Research Article

Green Synthesis of Silver Nanoparticles Using Acacia Nilotica Leaf Extract and Its Antibacterial and Anti Oxidant Activity

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ABSTRACT
The silver nanoparticles (AgNPs) synthesized using leaf extracts as reducing and stabilizing agent are reported and evaluated for antibacterial activity and antioxidant activity. The data revealed that the rate of formation of silver nanoparticles increased significantly in solvent with increasing temperature. The nature of AgNPs synthesized was analyzed by UV–vis spectroscopy, X-ray diffraction and scanning electron microscopy. The silver nanoparticles were with an average size of 20–25 nm and mostly spherical. The antibacterial potential of synthesized AgNPs was compared with that of aqueous,acetone ethanol and methanol extracts by well diffusion method. Here the microorganisms were used une gram negative gram positive and one fungus. Thus AgNPs showed broad spectrum antibacterial activity at lower concentration and may be a good alternative therapeutic approach in future, and antioxidant activity.

Keywords: Nanosilver; *acacia nilotica* leaf extract; Antibacterial activity;antioxidant activity.

INTRODUCTION

Since the starting of medicinal activities human being want to search new drugs for the present diseases and based on his daily food mainly composed of vegetables, reads to the discover of different medicinal plants. According to World Health Organization (WHO), medicinal plants would be the best source to obtain variety of drugs. About 80% of individuals from developed countries used traditional medicines, which have compounds derived from medicinal plants. Those plants used should be investigated to better understand their properties, safety and efficiency. (Arunkumar and Muthuselvam, 2009)

Medicinal plants are those plants which show antimicrobial, antifungal, antiviral or insecticidal activities. From the best known and used medicine shows the high level of usage of those plants and while traditional medicinal plants are often cheaper, locally available and easily consumable, raw or as simple medicinal preparations. (Cowan et al, 1999).

ACACIA NILOTICA

(KARUVELAI: Tamil name and KIKAL : Hindi name).

TAXONOMICAL CLASSIFICATION

KINGDOM	Plantae
SUBKINGDOM	Tracheobionta
SUPER DIVISION	spermatophyta
DIVISION	Magnoliophyta
CLASS	magnoliopsida
SUBCLASS	rosidae
ORDER	Fabales
FAMILY	Fabaceae
GENUS	*Acacia*
SPECIES	*nilotica*

ACACIA PLANT

Acacia known commonly as acacia, thorn tree, whistling thorn, or wattle, is a genus of shrubs and trees belonging to the subfamily Mimosoideae of the family Fabaceae, described by the Swedish botanist Carl Linnaeus in 1773 based on the African species *Acacia nilotica*. Many non-Australian species tend to be thorny, whereas the majority of Australian acacias are not. All species are pod-bearing, with sap and leaves often bearing large amounts of tannins and condensed tannins that historically found use as pharmaceuticals and preservatives.

The generic name derives from (*akakia*), the name given by early Greek botanist-physician Pedanius Dioscorides (middle to late first century) to the medicinal tree *A. nilotica* in his book *Materia Medica*. This name derives from the Greek word for its characteristic thorns, The species name *nilotica* was given by Linnaeus from this tree's best-known range along the Nile river.

The genus *Acacia* previously contained roughly 1,300 species, about 960 of them native to Australia, with the remainder spread around the tropical to warm-temperate regions of both hemispheres, including Europe, Africa, southern Asia, and the Americas. However, in 2005, the genus was divided into five separate genera under the tribe "Acacieae". The genus *Acacia* (*sensu stricto*) was retained for the majority of the Australian species and a few in tropical Asia, Madagascar, and Pacific Islands. Most of the species outside Australia, and a small number of Australian species, were reclassified into *Vachellia* and *Senegalia*. The two final genera,*Acaciella* and *Mariosousa*, each contains about a dozen species from the Americas. (Quattrocchi and Umberto,2000).

1.6 ACACIA NILOTICA SPECIES

Acacia nilotica is a shrub or tree belonging to the family Leguminosae. It is widely distributed in Kenya and is widely used for medicinal purposes in both human and veterinary medicine in resource-poor rural and urban households. The decoction of its stem barks is used against diarrhoea and eye problems in livestock, stomachache, malaria, coughs, primary infection of syphilis, sterility, and pneumonia in human being. (Kokwaro, 1976)

It is a low, branched tree with a more or less spherical crown. Black bark on stem becomes ash-grey to light brown on the branches, bearing small, short, sharply hooked spines in pairs. It has a shallow but extensive root system radiating from the crown, allowing the plant to exploit soil moisture and nutrients from a large volume of soil. The roots rarely penetrate more than 1 m. leaves characterized by 2 pairs of pinnulae, each with a single pair of leaflets. Leaflets elliptic 0.6-2 cm long and 0.6-1.2 cm wide, glabrous and highly coloured beneath.

Today, traditional medicinal practices form an integral part of complementary or alternative medicine. Although their efficacy and mechanism of action have not been tested scientifically in most cases, these simple medicinal preparations often mediate beneficial responses due to their chemical constituents.

The aim of this study is to assess the phytochemicals present in *Acacia nilotica* and examine their anti microbial effects.

Nowadays different new diseases are developed due to resistance of causal agents; these call researchers to get new medicine responsible of new causal agent. One of the best ways of achieving this is the analysis of phytochemicals and study of their effects on different microbes.

One of plants is used in this study *acacia nilotica* which is well known and used as medicinal plant in different areas of the world.

MEDICINAL USES OF ACACIA NILOTICA

Siddha Medicine is one of the oldest medical systems known to mankind. Contemporary Tamizh literature holds that the system of Siddha medicine originated in Southern India, in the state of Tamil Nadu, as part of the trio Indian medicines - ayurveda, siddha and unani. Reported to have surfaced more than 10000 years ago, the Siddha system of medicine is considered one of the most ancient traditional medical systems. (The *Hindu*, 2010).

SILVER NANOPARTICLES

Silver nanoparticles are nanoparticles of silver, i.e. silver particles of between 1 nm and 100 nm in size. While frequently described as being 'silver' some are composed of a large percentage of silver oxide due to their large ratio of surface-to-bulk silver atoms.

Silver nanoparticles have unique optical, electrical, and thermal properties and are being incorporated into products that range from photovoltaics to biological and chemical sensors. Examples include conductive inks, pastes and fillers which utilize silver nanoparticles for their high electrical conductivity, stability, and low sintering temperatures. Additional applications include molecular diagnostics and photonic devices, which take advantage of the novel optical properties of these

nanomaterials. An increasingly common application is the use of silver nanoparticles for antimicrobial coatings, and many textiles, keyboards, wound dressings, and biomedical devices now contain silver nanoparticles that continuously release a low level of silver ions to provide protection against bacteria.

MATERIALS AND METHODS

SAMPLE COLLECTION

Fresh leaves of *Acacia nilotica* were collected in Tamil nadu, Tanjavur district, in garden near Sarafogi College.

The plant materials were dried until all the water molecules evaporated and plants became well dried for grinding. After drying, the plant materials were ground well using mechanical blender into fine powder labeling for future use.

PREPARATION OF PLANT EXTRACTS

Crude plant extracts were prepared by Soxhlet extraction method. About 20 g of powdered plant leaves was uniformly packed into a thimble and extracted with 180 ml of different solvents separately. Solvents used were water, methanol, ethanol and acetone. The process of extraction till the solvent in siphon tube of an extractor became colorless. After that the extracts were taken in beakers and kept on a hot plate and heated at 30 – 40^{o}C till all the solvent got evaporated. Dried extracts were kept in a refrigerator at 4^{o}C for their future use in phytochemical analysis.

ETHANOLIC EXTRACT

It was prepared by packing 20g of powdered plant leaves into a thimble and 180 ml of ethanol was used as extract. Liquid extract obtained was dried on petri dish.

METHANOLIC EXTRACT

It was prepared by separating funnel where 20g of plant material was used by dissolving it into 180 ml of methanol. After two days extract was obtained and kept for future analysis.

ACETONE EXTRACT

It was prepared by using 20g of plant material which dissolved into 180ml acetone by using separating funnel. After two days extract was obtained and kept for future analysis.

Extracts were filtered and concentrated at room temperature. After completion of solvent these extracts are kept for future uses.

ANTIBACTERIAL STUDY

MICRO ORGANISMS

In this study both gram positive (*Staphylococcus aureus*) and gram negative (*Eschelichia coli*) bacteria were used to determine antibacterial activity of different alcoholic extracts of plant *Acacia nilotica*.

INNOCULUM PREPARATION

Bacteria broth was prepared by dissolving 1.3 g of nutrient broth in 100 ml of distilled water. Then, took loopful of bacteria culture from the slant and inoculate bacteria into broth medium. Incubation took place for 18-24 hrs at 37^{o}c.

DETERMINATION OF ANTIBACTERIAL ACTIVITY

During this study antibacterial activity of *Acacia nilotica* extracts were carried out by a modified well agar method. Mueller Hinton agar plates were swabbed with 24 hrs old broth culture of selected bacteria. Consequently, using sterile borer, well of 0.6 cm diameter was made into each Mueller Hinton agar 4 wells were made and 40 micro liter of each extract was filled into the well.

The control antibiotic (Tetracycline) was used to compare each extract activity, and then the plates were incubated for 24 hrs at 37 ^{o}c. Results were recorded by measuring the diameter of inhibitory zone by using a transparent meter rule at the end of 24 hrs.

ANTIFUNGAL STUDY

MICRO ORGANISM

For this study, fungal strain, *Aspergillus niger* fungi was used to determine antifungal activity of different extracts of plant *Acacia nilotica*.

INNOCULUM PREPARATION

Potato dextrose broth was prepared by dissolving 3.9g of potato dextrose broth into 100 ml of distilled water. Took a loopful of fungal culture from the slant and inoculate fungi in broth medium. Then incubate the culture broth for 48 hrs at 37^{o}c.

DETERMINATION OF ANTIFUNGAL ACTIVITY

In study antifungal activity of *Acacia nilotica* extracts was carried out by a modified well agar method. Mueller Hinton agar plates were swabbed with 24 hrs old broth culture of selected fungi strain (*Asperigullus niger*). Consequently, using sterile borer, well of 0.6 cm diameter was made into each Mueller Hinton agar 4 wells were made and 40 micro liter of each extract was filled into the well.

The control antibiotic (Clotrimazole) was used to compare each extract activity, and then the plates were incubated for 24 hrs at 37 ^{o}c. Results were recorded by measuring the diameter of inhibitory zone by using a transparent meter rule at the end of 24 hrs

ANTIOXIDANT ACTIVITY ASSAY

To determine the reducing power assay of Plant Sample by Yildrim *et al.,* Method, 2001.

Reagents Required

Phosphate buffer, Potassium Ferric Cyanide, Trichloro acetic acid and Ferric Chloride

Procedure

Take different concentration of plant extract was mixed with phosphate buffer (2.5 ml 0.2 M, pH 6.6) and potassium ferricyanide (2.5 ml). The mixture was incubated at 50^{o}C for 20 minutes. A portion (2.5 ml) of Tricholoroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5ml) was mixed with distilled water (2.5ml) and Ferriccloride (0.5ml, 0.1%) and read the absorbance measured at 700nm. Increased absorbance of the reaction mixture indicates stronger reducing power. The activity was compared with ascorbic acid standard.

Calculation

Percentage scavenging activity =

$$\frac{A_{control} - A_{test}}{A_{control}} = \times 100$$

Where $A_{control}$ is the absorbance of the control A_{test} is the absorbance in the presence of the sample.

SILVER NANOPARTICLES SYNTHESIS

During this study plant extract was used to reduce silver nitrate in order to get silver nanoparticles.

Aqueous extract was prepared by boiling 25 g of plant leaves with 100 ml of distilled water during 20 min color change indicate the formation of extract, the obtained extract was filtered by using whatman filter paper number one. This is followed by centrifugation to remove heavy biomolecules.

Silver nitrate solution was prepared and adjusted to 1x10^{-3}M, 100 ml of solution was mixed with 5ml of plant extract, after 12hrs show color change.

For analysis and characterization of formed nanoparticules UV visible spectrophotometer was used to characterize transiformation and stability of nanoparticles, fourier transform infra red was used to determine the stability of nanoparticles also scanning electron microscope was used to determine the shape of nanoparticles.

RESULTS AND DISCUSSION

ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY

The bacteria culture of *E. coli* and *staphylococcus aureus* in petriplates were incubated along with were checked for growth inhibition zones of organism after 24 hrs, the antibacterial activity of ethanolic, methanolic and acetone extracts of plant A*cacia nilotica* was studied. Antibacterial activity of dried leaves extract and their efficiency were quantitatively assessed using agar well diffusion methods by measuring the diameter of growth of inhibition zone.

Antibacterial activity of extracts and their efficiency were assessed using agar well diffusion methods by measuring the zone of inhibition diameter. The results showed that ethanolic extract is more powerful than other extracts, where it was most active against *E.coli and staphylococcus aureu*s. The antifungal culture of *Aspergillus niger* in petriplate was along with the test were checked for growth

inhibition zone of organisms after 48 hrs, the fungal activity of ethanolic, methanolic and acetone extracts of plant *Acacia nilotica* were studied.

The ethanolic extract of *Acacia nilotica* show the maximum zone of inhibition against *Aspergillus niger* which is 13mm while the acetonic extract show the minimum one which is 10mm.

The minimum inhibition zone was evaluated using ethanolic extract with different concentrations here 30%, 50% and 80% were chosen to be used. The 80% concentration showed maximum zone of inhibition in both *E. coli and staphylococcus aureus* wich is 34 mm.

When we compare all extracts ethanolic extracts showed high zone of inhibition against methanolic and acetone.

The culture of *Aspergillus Niger* was used for the antifungal activity test where the zone of inhibition was evaluated after 48 hrs, in all extracts here ethanolic extract showed high zone of inhibition which is 16 mm. different concentrations were used to evaluate the minimum zone of inhibition where 80% concentration of ethanolic extract show high zone of inhibition 28 mm. and 30% show the minimum one 20%.

The comparison in strain shows that in gram negative *E. coli* the minimum zone of inhibition was observed on acetonic extracts which is 23mm while the maximum was 25mm on methanolic extract, in gram positive *Staphylococcus aureus* the minimum zone of inhibition was observed on methanolic extract and was 13mm and the maximum one was 18mm on ethanolic extract. When compared to the ethanolic, methanolic and acetonic extracts, ethanolic extract showed the highest zone of inhibition among the organisms, as presented in figure 11 and 12 as well as in table 2.

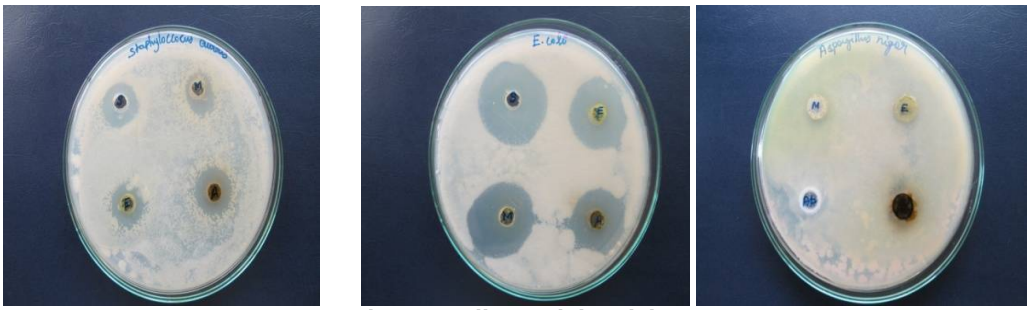


Fig. 1: Antibacterial activity

Fig. 2: Ethanolic extract with different concentrations

Table 1: Antimicrobial Activity

S NO	NAME OF ORGANISM	ZONE OF INHIBITION(mm)			
		S	30%	50%	80%
1	*E.coli*	38	28	32	
2	*Staphylococcus aureus*	38	29	32	34
3	*A.niger*	30	20	25	28

MINIMUM INHIBITORY CONCENTRATION

The present study indicates that the ethanolic extract of *Acacia nilotica* significantly suppress the growth of selected bacteria. The ethanolic extract of *acacia nilotica* was most active against the microorganisms *Bacillus subtilis* and *Escherichia coli*. The maximum inhibition zone was obtained in *E. coli* 26 mm and the minimum inhibition zone was methanolic extract found in *staphylococcus aureus* which is 1 mm.

The minimum inhibitory concentration of the extracts of *Acacia nilotica* against various pathogens was performed. The MIC of ethanol extract was low [2.5mg./ml] as compared to other extracts [10 mg/ml].The lower MIC is an indication of high effectiveness of extract. As showed in table: 3.

Table 2: Minimum inhibitory concentration of the extracts against pathogens

S. no	Name of organism	MIC(mg/ml)		
		Ethanolic extract	Methanolic extract	Acetone extract
1	*E. coli*	2.5 mm	9 mm	4 mm
2	*Staphylococcus aureus*	5 mm	10 mm	7 mm
3	*A.niger*	3 mm	10 mm	8 mm

ANTIOXIDANT ACTIVITY ASSAY

Antioxidant is a substance that prevents or slows the breakdown of others substance by oxygen, they are chemical substances that donate an electron to the free radicals and convert it into harmless molecules.

Natural antioxidants that are present in different plants and spices are responsible for inhibiting or preventing the deleterious consequences of oxidative stress. Spices and plants contain free radical scavengers like polyphenols, flavonoids and phenolic compounds. In the present study, we have evaluated the free radical scavenger activity of ethanolic extract of *Acacia nilotica* with different concentrations.

Reducing power assay method is based on the principle that substances, which have reduction potential, react with potassium ferricyanide (Fe3+) to form potassium ferrocyanide (Fe2+), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm.

In the present study antioxidant activity was performed ethanolic extract of *Acacia nilotica* using different concentrations (30%, 50% and 80%) and different quantity of ascorbic acid. (0.5And 1.0 ml).

Etahonolic extract at 30% of concentration with 1.0 ml shows high antioxidant activity compare to other extracts and concentrations, as presented in table: 4 and figure 13.

Table 3: In-Vitro Antioxidant activity of Plant extract by Reducing Power Scavenging Activity

Sample Extracts	Inhibition values in %	
	0.5 ml	1.0 ml
30% Ethanolic extract	53.2	91.6
50% Ethanolic extract	54.3	69.3
80% Ethanolic extract	37.4	61.4

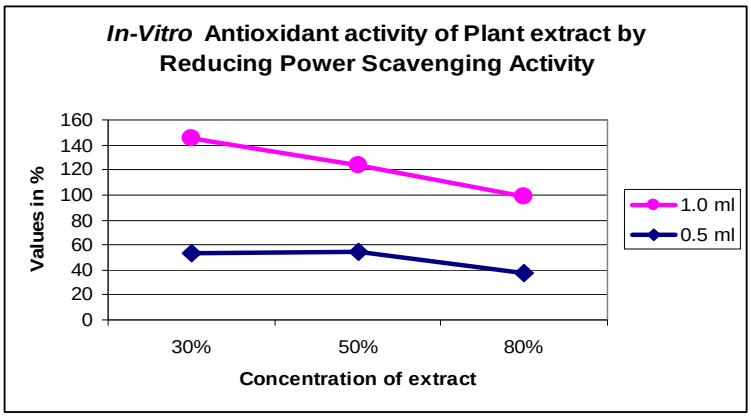


Fig. 3: in-vitro antioxidant activity by reducing power scavenging activity

SILVER NANOPARTICLES SINTHESIS

The synthesis of silver nanoparticles and their utilization in diverse areas become an area of research and investigation during the last two decades because of their unique optical, physical, chemical and magnetic properties compare to the bulk sold. Green synthesis of silver nanoparticals shows the rapid and eco friendly advantages compare to the other methods. During this study aqueous extract of *Acacia nilotica* was used to reduce silver nitrate ($AgNO_3$) into silver nanoparticles. The results are presented in figure 14 to 16.

Fig. 4: plant extracts

Fig. 5: silver nitrate solution

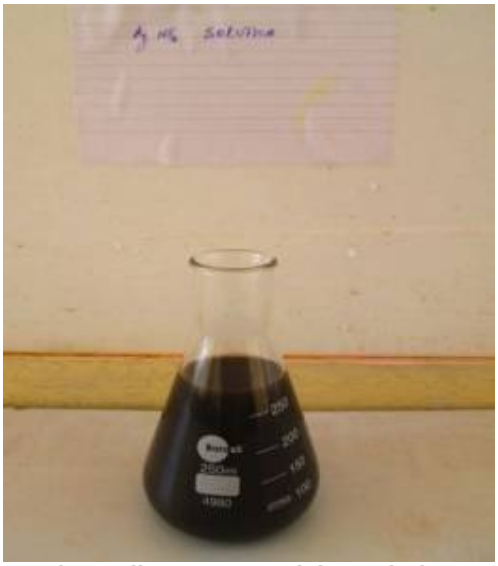


Fig 6: silver nanoparticles solution

UV-visible absorbance study showed that the addition of *Acacia nilotica* leaf extract to silver nitrate solution resulted in color change of solution from transparent to brown-black due to the production of silver nanoparticles (Figure: 6).

The SPR of silver nanoparticles produce a peak centred at 300 nm, (figure: 7). This indicates the reduction of silver nitrate to silver nanoparticles . it was observed that the reduction started at the start of the reaction and continue rapidly till the end of reaction, and showing the rapid biosynthesis of silver nanoparticles.

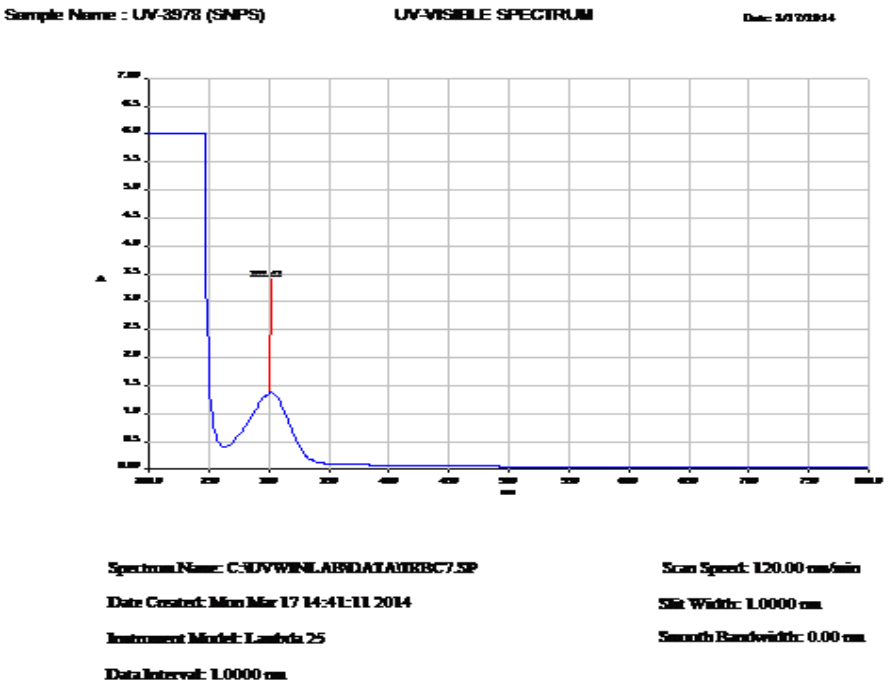


Fig. 7: UV- vis spectrum of silver nanoparticles

Results of FT-IR study of biosynthesis silver nanoparticles using *Acacia nilotica* extract showd sharp absorption peak located 3421, 2922, 2361, 1624, 1384 and 1060 cm^{-1} as showed on figure 8 the peak on1624 cm^{-1} may be assigned to the amide I bond of proteins arising from carbonyl stretching in

proteins and the peak 3421 cm^{-1} is arising to OH stretching in alcohols and phenolic compounds. The absorption peak at 1624 cm^{-1} is close to that reported for native proteins, which suggests that proteins are interacting with biosynthesized silver nanoparticles and also their secondary structure was not affected during reactionwith Ag^{+} ions or after binding with Ag^{0} nanoparticles. This spectroscopic study confirms that the carbonyl group of amino acid residues has a strong binding ability with silver, suggesting the formation of layer covering silver nanoparticles. These results confirm the presence of possible proteins acting as reducing and stabilizing agents.

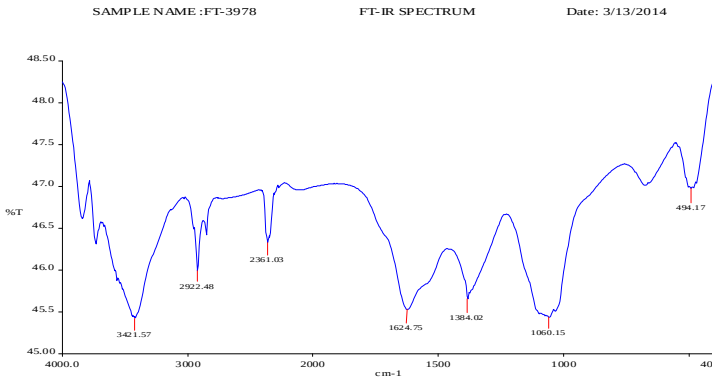
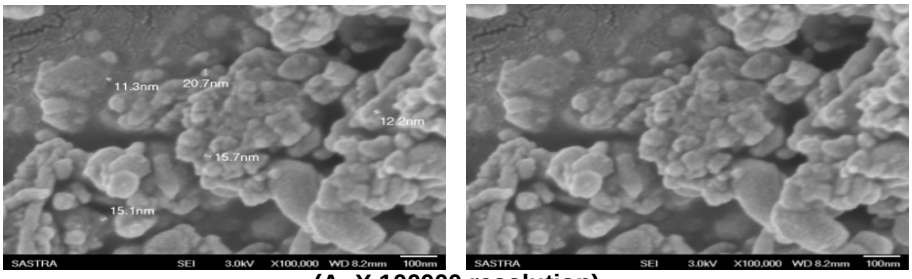
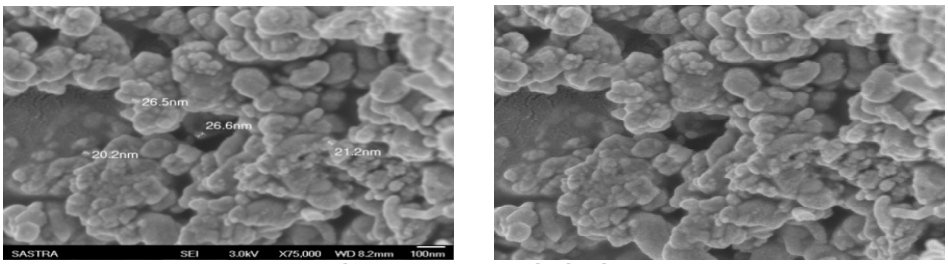


Fig. 8: FT-IR spectrum of silver nanoparticles

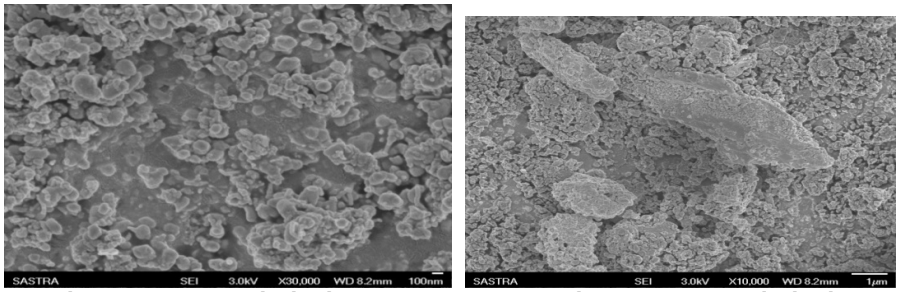
SEM analysis of silver nanoparticles shows uniformly distributed AgNPs on the surface of the cells; silver nanoparticles were spherical in shape with particle size range 10 to 50 nm. The larger one may be due to the aggregationof the smaller ones. Figure 9(A to D) shows the results of SEM with different resolutions were presented.



(A: X 100000 resolution)



(B: X 75000 resolution)



(C: X 50000 resolution) **(D: X10000 resolution)**

Fig. 9: SEM analysis of silver nanoparticles in different resolutions

5.5 DISCUSSION

Medicinal plants show their capacity to heal different infection and this was achieved by using different solvent in order to assess the phytochemicals, Extraction methods used pharmaceutically involves the separation of medicinally active portions of plant tissues from the inactive/inert components by using selective solvents (Kumar, 2011). During extraction, solvents diffuse into the solid plant material and solubilize compounds with similar polarity. In this study three solvents ware used (ethanolic, methanolic and acetone).

Secondary metabolites are the classes of compounds which are known to show curative activity against several ailments in man, and therefore could explain the use traditional of medicinal plant for the treatment of some illnesses.

There are a chemical compounds (phenolic compounds, alkaloids, terpenoids, steroids, quinones, saponins, etc) with complex structures and with more restricted distribution than primary metabolites. They are not indispensable for the plant that contains them; at least their metabolic functions have not been discovered yet.

According to Yaole(2010), phenolic compounds is one of the most numerous groups of substances in plant kingdom ranging from simple molecules, such as phenolic acids, to complex compounds, such as tannins. Large groups of phenolic compounds comprises: simple phenols (catechol, resorcinol, etc..), phenolic acids, stilbene (resveratrol, etc..), flavonoids (quercetin, cyanidin, etc..), biflavonoids (ormocarpine, etc..), proanthocyanidins (epicatechin), tannins, coumarins and anthraquinones. In this study selected phytochemicals were evaluated using standard techniques and the results were presented in table: 1 those compounds are known as phytochemicals.

Acacia nilotica has a wealth of medicinal uses. It is used for stomach upset and pain, the bark is chewed to protect against scurvy, an infusion is taken for dysentery and diarrhea. The pods are desirable as fodder for cattle, and the leaves, young shoots and young pods are thought to aid milk production. As reported by WHO, here it was selected to be used in this study.

This study shows minimum inhibitory concentration of the extracts against various pathogens used in this study. The MIC of ethanol extract was low [2.5mg./ml] as compared to other extracts [10 mg/ml].The lower MIC is an indication of high effectiveness of extract. It shows also antimicrobial effect on gram positive and gram negative bacteria as well as antifungal activity.

In this study plant extract shows antioxidant activity, these results suggest that the level of antioxidant activity in *Acacia nilotica* varies to a great extent. It also suggests that phenolics in this plant provide substantial antioxidant activity. Upon achievement of this survey, and using more samples, appears to be a rich and interesting source for supplementary ethnomedicinal and phytochemical studies.

In recent years, like other technology developments,nanotechnology also expected to grow based on their demand and its wider applications and the number of research being conducted in this field is rapidly growing throughout the world as reported in different scientific journals. Nanotechnology deals with the development of nanometer sized materials. In the field of nanotechnology different concepts of engineering, electronics, and material science are applied in molecular or submicron level. Particles with a size up to 100 nm are usually referred as nanoparticles and they exhibit completely new properties based on their size, distribution and morphology. In the nanoscale level the properties of the materials are different from that of their bulk materials and the increased surface area of these nanoparticles is mainly responsible for their different chemical,optical, and mechanical properties as showed by Satyavani (2011).

The extract of *Acacia nilotica* used in this study show the capacity of reducing some compounds and formation of nanoparticles the one formed is silver nanoparticles. The formation of stable silver nanoparticles from $AgNO_3$ gave mostly spherical particles with a diameter ranging from10 to 50 nm.

CONCLUSION

Plants have been evaluated as rich source of medicines due to their production of wide range of bioactive molecules which are classified as secondary metabolites or phytochemicals, most of which act as chemical defense against predation and infection.

Acacia nilotica is commonly known as medicinal plant in different area of the world.

- Different extracts of *Acacia nilotica* leaves showed presence of essential phytochemicals where the test shewed positive results on alkaloids,steroids, saponnins and terpenoids
- In the different three extracts (ethanolic, methanolic and acetone) ethanolic extract of *Acacia nilotica* leaves showed maximum zone of inhibition effect than methanolic and acetone extracts of *acacia nilotica* leaves.
- The 80% concentration of ethanolic extract exhibited high effect against bacteria and fungi than 30% and 50 % ethanolic extract.
- Ethanolic extracts of *Acacia nilotica* leaves showed high antioxidant activity in the concentration of 30%.
- Plant extract shows the antioxidant activity with different concentrations.
- Plant extract show capacity of reducing different compounds and give formation of nanoparticles.

REFERENCES

1. Apply nanotech to up industrial, agri output, (2012)The Daily Star (Bangladesh), 17 April.
2. Arunkumar S and Muthuselvam. Analysis of phytochemical constituents and antimicrobial activities of Aloe vera L. against clinical pathogens. World J Agril Sc. 2009;5(5):572–576.
3. Awwad AM and Salem NM. Green synthesis of silver nanoparticles by mulberry leaves extract. Nanosci Nanotechno. 2012;2:125–128
4. Awwad AM, Salem NM and Abdeen A. Biosynthesis of silver nanoparticles using Olea europaea leaves extract and its antibacterial activity. Nanosci Nanotechno. 2012;2:164–170 doi:10.1186/2228-5547-4-29
5. Banso A. Phytochemical and antibacterial investigation of bark extracts of Acacia nilotica. J Med Plants Res. 2009;3(2):082-085.
6. Binnig G and Rohrer H. Scanning tunneling microscopy. IBM Journal of Research and Development. 2012;30: 4.
7. Born D and Barron ML. Herb use in pregnancy: what nurses should know. 2005.
8. Chaubal R and Tambe A Isolation of new straight chain compound from Acacia nilotica. Ind. J Chem. 2006;45(B):1231-1233.
9. Cowan MM, Hawrelak JA, Cattley T and Myers SP. Clinical. Microbiology Rev. 1999;12:564.
10. Cristina Buzea, Ivan Pacheco and Kevin Robbie. "Nanomaterials and Nanoparticles: Sources and Toxicity. Biointerphases. 2007;2(4):MR17–71.doi:10.1116/1.2815690
11. Drexler and Eric K. Engines of Creation: The Coming Era of Nanotechnology. Doubleday. 1986. ISBN 0-385-19973-2.
12. Drexler and Eric K. Nanosystems: Molecular Machinery, Manufacturing, and Computatin. New York: John Wiley & Sons. 1992.
13. Duganath N. Evaluation of antidenaturation property and anti-oxidant activity of traditionally used medicinal plants. Int. J. Pharma. Bio Sciences. 2010;V1(2):1-7.
14. Harborne JB. Phytochemicals methods.Chapman and Hall Ltd, London: 1973;49–188.
15. Horborne JB. Phytochemical methods. 1998;3:20-25.
16. Kahn and Jennifer. Nanotechnology. National Geographic. 2006;98–119.
17. Khan R. Antimicrobial Activity of Five Herbal Extracts Against Multi Drug Resistant (MDR) Strains of Bacteria and Fungus of Clinical Origin. Molecules. 2009;14(2):586-597.
18. Kokwaro O. Medicinal plant of East Africa. Nairobi, Dar es Salaam: East African Literature Bureau Kampala. 1976.
19. Kremers and Edward. Kremers and Urdang's History of Pharmacy. 1986.
20. Kushi LH, Doyle C and McCullough M. American Cancer Society guidelines on nutrition and physical activity for cancer prevention: Reducing the risk of cancer with healthy food choices and physical activity. CA Cancer J Clin. 2012;62:30-67. Accessed at http://onlinelibrary.wiley.com/doi/10.3322/caac.20140/full on May 4,.
21. Lai PK and Roy J. Antimicrobial and chemopreventive properties of herbs and spices. Curr Med Chem. 2004;11(11):1451–60.
22. Mashram N. Antimicrobial activity of methanol extracts of medicinal plants against bacterial species. Int Res J. 2009;(3 &4):147-150.
23. Müller JL. Love potions and the ointment of witches: historical aspects of the nightshade alkaloids. 1998.

24. Yeole NB, Sandhya P, Chaudhari PS and Bhujbal PS. International Journal of PharmTech Research. 2010;2:385-389
25. Ncube NS, Afolayan AJ and Okoh AI. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. African Journal of Biotechnology. 2008;7(12):1797-1806.
26. Pande MB. Note on the nutritive value of babul (Acacia nilotica L.) seeds (extracted). Indian J Anim Sci. 1981;51(1):107-108.
27. Perez C, Pauli M and Bazevque P. An antibiotic assay by the agar well diffusion method. Acta Biologiae et Medicine Experimentalis. 1990;15:113-115.
28. Prasad SK. Modern Concepts in Nanotechnology. Discovery Publishing House. 2008;31–32.
29. Quattrocchi and Umberto. CRC World Dictionary of Plant Names. 1 A-C. CRC Press. 2000;6. ISBN 978-0-8493-2675-2
30. Quattrocchi and Umberto. CRC World Dictionary of Plant Names. 2000.
31. Rodgers P. Nanoelectronics: Single file. Nature Nanotechnology.doi:10.1038/nnano. 2006.
32. Saini Rajiv, Saini Santosh, Sharma and Sugandha. Nanotechnology: The Future Medicine. Journal of Cutaneous and Aesthetic Surgery. 2010;3(1): 32–33.doi:10.4103/0974-2077.63301
33. Satyavani K, Gurudeeban S and Balasubramanian TR. J. Nanobiotechnology. 2011;9:43.
34. Saxena A, Tripathi RM, Zafar F and Singh P. Green synthesis of silvernanoparticles using aqueous solution of Ficus benghalensis leaf extract and characterization of their antimicrobial activity. Mater Letters. 2012;67:91–94
35. Singh B.N. Prakash D and Singh HB. Antioxidant power of Acacia s pecies, Online Publication from www.herbication.com. 2009.
36. Singh Rajbir and Singh Bikram. Anti-free radical activities of kaempferol isolated from Acacia nilotica (L.) W illd Ex Del. Toxicology in Vitro. 2003;22(8):1965–1970.
37. Singh Rajbir, Singh Bikram and Singh Sukhpreeet. Anti-free radical activities of kaempferol isolated from Acacia nilotica(L.) Willd. Ex. Del. Toxicology in Vitro. 2008;22(8):1965–1970.
38. Sofowra A. Medicinal plants and Traditional medicine in Africa, Spectrum Books Ltd, Lbadan, Nigeria: 1993;191-289,
39. Solomon GO and Shittu GA. In vitro antimicrobial and phytochemical activities of Acacia nilotica leaf extract. J Med Plants Res. 2010;4(12):1232-1234.
40. Stepp, John R and Moerman Daniel E. The importance of weeds in ethnopharmacology. Journal of Ethnopharmacology. 2001;75(1):19–23.
41. Sultana B, Anwar F and Przybylski R. Antioxidant activity of phenolic components present in barks of Azadirachta indica, Terminalia arjuna, Acacia nilotica, and Eugenia jambolana Lam. 2007.
42. Sumner and Judith. The Natural History of Medicinal Plants. Timber Press. 2000.
43. Tapsell LC, Hemphill I, Cobiac L. "Health benefits of herbs and spices: the past, the present, the future. Med J Aust. 2006;185 (4 Suppl): S4–24.
44. Team visits Government Siddha Medical College. The Hindu, Saturday, 20 Feb. 2010.
45. Trease GE and Evans WC. Pharmacognosy, 11th Edn, Bailliere Tindall, London. 1989;45-50,
46. Yildirim A, Mavi A and Kara A. J Agric Food Chem. 2001;49:4083-4089.