Research Article **CODEN: AJPCFF** **ISSN: 2321 – 0915**

Asian Journal of Phytomedicine and Clinical Research

Journal home page: www.ajpcrjournal.com

BIO-INSPIRED SYNTHESIS OF SILVER NANOPARTICLES AND ANTIMICROBIAL ACTIVITY IN ANISOMELES *MALABARICA PLANT*

N. Packialakshmi[*1] and H. M. Nilofer Nisha[2]

*[1]PG and Research Department of Microbiology, Jamal Mohamed College (Autonomous), Tiruchirappalli, Tamilnadu, India.
[2]Research Scholar, PG and Research Department of Microbiology, Jamal Mohamed College (Autonomous), Tiruchirappalli, Tamilnadu, India.

ABSTRACT

Biologically synthesized silver nanoparticles (SNPs) are widely used in the field of medicine. The silver nanoparticles formation was confirmed by the colour change of plant extracts (SNPs). Phytosynthesized silver nanoparticles were tested for antibacterial activities using disc diffusion method. The test cultures *Proteus, Pseudomonas, Klebsiella, Bacillus* and *E.coli* species of bacteria were used. The microbial property of silver nanoparticles was analyzed by measuring the inhibition zone. The SNPs synthesized from leaf and boiled leaf extracts of *Anisomeles malabarica* showed the growth of *Pseudomonas* species were inhibited maximum zone of inhibition. FTIR Spectroscopic analysis reveals the presence of biomolecules in the reaction medium may be responsible for the reduction of silver ions. The results indicate that the silver nanoparticles may have an important advantage over conventional antibiotics.

KEYWORDS

Antimicrobial activity, Medicinal plant, Silver nanoparticles and FTIR.

Author of Correspondence:
Packialakshmi N,
PG and Research Department of Microbiology,
Jamal Mohamed College (Autonomous),
Tiruchirappalli, Tamilnadu, India.

Email: packia_lakshmi_1977@yahoo.com.

INTRODUCTION

Nanotechnology is now creating a growing sense of excitement in the life sciences especially biomedical devices and Biotechnology. Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. The silver nanoparticles have various and important applications. Historically, silver has been known to have a disinfecting effect and has been found in applications ranging from traditional

medicines to culinary items. It has been reported that silver nanoparticles (SNPs) are non-toxic to humans and most effective against bacteria, virus and other eukaryotic micro-organism at low concentrations and without any side effects. Moreover, several salts of silver and their derivatives are commercially manufactured as antimicrobial agents. In small concentrations, silver is safe for human cells, but lethal for microorganisms. Antimicrobial capability of SNPs allows them to be suitably employed in numerous household products such as textiles, food storage containers, home appliances and in medical devices. The most important application of silver and SNPs is in medical industry such as tropical ointments to prevent infection against burn and open wounds. Biological synthesis of nanoparticles by plant extracts is at present under exploitation as some researchers worked on it and testing for antimicrobial activities. For the last two decades extensive work has been done to develop new drugs from natural products because of the resistance of micro-organisms to the existing drugs. Nature has been an important source of a products currently being used in medical practice.

Plant have been an integral part of life in many local for food and medicine both. India has more than 3000 years of medicinal heritage based on medicinal plants. Medicinal plants are widely used by all sections of the population either directly as folk remedies are indirectly in the preparation of pharmaceuticals. Out of nearly 17,000 higher plants recorded in india, 7500 are reported to be in medicinal use by the rural and tribal communities[1]. The Lamiaceae (Labiatae) is one of the most diverse and widespread plant families in terms of ethnomedicine and its medicinal value is based on the volatile oils concentration[2]. The Lamiaceae plant family is one of the largest families among the dicotyledons, many species belonging to the family being highly aromatic, due to the presence of external glandular structures that produce volatile oil[3]. *Anisomeles malabarica* is a medicinal plant that has been used as a folkloric medicine to treat amentia, anorexia, fevers, swelling, rheumatism. The herb is reported possess anticancer, allergenic, antihelmintic, antibacterial, antiplasmodial and antiperotic properties[4]. *Anisomelic* acid, is one of the major compounds in *Anisomeles malabarica (L.) R. Br.*, is a cembrane type diterpenoid, which can be synthesized chemically. *Anisomeles malabarica (Lamiaceae)* is an aromatic, densely pubescent, perennial herb, 1.2-2 m in height. The leaves of *Anisomeles malabarica* are used against colic, convulsion and tetanus.

MATERIALS AND METHODS

Sample Collection

The plant samples were collected from the Theekalamalai near Vaiyampatti, dry rocky region of Trichy district, Tamilnadu. The plants were identified in Botany Department of Jamal Mohammed College in Tiruchirapalli. The leaves were separated from the collected plant and dried under shade. After drying it was pulverized to powder in a mechanical grinder for further studies.

Preparation of Plant Extracts

10 gm of plant powder was weighed and it is mixed with 100 ml of water. The extraction was carried out in a shaker for 24 hours. The solution was filtered through Whatsman no.1 filter paper. The filtered samples were collected in a conical flask. The obtained extract was used for the synthesis of silver nano particles.

Preparation of silver nitrate solution

1 mM silver nitrate solution was prepared by the concentration of 0.0169 gm in 100 ml double distilled water and stored.

Metal-plant extracts interaction

90 ml of silver nitrate solution was taken in conical flask. To this add 10 ml of leaf and boiled leaf extract. The color change of the silver nitrate solution was found from colorless to dark brown. Incubate the conical flask at room light for 24 hours.

Concentration of phyto nano particles

After 24 hours incubation, the color change was observed. This indicates that the silver nano particles were synthesized from leaves and boiled leaf with the help of aqueous solution. Then this solution was taken in centrifuge tube and it was centrifuged at 10,000 rpm for 20 minutes. The pellets were taken

after centrifugation and it were air dried. The pellets were used for further testing phase contrast microscope analysis and antimicrobial activity.

Phase contrast microscope

Thin film of the sample were prepared on a glass slide by dropping a very small amount of sample. Allowed to air dry and observed under phase contrast microscope.

Antimicrobial activity

The silver nanoparticles synthesized and aqueous extract of *Anisomeles malabarica* extract were tested for antimicrobial activity by agar disc diffusion method against pathogenic bacteria. Test microorganisms selected for antimicrobial activity are *Staphylococcus aureus, Bacillus sp, Proteus vulgaris, Klebsiella sp* and *Escherichia coli*. The strains were obtained from MTCC, Chandigarh in India and maintained on agar slants.

Disc Diffusion Method

The disc diffusion method provides a simple and reliable test in routine clinical bacteriology in order to find out the effect of a particular substance on a specific bacterium. This method consists of impregnating small circular discs of standard sterile disc with given amount of a chosen concentration of plant extract. Muller Hinton agar (MHA) plates were prepared. Overnight nutrient broth culture of test organisms were seeded over the MHA plates. A sterile cotton swab is used to make a lawn. The discs which had been impregnated with different extracts of leaf and boiled leaf (silver nanoparticles synthesized and aqueous extract) were placed on the MHA with the control disc and subjected to antibacterial screening. The plates were then incubated at 37^0 C for 18-24 hours. After the incubation the plates were examined for inhibition zone.

IR spectrum analysis

FTIR relies on the fact that the most molecules absorb light in the infra red region of the electromagnetic spectrum. This absorption corresponds specifically to the bonds present in the molecule. The frequency ranges are measured as wave numbers typically over the range 4000-600 cm^{-1}. The compounds were analysed using shimaze IR affinity I instrument.

RESULTS

Antibacterial activity was assayed *invitro* by disc diffusion method against five bacterial strains. The antimicrobial activity of the aqueous extracts of *Anisomeles malabarica* was more effective against *Pseudomonas aeruginosa, Bacillus subtilis Proteus vulgaris, Klebsiella sp and E.coli.* The bacterial strains revealed the zone of Inhibition (Table No.1 and Figure No.2). The tested plant extract, aqueous extract of *Anisomeles malabarica* showed antibacterial activity against most of the bacterial species. Best zone of inhibition was produced by aqueous leaf extract of *Anisomeles malabarica, Pseudomonas aeruginosa* (15 mm) and least was produced against *E.coli and Klebsiella sp* (12 mm). Best zone of inhibition was produced by aqueous boiled leaf extract *Pseudomonas aeruginosa* (14 mm) and least was produced against *Proteus sp, Bacillus sp, E.coli and Klebsiella sp* (13 mm).

The chi-square values obtained respectively which was less than the calculated table value. $X^2(0.05)=3.841$ at 5% level of significance. Above results lead to the conclusion that the data was consistent with the hypothesis, the diameter of inhibition zone obtained from the observed data showed the similarities with experimental data.

The green synthesis of silver nanoparticles through plant extracts were carried out. It is well known that silver nanoparticles exhibit yellowish - brown color in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles. The appearances of yellowish-brown color in the reaction vessels suggest the formation of silver nanoparticles (SNPs) (Figure No.3 and 4). Silver nitrate is used as reducing agent as silver has distinctive properties such as good conductor, catalytic and chemical stability. The aqueous silver ions when exposed to herbal extracts were reduced in solution, there by leading to the formation of silver hydrosol. The time duration of change in color varies from plant to plant. *Anisomeles malabarica* synthesized silver nanopartcles within 10 minutes.

The synthesis of SNPs had been confirmed by visual observation of phase contrast microscope (Figure No.5 and 6).

Antibacterial study on pathogen opens a door for nanotechnology applications in medicine. Biological synthesis of metal NPs is a traditional method and the use of *Anisomeles malabarica* plant extracts has a new awareness for the control of disease, besides being safe and no phytotoxic effects. The tested plant extract, silver nanoparticle synthesis *extract* of *Anisomeles malabarica* showed antibacterial activity against most of the bacterial species. Best zone of inhibition was produced by silver nanoparticle synthesis of leaf extract of *Anisomeles malabarica, Pseudomonas aeruginosa* (19 mm) and least was produced against *Bacillus sp and Klebsiella sp* (12 mm). Best zone of inhibition was produced by boiled leaf extract *Pseudomonas aeruginosa* (20 mm) and least was produced against *Bacillus sp, E.coli and Klebsiella sp* (13 mm). The minimum zone was observed against *Proteus sp* (12 mm) (Figure No.1).

The biologically synthesized silver nanoparticles using medicinal plants were found to be highly toxic against different pathogenic bacteria of selected species. The SNPs and extracts of *Anisomeles malabarica* leaf and boiled leaf extract shows highest antibacterial activity was observed against *Pseudomonas species* showed in Table No.1 and Figure No.1 and 2.

The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The results of FTIR peak values and functional groups were represented in Table No.2, 3 and 4 and Figure No.7 and 8. The presence of various functional groups of different compounds was found. FTIR spectroscopy is proved to be a reliable and sensitive method for detection of biomoleclar composition.

The absorption spectra of sample in leaf. The dominant band in case of leaf was observed at 1325.10, 1348.24, 1525.69, 1564.27 and 1658.78 cm^{-1} represents nitro compounds. The band at 1442.75, 1463.97, 1481.33 and 1932.62 cm^{-1} was due to alkanes. The band at 451.34, 484.13, 651.94 and 677.01 cm^{-1} represents halogen compound. The peak at 1381.03 and 3697.54 cm^{-1} represents alcohols. The peak at 1811.16 and 1855.52 cm^{-1} indicates acid anhydrides. The band at 1876.74 and 1901.81 cm^{-1} was due to the presence of acid halides. The peak at 2852.72 and 2922.16 cm^{-1} indicates carboxylic acids. The band at 1406.11cm^{-1} shows phenols. The peak at 840.96cm^{-1} shows c-H stretching (aromatic). The band at 1020.34 cm^{-1} show C-O stretching represents the presence of ethers. The peak at 3782.41 cm^{-1} represents amines. The band at 1743.65 cm^{-1} indicates lactams compounds.

The absorption spectra of the boiled leaf sample are shown in Figure No.4. The band at 1265.30, 1323.17, 1379.10, 1523.76, 1598.99 and 1658.78 cm^{-1} attributed to nitro compounds. The band at 516.92, 603.72, 673.16 and 1157.29 cm^{-1} represent halogens. The absorption band at 1782.23, 1807.30 and 1853.59 cm^{-1} represent acid anhydrides. The peak at 2852.72 and 2922.16 indicates O-H stretching of carboxylic acids. N-H stretching was found to be present due to the appearance of absorption peak at 3425.58 and 3697.54 cm^{-1}. The band at 1022.27 cm^{-1} was due to ether. The band at 1404.18 cm^{-1} represents phenol. The peak at 1440.83 cm^{-1} indicates alkane. The band at 1681.93 cm^{-1} represents amide. The band at 1741.72 cm^{-1} revealed the presence of C=O stretching of lactams. The band at 1874.81 cm^{-1} shows ester. The peak value at 2374.37 cm^{-1} show amino acids and 3697.54 cm^{-1} shows alcohol group.

Significant changes have been recorded in the functional groups in *Anisomeles malabarica* subjecting the sample for FTIR. This includes the presence of amine group present in leaf and boiled leaf.

DISCUSSION

The methanolic extract of leaves from *Anisomeles malabarica* was tested for *in vitro* antioxidant and antimicrobial activity using various free radical scavenging assays and antibacterial activity against clinically important bacterial strains. Free radical

scavenging assays such as hydroxyl, superoxide anion radicals, 2,2-diphenyl-1-picryl hydrazyl (DPPH) and 2,2'-azinobis-(3-ethyl-enzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assays were performed. The agar disk diffusion method was used to study the antibacterial activity of *A. malabarica* against five bacterial strains. It was observed that the extract effectively scavenged hydroxyl and superoxide anion radicals. It also scavenges DPPH and ABTS radicals. All the concentrations of leaf extract showed free radical scavenging and antioxidant power and the preventive effects were in a dose-dependent manner. Those various antioxidant activities were compared to standard antioxidants such as ascorbic acid, butylated hydroxytoluene (BHT) and a-tocopherol. Further, the extract showed inhibitory activity for Gram-positive and negative bacteria. Thus, the results obtained in the present study indicate that *A. malabarica* extract could be considered as a potential source of natural antioxidants and that could be used as an effective source against bacterial diseases[5].

Anisomeles indica plant was collected from Dr. Panjabrao Deshmukh Krushi Vidyapeeth Agriculture Farm, Nagpur district (Maharashtra state). This plant belongs to family -Lamiaceae and is commonly known as "Gopoli" which is an evergreen aromatic, perennial, erect herb and measuring about 1.5cm in height. The stem is quadrangular, the leaves are covered by hairs and inflorescence is of verticillasters type. In the present study anatomical characters and phytochemical analysis of the leaves are reported. Anatomical characters reveal the study of leaf anatomy. Phytochemical analysis confirmed the presence of alkaloids, tannin, glycosides, carotenoids and saponin in leaves. Alkaloids showed high scores while tannins showed moderate scores but saponins indicated low scores. Aromatic oil is found in 6% in 3gm of dry weight of powder of leaves of Anisomeles indica. The present investigations concluded that the leaves of Anisomeles indica contains alkaloids> tannins> saponins> Glycosides> Carotenoids> Polyuronoids in this order, and contains double percent amount of aromatic oil. These chemicals are widely used in Ayurvedic traditional medicinal system[6].

The objective of the present study was to review on few medicinally potential plants of Lamiaceae of Karnataka. Plants in this family, are herbs or shrubs often with an aromatic smell. They are common in the Mediterranean countries for the fact that some of them produce a high amount of essential oil that enables them to survive the hot summer season. Some examples from this family include Anisomeles, Colebrookea, Coleus, Hyptis, Leonotis, Leucas, Mentha, Ocimum, Oreganum and Salvia. These are important for medicinal, perfumery, culinary and ornamental purposes. Medicinal constituents include the strong aromatic essential oil, tannins, saponins and organic acids. The oil is obtained by steam distillation. In aromatherapy, the oil is used for its soothing effects. These plants have sedative, diuretic, tonic, antispasmodic, antifungal, antimicrobial, anti-inflammatory and antiseptic properties[7].

The rapid biological synthesis of silver nano particles using leaf extract of *Anisomeles malabarica* provides an environmental friendly, simple and efficient route for synthesis of nano particles. The bio reduced silver nano particles were characterized by using phase contrast microscopic technique. In the present study we found that leaf can be used as a good source for synthesis of silver nano particles and it has more antibacterial activity against various pathogenic organisms.

Table No.1: Antibacterial activity of aqueous and silver nanoparticles synthesis extract of Anisomeles malabarica [leaf and boiled leaf (zone of inhibition in mm)]

S.No	Sample	Bacterial Strains	Aqueous Extract		Nanoparticle extract	
			Leaf	Boiled leaf	Leaf	Boiled leaf
1	*Anisomeles malabarica*	*E.coli*	12	13	13	12
2		*Klebsiella sp*	12	13	12	13
3		*Pseudomonas sp*	15	14	19	20
4		*Bacillus sp*	13	13	12	13
5		*Proteus sp*	14	13	13	13

Table No.2: Infra red spectrum analysis by *Anisomeles malabarica* leaf powder

S.No	Peak value	Stretching	Interpretation
1	451.34	C-CI	Halogens
2	484.13	C-CI	Halogens
3	651.94	C-Cl	Halongens
4	677.01	C-Cl	Halogens
5	840.96	C-H	Aromatics
6	1020.34	C-O	Ethers
7	1325.10	N=O	Nitro compounds
8	1348.24	N=O	Nitro compounds
9	1381.03	C-O	Alcohols
10	1406.11	C-O	Phenols
11	1442.75	C-H	Alkanes
12	1463.97	C-H	Alkanes
13	1481.33	C-H	Alkanes
14	1525.69	N=O	Nitro compounds
15	1564.27	N=O	Nitro compounds
16	1589.34	C-H*	Aldehydes
17	1658.78	N=O	Nitro compounds
18	1687.71	C=O	Amides
19	1707.00	C=O	Ketones
20	1724.36	C=O	Ketones
21	1743.65	C=O	Lactams
22	1811.16	C=O	Acid anhydrides
23	1855.52	C=O	Acid anhydrides
24	1876.74	C=O	Acid halides
25	1901.81	C=O	Acid halides
26	1932.67	C=C	Alkanes
27	2852.72	O-H	Carboxylic acids
28	2922.16	O-H	Carboxylic acids
29	3697.54	O-H	Alcohols
30	3782.41	N-H Rocking	Amines

Table No.3: Infra red spectrum analysis by *Anisomeles malabarica* boiled leaf powder

S.No	Peak value	Stretching	Interpretation
1	516.92	C-Br	Halogens
2	603.72	C-Cl	Halogens
3	673.16	C-C	Ethers
4	1022.27	C-O	Halogens
5	1157.29	C-F	Nitro compounds
6	1265.30	N=O	Nitro compounds
7	1323.17	N=O	Nitro compounds
8	1379.10	N=O	Phenols
9	1404.18	C-O	Alkanes
10	1440.83	C-H	Nitro compounds
11	1523.76	N=O	Nitro compounds
12	1598.99	N=O	Nitro compounds
13	1658.78	N=O	Amides
14	1681.93	C=O	Lactams
15	1741.72	C=O	Acid anhydrides
16	1782.23	C=O	Acid anhydrides
17	1807.30	C=O	Acid anhydrides
18	1853.59	C=O	Esters
19	1874.81	C=O	Esters
20	2374.37	N-H	Amino acids
21	2852.72	O-H	Carboxylic acids
22	2922.16	O-H	Carboxylic acids
23	3425.58	N-H	Amines
24	3697.54	O-H	Alcohols
25	3780.48	-N-H Rocking	Amines

Table No.4: Interpretation of infra red spectrum analysis by *Anisomeles malabarica* leaf and boiled leaf powder

S. No	Peak value	Stretching	Interpretation
1	1658.93	N=O	Nitro compounds
2	2852.72	O-H	Carboxylic acids
3	2922.16	O-H	Carboxylic acids
4	3425.58	N-H	Amines
5	3697.54	O-H	Alcohols

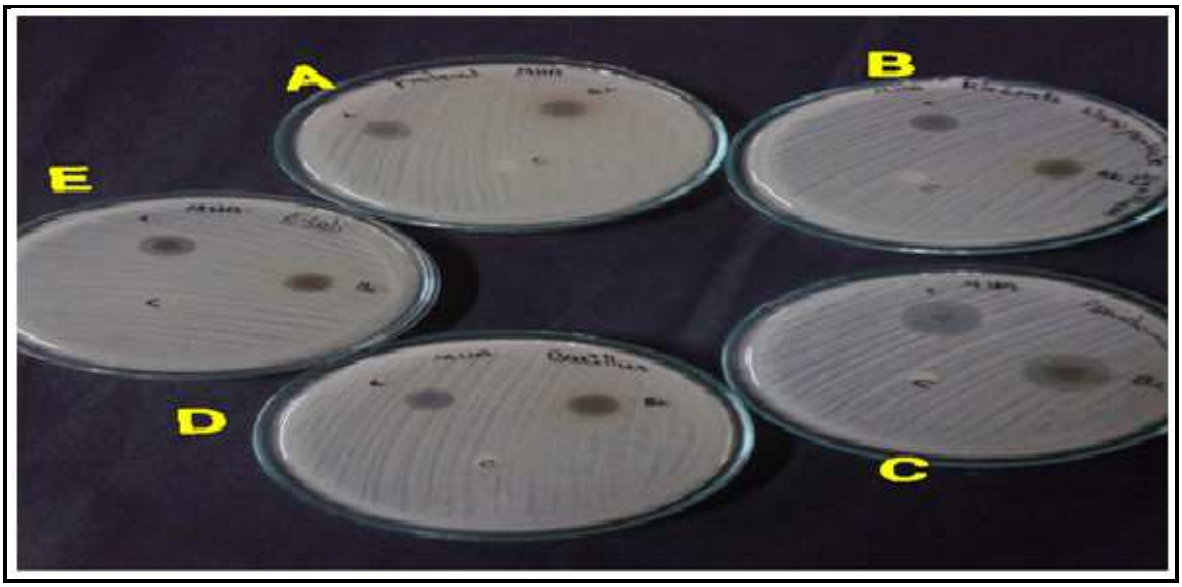

Figure No.1: Zone Inhibition formed by aqueous extract of *Anisomeles malabarica* Leaf and Boiled leaf. A-*Proteus sp, B-Klebsiella sp, C-Pseudomonas aeruginosa, D-Bacillus sp, E-E.coli*

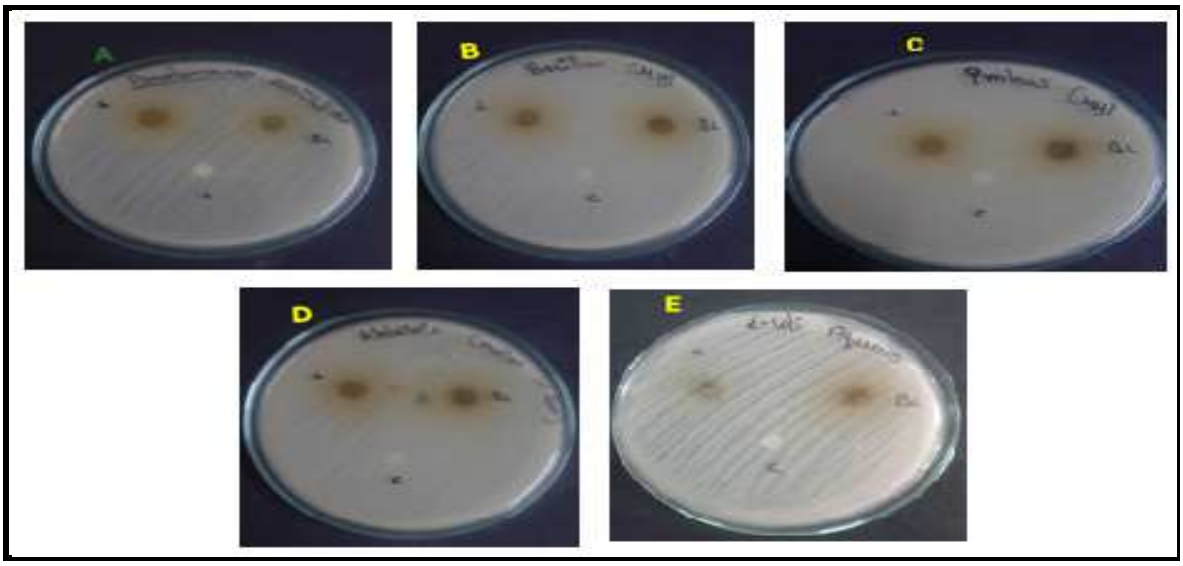

Figure No.2: Zone Inhibition formed by nano particles extract of *Anisomeles malabarica* Leaf and Boiled leaf. *A- Pseudomonas aeruginosa, B- Bacillus sp, C- Proteus sp, D- Klebsiella sp, E- E.coli Proteus sp*

Figure No.3: Synthesis of silver nanoparticles from *Anisomeles malabarica crude extract (leaf)* A-Zero (hrs), B-12 (hrs), C-24(hrs), D-Control

Figure No.4: Synthesis of silver nanoparticles from *Anisomeles malabarica crude extract (boiled leaf)* A-Zero (hrs), B-12 (hrs), C-24(hrs), D-Control

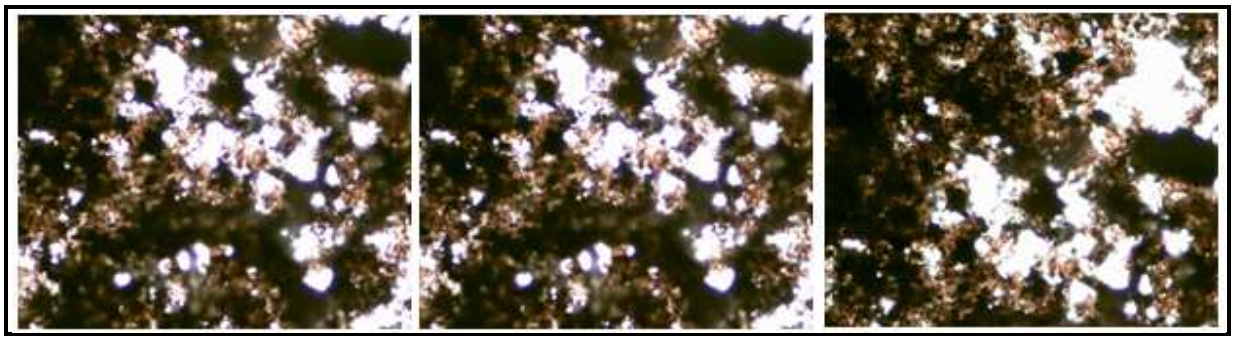

Figure No.5: Synthesis of silver nanoparticles observed under phase contrast microscope (leaf)

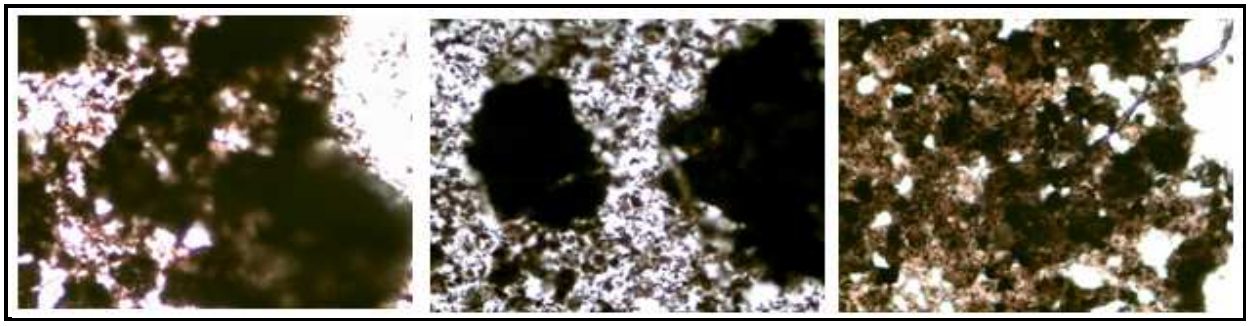

Figure No.6: Synthesis of silver nanoparticles observed under phase contrast microscope (boiled leaf)

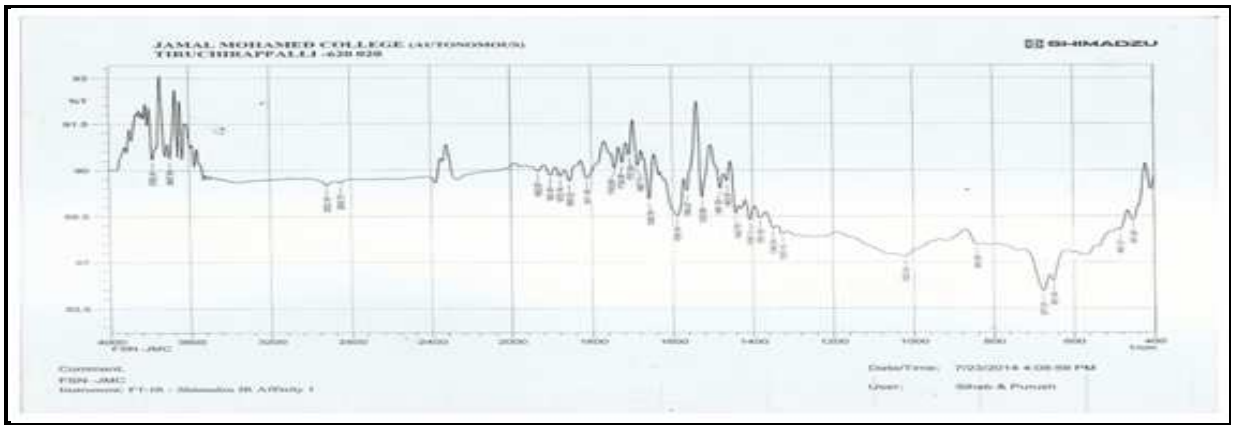

Figure No.7: Infrared spectrum analysis by silver nanoparticles in Anisomeles malabarica crude leaf extract

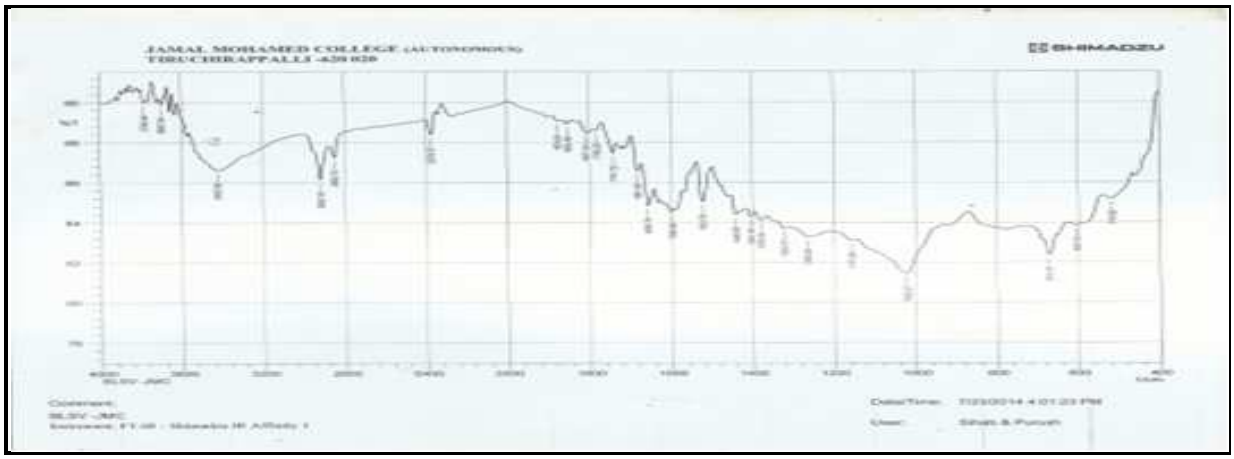

Figure No.8: Infrared spectrum analysis by silver nanoparticles in Anisomeles malabarica crude boiled leaf extract

CONCLUSION

The study includes the synthesis of silver nano particles from the leaves and boiled leaf of *Anisomeles malabarica* and its antibacterial activity was confirmed by disc diffusion method. The reduction of metal ions through the leaves extracts leading to the formation of silver nano particles of fairly well defined dimensions. The silver nano particles have potential applications in the biomedical field and this simple procedure has several advantages such as cost effectiveness, compatibility for medical and pharmaceutical applications as well as large scale commercial production. Thus it can be concluded that *Anisomeles malabarica* posses more activity against pathogenic microorganisms and further studies reveal its other activities.

ACKNOWLEDGEMENT

The authors are thankful to the Secretary, Principal and Management of Jamal Mohamed College (Autonomous), Tiruchirappalli for offering facilities to carry out this study.

REFERENCES

1. Karuppusamy S. Medicinal plant used by paliyan tribes of sirumali hills of southern India, *Natural product radiance*, 6(5), 2007, 436-445.
2. Sarac N and Ugur A. Antimicrobial activities and usage in folkloric medicine of some Lamiaceae species growing in Mugla Turkey,

European Asian Journal of Biological Science, 1, 2007, 28-34.

3. Giuliani C and Maleci Bini L. Insight into the structure and chemistry of glandular trichomes of Labiatae, with emphasis on subfamily Lamioideae, *Plant systematic and Evolution,* 276, 2008, 199-208.
4. Abdul Abudz Zahir *et al*. Evaluation of antileishmanial activity of South Indian medicinal plants against *Leishmania donovani*. *Experimental parasitolog,* 132, 2012, 180-184.
5. Boobalan Raja and Jeganathan Manivannan. Evaluation of in vitro antioxidant and antimicrobial potential of methanolic leaf extract of *Anisomeles malabarica (Linn.), Journal of Pharmacy Research,* 3(7), 2013, 1471-1474.
6. Ulhe S K and Narkhede S D. Histological and Phytochemical studies on aromatic plant, Anisomeles indica (L.) of family Lamiaceae (M.S.) India, *International Journal of Life Science*, 1(4), 2013, 270-272.
7. Venkateshappa S M and Sreenath K P. Potential Medicinal Plants of *Lamiaceae, American International Journal of Research in Formal, Applied and Natural Sciences*, 3(1), 2013, 82-87.