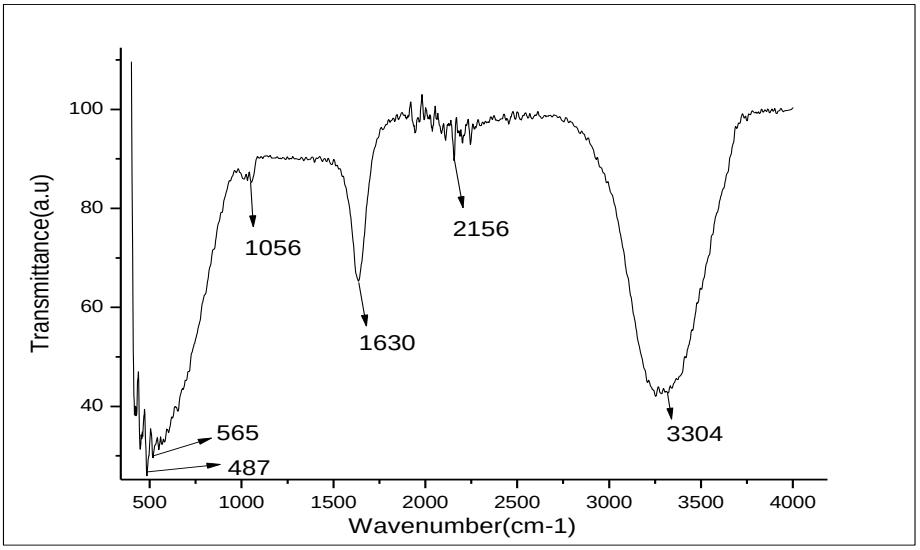


Fig.4. (b) FT-IR spectrumum of *Sphagneticola trilobata* flower extracts.

Scanning Electron Microscopy (SEM) of silver nanoparticles:

The formation of AgNPs in the SEM image (Fig. 5) has shown separate AgNPs as well as particle agglomeration. This indicates, the particle size is irregular and shape of the particles has spherical in morphology with an average size of 23.95 nm ranging from 22 to 26 nm.

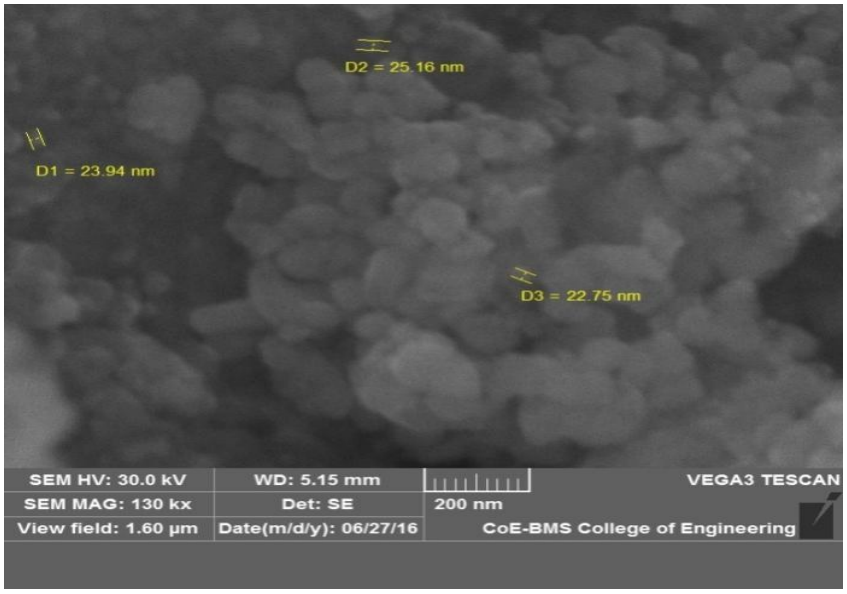


Fig. (5) SEM images of synthesized *Sphagneticola trilobata* AgNPs.

X-ray diffraction.

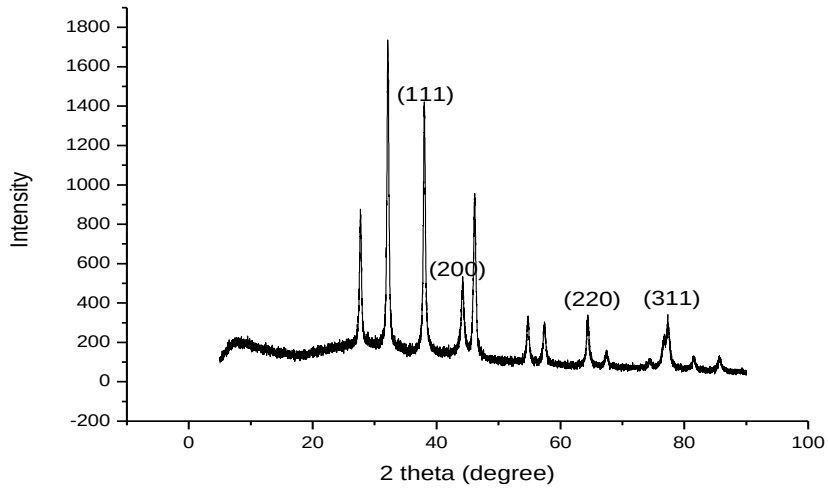


Fig. (6) XRD pattern of synthesized *Sphagneticola trilobata* AgNPs.

X-ray diffraction pattern (XRD) was recorded for the synthesized AgNPs. The diffraction peaks at 2θ= 38.16°, 44.27°, 64.41° and 77.27° were indexed with the planes (111), (200), (220) and (311) for the fcc lattice of obtained silver as per the Joint Committee on Powder Diffraction Standards (JCPDS) card no. 4-783 was matched with database. The average size (D) of synthesized Silver nanoparticles was found to be 24 nm as calculated by using Debye-Scherer formula.

Antibacterial Assay

The synthesized AgNPs by the flower extracts of *Sphagneticola trilobata* have a significant antibacterial activity against *E-coli* followed by *Psedomonus aerogenes, Staphylococus aureus,* and *Klebsiella aerogenes* (Fig.7; Table 2).

E-coli	***Pseudomonas aeruginosa***	***Klebsiella aerogenes***	***Staphylococus aureus***

Fig 7: Antibacterial activity of AgNPs synthesized by flower extracts of *Sphagneticola Trilobata*

Table.2 Antibacterial zone formation.

		Zone of Inhibition (in mm)			
S.No	**Strains**	**(1) Control**	**(2) Standard**	**(3) AgNPs**	**(4) Flower Extract**
1	*E-coli*	_	13mm	25mm	_
2	*Pseudomonasaerogenes*	_	11mm	24mm	_
3	*Klebsiellaaerogenes*	_	13mm	22mm	_
4	*Staphylococus aureus*	_	12mm	22mm	_

Control - sterile distilled water, AgNPs - Silver Nanoparticles, Standard -Taxim, Flower Extract - *Sphagneticola trilobata* flower Extract.

V. CONCLUSION

The present work a simple ecofriendly approach for the green synthesis of stable AgNPs using *Sphagneticola trilobata* flowers extracts at room temperature is reported. The formation of AgNPs was identified by the change of color of *Sphagneticola trilobata flower* extracts and the synthesized AgNPs were characterized by UV-Visible spectroscopy, XRD, FT-IR and SEM, which confirms the formation of AgNPs. The synthesized AgNPs which shown significant anti-bacterial activity against four tested bacterial strains. It can be concluded that the AgNPs may supply with large potential applications as a better catalytic activity and also in medical field.

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REFERENCES

1. Monaliben Shah, Derek Fawcett, Shashi Sharma, Suraj Kumar Tripathy and Gerrard Eddy Jai Poinern. **"**Green Synthesis of Metallic Nanoparticles via Biological Entities". Materials 2015, 8, 7278–7308; doi: 10.3390/ma8115377.
2. Daniel, M.C.; Astruc, D. Gold nanoparticles: Assembly, supramolecular chemistry, quantum - size-related properties, and applications toward biology, catalysis, and nanotechnology. J. Chem. Rev. 2004, 104, 293–346.
3. Krolikowska, A.; Kudelski, A.; Michota, A.; Bukowska, J. SERS studies on the structure of thioglycolic acid monolayers on silver and gold. Surf. Sci. 2003, 532, 227–232.
4. Zharov, V.P.; Kim, J.W.; Curiel, D.T.; Everts, M. Self-assembling nanoclusters in living systems: Application for integrated photothermal nanodiagnostics and nanotherapy, Nanomed. Nanotechnol. Biol. Med. 2005, 1, 326–345.
5. Kumar, A.; Mandal, S.; Selvakannan, P.R.; Parischa, R.; Mandale, A.B.; Sastry, M. Investigation into the interaction between surface-bound alkylamines and gold nanoparticles. Langmuir 2003, 19, 6277–6282.
6. Bogunia-Kubik, K.; Sugisaka, M. From molecular biology to nanotechnology and nanomedicine. BioSystems 2002, 65, 123–138.
7. Gavhane A.J., Padmanabhan P., Kamble S.P., & Jangle S.N. (2012) Synthesis Of Silver Nanoparticle Using Extract Of Neem Leaf And Triphala And Evaluation Of Their Antimicrobial Activities. Int. J. Pharm. Bio. Sci. 3: 88-100.
8. Wijnhoven, S.W.P., Peijnenburg, W.J.G.M., Herberts, C.A., Hagens, W.I., Oomen, A.G., Heugens, E.H.W., Roszek, B., Bisschops, J., Gosens, I., Van de Meent, D., Dekkers, S., deJong, W.H., Van Zijverden, M., Sips, A.J.A.M., Geertsma, R.E., 2009. Nano-silver: a review of available data and knowledge gaps in human and environmental risk assessment. Nanotoxicology 3, 109.
9. Jain, P.K., Huang, X.H., El-Sayed, I.H., El-Sayed, M.A., 2008. Noble metals on the nanoscale: optical and photothermal properties and some applications in imaging, sensing, biology, and medicine. Acc. Chem. Res. 41, 1578–1586.
10. Park, K., Seo, D., Lee, J., 2008. Conductivity of silver paste prepared from nanoparticles. Colloids Surf., A 313, 351.
11. Ayyakannu Arumugam, Shanmugam Gowri and Kasi Gopinath. "Phytosynthesis of silver nanoparticles using Pterocarpus santalinus leaf extract and their antibacterial properties". Journal Of Nanostructure in Chemistry, journal of nanochem, 2013, 3:68, 1- 7.
12. Mahipal S. Shekhawat, M. Manokari, N. Kannan, J. Revathi, R. Latha. "Synthesis of silver nanoparticles using *Cardiospermum halicacabum* L. leaf extract and their characterization". The Journal of Phytopharmacology 2013; 2(5): 15-20.
13. Dulen Saikia. "Green Synthesis and Opticalcharacterizations Of Silver Nanoparticles". *International Journal of Latest Research in Science and Technology* Volume 3, Issue 2: Page No.132-135, March-April, 2014.
14. Supraja S, Mohammed Ali S, Chakravarthy N, Jayaprakash Priya A,Sagadevan E, Kasinathan M.K , Sindhu S, Arumugam P. "Green Synthesis Of Silver Nanoparticles From Cynodon Dactylon Leaf Extract". International Journal of ChemTech Research CODEN (USA): Vol.5, No.1, pp 271-277, Jan-Mar 2013.
15. Willy Shah, Ujwala Patil and Abhishek Sharma. "Green synthesis of silver nanoparticles from stem bark extract of *Terminalia tomentosa* Roxb. (Wight & Arn.)". Der Pharma Chemica, 2014, 6(5):197-202.

16. P. Shanmuga Praba, V. S.Vasantha, J . Jeyasundari and Y. Brightson Arul Jacob. "Synthesis of Plant-Mediated Silver Nanoparticles Using *Ficus Microcarpa* Leaf Extract and Evaluation Of Their Antibacterial Activities".Eur. Chem. Bull., 2015, 4(3), 116-120.
17. Ambili Reveendran, Sanoj Varghese, K.Viswanathan. "Green Synthesis of Silver Nano Particle Using Hibiscus Rosa Sinensis".IOSR Journal of Applied Physics. Volume 8, Issue 3 Ver. II (May. - Jun. 2016), PP 35-38.
18. Sangeetha arun , Saraswathi U and Singaravelu. "Green synthesis of silver nanoparticles using a mangrove *Excoecaria agallocha"*.International Journal of Pharmaceutical Science Invention: Volume 3 Issue 10, October 2014, PP.54-57.
19. M.P. Arulmoorthy, S.Vasudevan, R.Vignesh, A.C.Rathiesh and M. Srinivasan. "Green Synthesis Of Silver Nanoparticles Using Medicinal Sand Dune Plant *Ipomoea Pescaprae* Leaf Extract".IJSIT, 2015, 4(4), 384-392.
20. Mostafa M.H. Khalil , Eman H. Ismail, Khaled Z. El-Baghdady,Doaa Mohamed. "Green Synthesis of Silver Nanoparticles Using Olive leaf Extract and Its Antibacterial Activity". Arabian Journal of Chemistry (2014) 7, 1131–1139.
21. M. Maldini , S. Sosa , P. Montoroa, A. Giangaspero , M.J. Balick, C. Pizzaa,∗, R. Della Loggia. "Screening of the topical anti-inflammatory activity of the bark of Acacia cornigera Willdenow, Byrsonima crassifolia Kunth, Sweetia panamensis Yakovlev and the leaves of *Sphagneticola trilobata* Hitchcock". Journal of Ethnopharmacology 122 (2009) 430–433.

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