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GREEN SYNTHESIS OF SILVER NANOPARTICLES FROM *SOLANUM MURICATUM* LEAF EXTRACTS AND ITS ANTIBACTERIAL ACTIVITY

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ABSTRACT
Silver nanoparticles when compared with other metallic nanoparticles, it has excellent medical and nonmedical properties. The synthesis of silver nanoparticles (AgNPs) study was reported, by using the *Solanum muricatum* leaf extracts. This study is simple and economical procedure and was adopted for silver nanoparticles synthesis. Extensive work has been done to explore the potential promises of the plant system in the synthesis of silver nanoparticles. It is a chemical compound that protects cells against the effect of free radicals. Antioxidant was done using reducing power assay. A biological material for nanoparticles production from *Solanum muricatum* leaf extract was processed using methanol extract. Synthesized nanoparticles were characterized by ultraviolet (UV) spectroscopy, Fourier Transform Infra-Red (FTIR) spectroscopy and Scanning electron microscopy (SEM). The antimicrobial activity against Gram-positive bacteria and Gram-negative bacteria E. coli was showed by silver nanoparticles. However, the silver nanoparticles were more effective against bacteria. The generation of silver nanoparticle was very rapid and cost-effective method for this present study. The ratio of water is needed to optimize downstream processing.

KEYWORDS: Phytochemical analysis, Antioxidant activity, FTIR, SEM, UV spectroscopy.

INTRODUCTION

Nanotechnology is a promising interdisciplinary field of research, which has very wide applications including medicine, food conservation, electronics, chemical sensors (Alessio Massironia *et al*., 2019). They are also used for environmental applications because of their potent antimicrobial activity against bacteria, viruses and fungi (Wallance, R, *et al*., 2019). Synthesizing silver nano-particles by plants is a major advantage in the broad variety of metabolites that can aid in the reduction of silver ions and are quicker than microbes in synthesis. In most cases, they are easily available and non-toxic. When the main mechanism of action is redox reaction then it is a kind of bottom up approach in the biosynthesis of nanoparticles (Sapna *et al*., 2019). Hence, the plant extracts tentatively offer a route for large scale production of commercially attractive nanoparticles by synthesis (Jerushka *et al*., 2018)

Phytochemical mediated metal nanoparticle synthesis is effective, economical and environmentally friendly. Antioxidants which occur under the influence of atmospheric oxygen or reactive oxygen species. These compounds are capable to either delay or inhibit the oxidation process. They are used for the stabilization of polymeric products, of petrochemicals, foodstuffs, cosmetics and pharmaceuticals (Aurelia Magdalena Pisoschi and Gheorghe Petre Negulescu, 2011) The pathogenesis of several degenerative diseases like atherosclerosis, neuro degenerative diseases like Parkinson's syndrome, cancer, diabetes, arthritis, aging, ischemia and liver disorders plays a major role by oxygen free radicals and its metabolites (Gowri, R. and Madhavan, V., 2013). In recent decades, many researches are interested in medicinal plants for evaluation of antioxidant phytochemicals such as phenols, flavonoids and tannins which have received more attention for their potential role in prevention of diseases (Jamuna Senguttuvan *et al*., 2014). The antimicrobial applications were incorporated because of the unique properties of silver nanoparticles such as biosensor materials, composite fibres and electronic components (Iravani *et al*., 2014).

MATERIALS AND METHODS

Sample collection and extraction

The fresh plant leaves of *Solanum muricatum* were harvested from Yercaud, Salem District, Tamil Nadu. The leaf material from the stem was separated, washed under running tap water and air dried to remove residual debris. The leaves were shade dried and homogenized to fine powder, *Solanum muricatum* was divided into two parts. Each part having 5 g of *Solanum muricatum* leaf extract powder was treated with 80% of 150 ml methanol and ethyl acetate, and subjected to Soxhlet apparatus at 70^0C. In siphon tube of extractor, the process was continued for 10 hours till the solvent became colourless and the extract was filtered using Whatman filter paper no: 42 and the solvent was kept in a hot plate till it got evaporated. The dried extract was stored in the refrigerator at 4ºC.

Phytochemical screening

Phytochemical analysis such as detection of alkaloids, flavonoids, steroids, terpenoids, anthraquinones, phenols, saponins, tannins, carbohydrates, oils and resins were carried out for *Solanum muricatum* extract as per standard

methods described by Brain and Turner 1945 and Evans 1996.

Antioxidant

50µl, 250µl, 500µl, 750µl, 1000µl of extract was mixed with 2ml of 10 mg potassium ferricyanide, 0.2 ml of 0.2% phosphate buffer. The mixture was incubated in a water bath for 20 min at 50^0C followed by addition of 2 ml of 100 mg tricholoroacetic acid and centrifuge the mixture at 2000 rpm for 10 min. Supernatant was collected. Then 2ml of supernatant was taken in a test tube, 2 ml of distilled water and 0.4 ml of 0.1% ferric chloride were added, at 700nm the absorbance was measured, using UV spectrophotometer (Natasha Anwar *et al*., 2018).

Synthesis of silver nanoparticles

1 ml of plant extract was added to 9 ml of 0.9% silver nitrate solution in 250 ml beaker and stirred twice for 5 min using magnetic stirrer at room temperature. The change in colour of the solution a1 week later indicated the reduction of silver nitrate into AgNP's. After the completion of the reaction, the solution was centrifuged at 5000 rpm for 15mins and the pellet was collected. The pellets were washed thrice using 5 ml of deionized water and centrifuged at 5000 rpm for 15 min. Further the pellets were dried in hot air oven at 80^0C for 5 hours.

Antimicrobial susceptibility test

The antimicrobial activity was performed by disc diffusion method using Muller Hinton Agar for the following gram positive and gram-negative bacteria- *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Klebsiella pneumonia.* The discs were placed on the surface of medium of 6mm with 30µl and 60µl sample for each plate. Incubate the plates at 37 ^{0}C for 24 hours. The disc were measured using transparent ruler in millimetre and the zones of inhibition were formed around it (Raja, A. *et al*., 2018) and (Redina Sapam *et al*., 2018).

CHARACTERIZATION OF SILVER NANO-PARTICLES

UV-Vis Analysis

UV-Vis spectrophotometer (Perklin-Elmer, Lamda 35, Germany), was used to measure the optical density of silver nanoparticles from range 350-600nm (Alessio Massironia *et al*., 2019) and (Sapna, G. *et al*., 2018) 24 hours later. The pure Ag+ ions reduction was monitored by using UV-Vis spectrum. Sample was prepared by diluting the solution in deionized water in the ratio 1:10.

FTIR analysis

Silver synthesized was characterized Bruker Optics Fourier Transform Infrared spectrometers (FTIR: Model ALPHA T) in the range of 4000-400 cm by using an inert KBr powder.

SEM Analysis

The morphological features and surface characteristics of silver nanoparticles of *solanum muricatum* were done using scanning electron microscopy (SEM). SEM micrographs of silver nanoparticle were given with different magnifications. The samples conductive were made by coating a thin layer of platinum. Characterization was done in the SEM at an accelerating voltage of 20 KV. The average size of the synthesized nanoparticles was in the range of 15-20 nm.

RESULTS AND DISCUSSION

Phytochemical analysis

The crude extract from the plant was investigated for the presence of various classes of phytochemicals by performing appropriate testes. The results observed were shown in the table 1. The methanol extract of *Solanum muricatum* leaf extract shows the maximum phytochemical when compared to the ethyl acetate solvent extract. Thus, indicates the fact that solvent nature also accounts for the biological activities. It is evident from the results of phytochemical analysis that alkaloids, flavonoids, steroids, phenols, saponins, tannin, carbohydrate are present methanolic extract of *Solanum muricatum*.

TABLE 1: Qualitative phytochemical analysis of *Solanum muricatum*

Phytochemicals	Observations	Extracts	
		Methanol	Ethyl Acetate
Alkaloids		+	-
Mayer's test	Cream color precipitate		
Flavonoids	Orange color precipitate	+	+
H_2SO_4 test			
Steroids	Violet to bluish Green color formation	+	-
Terpenoids	Red or brown precipitate	-	+
Arthroquinone	Mild Pink color	-	-
Phenols	Dark blue to Black color formation with	+	-
Ferric chloride test	White precipitate		
Saponin	Persistently stable	+	-
Tannin	Brownish green / Bluish black	+	-
Carbohydrates	Blue or green color	+	+
Oils & Resins	Filter paper test	-	+

(+) – Present (–) - Absent

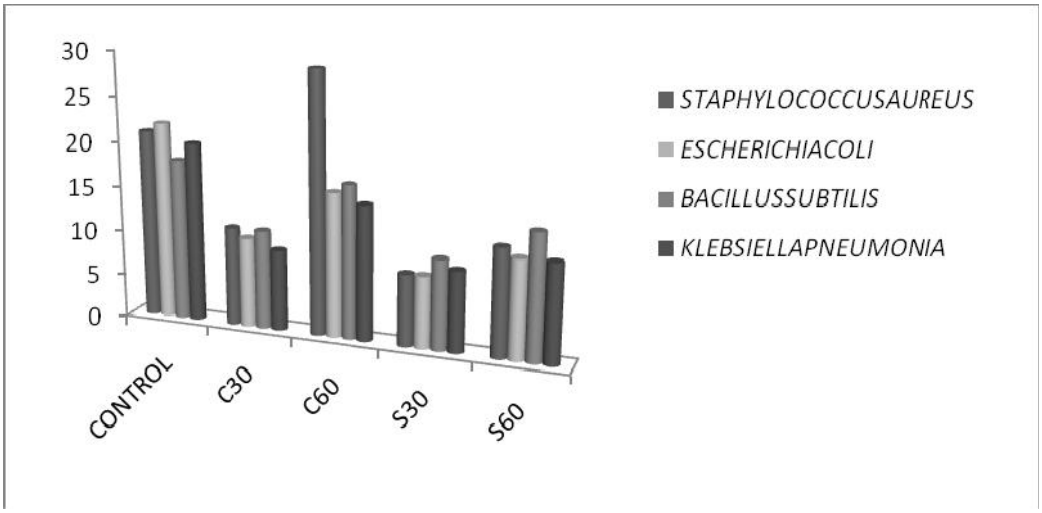
ANTIBACTERIAL ACTIVITY

The antibacterial activity of the crude and synthesis of methanol as solvent were studied in different concentration of each 30 µl and 60 µl against four bacteria *Staphylococcus aureus, Escherchia coli, Bacillus Subtilis and Klebsiella pneumonia*. The antibacterial activity of extracts and synthesis (AgNPs) were assessed in terms of inhibition zone of bacterial growth. The antibacterial activity results are presented in the table.

The antibacterial activities of the extracts increased linearly with increase in concentration of extracts (µl). This result shows that the antibacterial activity of *Staphylococcus aureus* was relatively sensitive than the other molecules, whereas in the synthesized extract *Bacillus subtilis* was more sensitive than the other microbes used. For the extract the inhibition zone ranges from 15 to 29 and synthesis inhibition zone ranges from 11 to 14 for 60 µl concentrations. For 30 µl concentration, the inhibition zones range from 9 to 11 and 8 to 10 for crude and synthesis.

TABLE 2: Antibacterial activity of *Solanum muricatum* methanol extract

Sl. No.	Microorganisms	Control	C30	C60	S30	S60
1.	*Staphylococcus aureus*	21	11	29	8	12
2.	*Escherichia coli*	22	10	16	8	11
3.	*Bacillus subtilis*	18	11	17	10	14
4.	*Klebsiella pneumonia*	20	9	15	9	11



GRAPH 1: Antibacterial activity of microbes

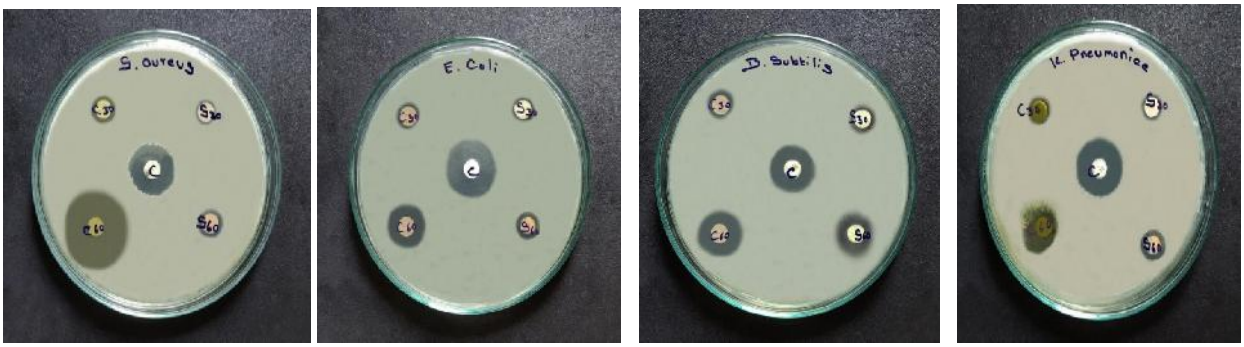


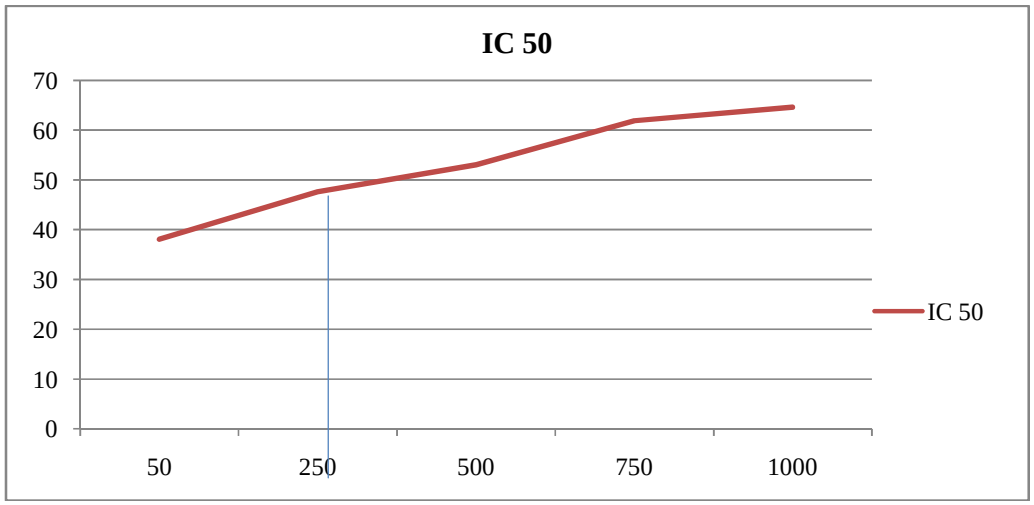
FIGURE 1: Antibacterial activity of *Solanum muricatum* Methanol extract with *Klebsiella pneumoniae, Staphylococcus aureus, Bacillus subtilis, Escherichia coli*

ANTIOXIDANT ACTIVITY

Reducing power assay method often used to determine the presence of antioxidant in the methanol extract. In this assay, the extract has the ability to reduce Fe^{3+} to Fe^{2+} was determined. The result showed the presence of antioxidant extract, by the reduction of ferricyanide complex to the ferrous cyanide and thereby the colour changes from green to blue. The absorbance was measured at 700nm. The absorbance is higher would be the reducing power. Reducing power of the extract increases as the concentration of the extract varies.

TABLE 3: Antioxidant Activity

Concentration	OD Value	IC 50	% IC 50
50	0.203	38.19	
250	0.217	47.62	
500	0.225	53.06	399.96
750	0.238	61.90	
1000	0.242	64.63	



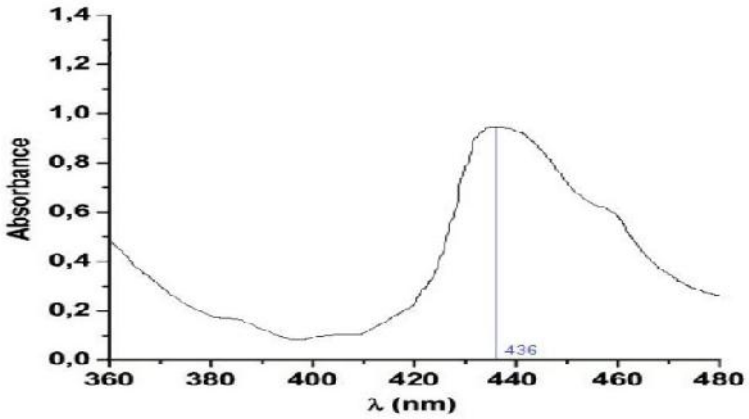
GRAPH 2: Antioxidant activity of *Solanum muricatum* with methanol extract

UV ANALYSIS

During exposure to plant extracts, the colour change was observed by the reduction of silver ions to silver nanoparticles. Surface Plasmon Resonance phenomenon was responsible for the colour change were obtained by Surface Plasmon Resonance phenomenon. The resonance flight waves were due to the combined vibration of electrons with metal nanoparticles that have free electrons, which give SPR absorption band. In case of *Solanum muricatum* the sharp bands of silver nanoparticles were absorbed around 350-600 nm. The silver nanoparticles show SPR peak at 430 nm from different literatures. This research paper shows that the SPR peak for *Solanum muricatum* was 436 nm.

So, this research paper conformed that the potential to reduce Ag ions into Ag nanoparticles by using *Solanum muricatum* leaf extract. With increasing time period, the intensity of absorbance peak increases. The characteristics colour variation was obtained due to the excitation of the SPR in the metal nanoparticles. The plots of absorbance at λ-max the plots of absorbance was 430 nm in the above fig versus absorbance.

More than 80 % of reductions of Ag+ ions were completed within 5 hours and it occur fairly rapid reduction of metal ions. After the addition of metal ions to the plant extract. After their synthesis, even 4 weeks the metal particles were absorbed to the state in solution[8]. By stability with time, there was no observable variation in the optical properties of the nanoparticle.



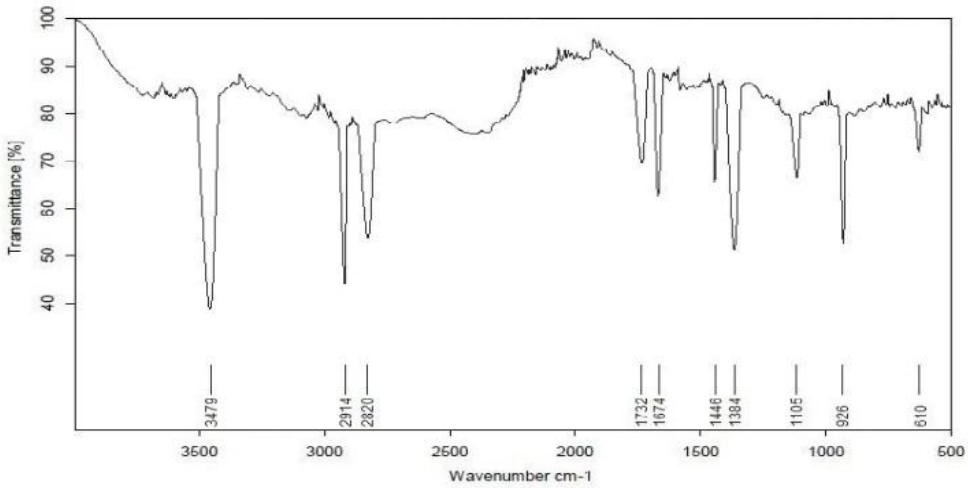
GRAPH 3: UV analysis of synthesized Methanol extract of *Solanum muricatum*

FITR ANALYSIS

The aqueous plant extract and biosynthesized silver nanoparticles in FTIR spectra are shown in figure. Using FTIR spectrum the reduction of Ag ions was detected for responsible metabolites. The presence of primary amines (weak to medium) with instinct absorption bands at 3479 cm^{-1}. At 2914 and 2820 cm^{-1} bands represent the presence of strong alkanes. In the biosynthesized solution, the region of 1732 cm^{-1} indicates the presence of aliphatic aldehydes (very strong). The primary amines (medium to strong) are assigned from the bands at 1674 cm^{-1}. The bands at 1446 cm^{-1} reveal the presence of vinyl terminal (medium). The absorption bands 1384 cm^{-1} corresponds to the acetates (strong). The bands at 1105 cm^{-1} belong to the aromatic esters (very strong). The bands at 926 cm^{-1} corresponds to the aromatic methane (strong). The bands at 610 cm^{-1} belongs to primary amines (medium)[1].

Table 4: FTIR analysis of synthesized methanol extract of *Solanum muricatum*

S.NO	Infrared Absorption Bands (wave number in cm^{-1})	Infrared band assignment
1	3479	Primary amines (Weak to Medium)
2	2914,2820	Alkanes (Strong)
3	1732	Aliphatic aldehydes (Very Strong)
4	1674	Primary amines (Medium to Strong)
5	1446	Vinyl terminal (Medium)
6	1384	Acetates (Strong)
7	1105	Aromatic esters (Very Strong)
8	926	Aromatic Methane (Strong)
9	610	Primary amines (Medium)



GRAPH 4: FTIR Analysis of synthesized Methanol extract of *Solanum muricatum*

SEM ANALYSIS

This Figure represent SEM analysis of purified silver nanoparticles. SEM provided with further insight into the morphology and size details of the silver nanoparticles. The size of the nanoparticle's ranges from 1-100 nm. The shape of the silver nanoparticles was in spherical, that shows the proteins which were bound to the surface of the nanoparticles. Due to the SEM measurements the longer silver nanoparticles were converted into smaller silver nanoparticles because of aggregation.

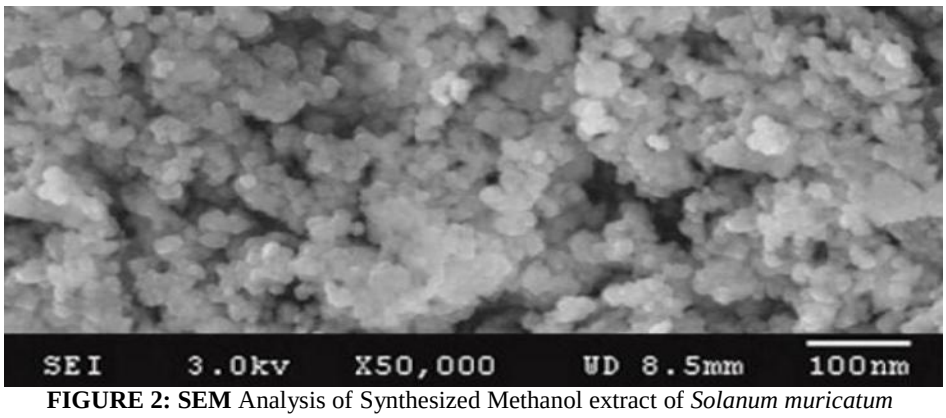


FIGURE 2: SEM Analysis of Synthesized Methanol extract of *Solanum muricatum*

CONCLUSION

The Nanotechnology is developing rapidly and more methods to obtain nanoscale particles are emerging continuously. The *Solanum muricatum* leaves extract provide environmentally friendly, simple and efficient route for synthesis of nanoparticles, by the rapid biological synthesis. These silver nanoprticles were analysed by different physical- chemical techniques and all the results showed that the synthesis takes place with good and reproducible results. Also, antimicrobial activity and antioxidant properties were investigated.

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REFERENCES

Abdul wadood, Mehreen Ghufran, Syed Babar Jamal, Muhammad Naeen, Ajmal Khan, Rukhsana Ghaffar and Asnad (2018). Phytochemical Analysis of Medicinal plants Occuring in local Area of Mardan. *Biochem Anal Biochem*, **2(144)**.

Alessio Massironia, Andrea Morellia, Lucia Grassib, Dario Puppia, Simona Braccinia,Giuseppantonio Maisettab, Semih Esinb, Giovanna Batonib, Cristina Della Pinac,Federica Chiellinia (2019). Ulvan as novel reducing and stabilizing agent from renewable algal biomass: Application to green synthesis of silver nanoparticles. *Carbohydrate Polymers*,**203,** 310-321.

Aurelia Magdalena Pisoschi and Gheorghe Petre Negulescu (2011). Methods for Total Antioxidant Activity Determination: A Review. *Biochem & Anal Biochem*, **1(106)**, 2161-1009.

Gowri R, Madhavan V (2013). Evaluation of Antioxidant activity of Ethanolic extract of Sphaeranthus amaranthoides Burm.f. *International Journal of Drug Development and Research*, **5(4)**, 320-329.

Iravani.S, Korbekandi.H, Mirmohammadi.S. V, and Zolfaghari.B (2014). Synthesis of silver nanoparticles: chemical, physical and biological methods. *Res Pharm Sci*. **9(6)**, 385-406.

Jamuna Senguttuvan, Subramaniam Paulsamy, Krishnamoorthy Karthika (2014). Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, *Hypochaeris radicata* L. for in vitro antioxidant activities. *Asian Pac J Trop Biomed*.**4(1)**, 359-367.

Jerushka S Moodley, Suresh baby, Naidu Krishna, Karen Pillay, Sershen and Patrick Govender, 2018. Green synthesis of silver nanoparticles from Moringa oleifera leaf extracts and it's antimicrobial potential. Vietnam Academy of science & Technology. *Advanced in Natural science : Nanoscience and Nanotechnology*, **9(1)** 015011**.**

Jeyanthi palanivelu et al (2018). *J. Pharma. Sci*. **7(9)**, 690-695.

Natasha Anwar, Mohib Shah, Samreen Saleem and Hadia Rahman (2018). Plant mediated sybthesis of silver nanoparticles and their biological applications. *Bull. Chem. Soc. Ethiop*. **32(3)**, 469-479. ISSN 1011-3924**.**

Pavle Z. Maškovića, Vesna Veličkovićb, Saša Đurovićc,d, Zoran Zekovićc, Marija Radojkovićc, Aleksandra Cvetanovićc, Jaroslava Švarc-Gajićc, Milan Mitiće, Jelena Vujić (2018). Biological activity and chemical profile of Lavatera thuringiaca L. extracts obtained by different extraction approaches. *Phytomedicine.* **38**, 118–124**.**

Raja.A, Ashokkumar.S, Pavithra Marthandam.R, Jayachandiran.J, Chandra Prasad Kathiwada, Kaviyarasu.K, Ganapathi Raman.R, Swaminathan.M *(2018). Journal of Photochemistry & Photobiology, B: Biology.* ***(18)30009-5,*** *1011-1344***.**

Redina Sapam, Partha Pratim Kalita, Manash Pratim Sarma, Nayan Talukdar, Harajyoti Das (2018). Screening of phytochemicals and determination of total phenolic ontent, anti-oxidant and antimicrobial activity of methanolic extract of piper nigrum leaves. Indo American Journal of Pharmaceutical Research. **8(02),**1354-1360.

Saba Pirtarighat, Maryam Ghannadnia, Saeid Baghshahi (2019). Green synthesis of silver nanoparticles using the plant extract of Salvia spinosa grown in vitro and their antibacterial activity assessment. *Journal of Nanostructure in Chemistry*, Volume **9(1)**, 1-9**.**

Sapna G. Yadav, Sudeep H Patil, Pratima Patel, Vineeetha Nair, Shahida Khan, Shivani Kakkar, Annika Durve Gupta (2018). Green synthesis of silver nanoparticles from plant sources and evaluation oftheir antimicrobial activity. *Themed Section : Science and Technology*,**(5)4**, 2395-1990, 133-139**.**

Sapna Malviya, Dr.Dwivedi.P (2019). Extracts of Ailanthus excels an Essential Medicine in Ayurveda: Pharmacological evaluationand preliminary screening of phytochemicals. *Journal of Drug Delivery & Therapeutics*. **9(1)**, 84-87.

Wallace R. Rolima, Milena T. Pelegrinoa, Bruna de Araújo Limac, Letícia S. Ferraza, Fanny N. Costaa, Juliana S. Bernardesd, Tiago Rodiguesa, Marcelo Brocchic, Amedea B. Seabra (2019). Green tea extract mediated biogenic synthesis of silver nanoparticles: Characterization, cytotoxicity evaluation and antibacterial activity. *Applied Surface Science*,**463**, 66-74.

Wu, M. WI, Duncan A.R, and Okunji.C.O,. J. ASHA (1999), Press Alexandra V.A, 457-462**.**

Indhumathi V, Perundevi S, Vinoja B.N, Manivasagan V, Saranya K and Ramesh Babu N.G (2018). Phytochemical screening and Antimicrobial Activity of fresh and shade dried leaves of Azadirachta Indica. *International of Innovations in Engineering and Technology (IJIET)*, **11(3),** 2319-1058, 27-32.