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SCREENING OF ANTIBACTERIAL ACTIVITY IN POLAR AND NON-POLAR FLOWER AND STEM EXTRACT OF *ANISOMELES MALABARICA* PLANT

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ABSTRACT

Anisomeles malabarica flower and stem were tested for their antibacterial properties against some pathogenic gram positive and gram negative bacteria. The growth of *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Bacillus subtilis* and *Proteus vulgaris* were significantly inhibited. The maximum zone of inhibition were found in *Staphylococcus aureus*, *Proteus vulgaris* and *Bacillus subtilis*. The FTIR spectroscopic studies revealed different characteristic peak values with various functional compounds in the crude (Flower and Stem). The FTIR method was performed on a spectrophotometer system, which was used to detect the characteristic peak values and their functional groups. The study revealed the presence of halogen, alkanes, amides, acid anhydride, ester, nitro compounds, amines and ketones. These findings have confirmed the use of this plant in treating of several bacterial infections both traditional and folk medicine in india.

Keywords: *Anisomeles malabarica,* Pathogenic organisms, Antibacterial activity and FTIR.

INTRODUCTION

Traditional and folk medicines play an important role in health services around the globe. About three quarter of the world's population relies on plants and plant extracts for health care. India has an extensive forest cover enriched with plant diversity. Over the past 20 years, there has been an increased interest in the investigation of natural material source of new antifungal and antibacterial agents. Different extracts and essential oil from traditional plants

have been tested to identify the source of therapeutic effects. A wide range of medicinal plants part is used as raw drugs with varied medicinal properties.

Anisomeles malabarica is a potent drug used in ayurveda and siddha systems of medicine. *Anisomeles malabarica* is the genuine source of sprikka. It is widely distributed in Srilanka, Malayan peninsula, Mauritius etc. In India, they are found in Deccan, North India, South carnatic region, Tamilnadu and Southern India. Plant is a shurb with tomentose, tetrangular stem. Leaves are simple, opposite, thick, aromatic and wolly. Flowers are purple in dense whorls of interrupted spikes. Stem and leaves are covered with wolly soft and white hairs.[1] It is a medicinal plant especially in India. The leaves and roots are used as an astringent, a carminative, febrifuge and a tonic. It is used to treat amentia, colic, fever, dyspepsia etc.

Pharmacognostic studies of *Anisomeles malabarica* have been reported by [2] Plants show characteristic anatomical features of *Lamiaceae* family such as quadrangular stem with glandular, curved trichomes. Plants yield an essential oil containing citral and geranic acid. It also contain macro cyclic diterpenedilactone-ovatodiolide with two beta unsaturated lacton emoeities. Two diterpenoid compounds, ovatodiolide and anisomelic acid have been isolated from *Anisomeles malabarica.* Plants are stomachic, bitter, aphrodistiac and intellect promoting.

In the present study deals with the antimicobial activity and functional groups present in these chemical constituents of plants are usually identified by FTIR. This helps in structure elucidation with other methods and gained importance to identify medicines . Initially, FTIR was used to elucidate the structure of isolated compounds. Identification and comparison of biomolecules of *Anisomeles malabarica* were done by using FTIR. This study creates a platform to screen many bio active components to treat various diseases.

MATERIALS AND METHODS

Sample Collection

The plant sample were collected from the Theekamalai near Vaiyampatti, dry rocky region of Pudukottai district in Tamilnadu. The plant were identified in Botany Department of Jamal Mohammed College in Tirchirapalli. The flowers and stems were separated from the collected plant and dried under shade. After drying it was pulverized to powder in a mechanical grinder for futher studies.

Preparation Of Plant Extracts

The different parts of *Anisomeles malabarica* plant were collected and dried at room temperature for 2-3 days and further dried at 60^{0} C. The dried flower and stems were extracted with solvents. Ethanol, methanol and petroleum ether extracts are separately prepared and incubated at room temperature for 48 hours with stirring at regular interval. The extracts were filtered with the whattmann filter paper and then dried by using rotary evaporator. The filtrate was stored in screw cap bottle at -20^{0} C for further use.

Antimicrobial activity

Detection of antimicrobial activity of *Anisomeles malabarica* by bioautography. Test microorganisms were selected for antimicrobial activity are *Escherichia coli MTCC 78 , Klebsiella pneumoniae MTCC 109, Staphylococcus aureus MTCC 96 , Bacillus subtilis MTCC 121* and *Proteus vulgaris.* The strains were obtained from MTCC, *Chandigarh* in India and maintained on agar slants.

Disc Diffusion Method

The disc diffusion method provide 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. Using sterile cotton swab to make lawn. The discs which had been impregnated with different extracts of Flower and stem 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.

Chi-Square Test (X^2)

In this study chi-square test was applied. The purpose of chi-square test (X^2) was to decide whether the set of observed data (Antibiogram of microorganisms) agrees with the standard antimicrobial disc suceptibility test (NCCLS, 2002).

IR SPECTRUM ANALYSIS[3]

FTIR relies on the fact that the most moleculas 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

The result of the antimicrobial activity of the fresh flower and stem of *Anisomeles malabarica* extracts is given in Table 1, 2, 3 and 4. The solvents were prepared as different concentrations compared with all the concentration 128µg/ml. Concentration gave the maximum zone of inhibition for all the extracts. The parts of the plants *Anisomeles malabarica,* the flower and stem produced best antibacterial activity. Polar extracts like ethanol and methanol to be the best solvent for extraction of antibacterial compounds from the *Anisomeles malabarica* plants. The maximum zone of inhibition was found in stem extract and least with flower extract.

Best zone of inhibition was produced by the ethanol flower extract, *Staphylococcus aureus MTCC 96* (15 mm) and least was produced against *Escherichia coli MTCC 78* (13 mm). The methanol extract revealed the maximum zone of inhibition was produced *Proteus vulgaris* (16 mm) and the least was produced against *Escherichia coli MTCC 78* (13 mm). The petroleum ether extract (non-polar) showed maximum zone of inhibition against *Proteus vulgaris* (13 mm) and absence of zone was observed against *Staphylococcus aureus MTCC 96.*

Best zone was produced by ethanol stem extract *Staphylococcus aureus MTCC 96* (21 mm), least was produced against *Bacillus sp., MTCC 121* and *Escherichia coli MTCC 78* (14 mm). The methanol extract revealed that maximum zone of inhibition was produced against *Staphylococcus aureus MTCC 96* (20 mm) and least was produced against *Escherichia coli MTCC 78* (12 mm). The maximum zone of inhibition in the petroleum ether stem extract revealed against Proteus vulgaris (16 mm), least was produced against *Escherichia coli MTCC* 78 (13 mm).

Overall, the antibacterial activity of the *Anisomeles malabarica* revealed that the best antibacterial activity was produced by stem extract followed by flower extract. All the extracts produced better zone of inhibition against *Staphylococcus aureus MTCC 96* (21 mm) and *Proteus vulgaris* (19 mm). The least zone of inhibition againt *Escherichia coli MTCC 78* (10 mm). In the present study to analyse the solvent extracts using both polar and non-polar

extracts. The stem gave the maximum zone of inhibition in the non polar extracts when compared to flower extracts Fig 1.

FTIR analysis for functional groups revealed the presence of various characteristic functional groups in the samples (Flower and Stem) of *Anisomeles malabarica*. The frequency range and functional group obtained from absorption spectra are shown in table 5 and 6 and Fig 2 and 3 . The absorption spectrum of sample in Flower are shown in fig 5. The dominant band in case of flower was observed at 1116.78, 1161.15, 1402.25, 1442.75, 2854.65 and 2924.09 cm^{-1} was due Alkanes compound. The band at 422.41, 576.72, 669.30, 1323.17 and 1382.96 cm^{-1} represents Halogens compound. The band at 1525.69 and 1654.92 cm^{-1} represents Amides. The peak value at 1564.27 cm^{-1} Nitro compounds. The peak at 1683.86 and 1878.67 cm^{-1} was due to the presence of ketones. The peak at 1743.65 cm^{-1} indicate Ester compound. The peak at 1805.37 and 1853.59 shows C=O stretching (Acid anhydrides). The band at 2270.22, 2339.65 and 2374.37 cm^{-1}shows Amino acid. The band at 3421.72 and 3759.26 cm^{-1}show O-H stretching represent the presence of Alcohol compound.

The absorption spectra of the stem sample are shown in fig. 6. The strong band in case of stem sample was observed at 1029.99, 1109.07, 1377.17, 1402.25, 1440.83, 2852.72 and 2922.16 $cm^{-1}$was attributed to Alkanes group. The band at 530.42, 617.22, 667.37,777.31, 1155.36 and 1246.02 $cm^{-1}$was due to Halogens group. The band at 1325.10 cm^{-1}revealed the presence of C-O stretching of Alcohol. The band at 1521.84 $cm^{-1}$was due to Nitro compound. The band at 1656.85cm^{-1} represent Ester. The band at 1739.79 cm^{-1} represent Aldehyde. The band at 1803.44 cm^{-1} was due to Acid an hydride. The peak at 2374.37 cm indicate N-H stretching of Amino acids. The peak value at 3423.65 cm^{-1} was due to Amine compounds. The peak at 3759.26 cm^{-1} resulted from the presence of Amide.

Table1- Antibacterial activity of polar extracts of *Anisomeles malabarica* flower powder (zone of inhibition in mm)

S.no	Sample	µg/ml	Bacterial strains	Ethanol		$X^2=(O-E)^2/E$	Methanol		$X^2=(O-E)^2/E$
				SV	OV		SV	OV	
1	Flower extract of *Anisomeles malabarica*	128µg	*E. coli*	22	13	3.681	22	13	3.681
2			*Klebsiella sp.,*	22	14	2.909	22	14	2.909
3			*Staphylococcus aureus*	22	15	2.227	22	14	2.909
4			*Bacillus sp.,*	22	14	2.909	22	14	2.909
5			*Proteus sp.,*	22	14	2.909	22	16	1.636

Table value $X^{2(0.05)=3.84}$, Chi - square value significance at 5% level
SV-Standard value and OV-Observed value

Table 2- Antibacterial activity of Non-polar extracts of *Anisomeles malabarica* flower powder (zone of inhibition in mm)

S.no	Sample	µg/ml	Bacterial strains	Petroleum ether		X2=[(O-E)2]/E
				SV	OV	
1	Flower extract of *Anisomeles malabarica*	128µg	*E. coli*	22	10	6.546
2			*Klebsiella sp.,*	22	12	4.545
3			*Staphylococcus aureus*	22	-	-
4			*Bacillus sp.,*	22	12	4.545
5			*Proteus sp.,*	22	13	3.681

Table value $X^{2(0.05)=3.84}$, Chi - square value significance at 5% level

SV-Standard value and OV-Observed value

Table 3- Antibacterial activity of polar extracts of *Anisomeles malabarica* stem powder (zone of inhibition in mm)

S.no	Sample	µg/ml	Bacterial strains	Ethanol		X2=[(O-E)2]/E	Methanol		X2=[(O-E)2]/E
				SV	OV		SV	OV	
1	Stem extract in *Anisomeles malabarica*	128µg	*E. coli*	22	14	2.909	22	12	4.545
2			*Klebsiella sp.,*	22	15	2.227	22	15	2.227
3			*Staphylococcus aureus*	22	21	0.045	22	20	0.181
4			*Bacillus sp.,*	22	14	2.909	22	18	0.727
5			*Proteus sp.,*	22	15	2.227	22	19	0.049

Table value $X^{2(0.05)=3.84}$, Chi - square value significance at 5% level

SV-Standard value and OV-Observed value

Table 4 - Antibacterial activity of Non-polar extracts of *Anisomeles malabarica* stem powder (zone of inhibition in mm)

S.no	Sample	µg/ml	Bacterial strains	Petroleum ether		X2=[(O-E)2]/E
				SV	OV	
1	Stem extract of *Anisomeles malabarica*	128 µg	*E. coli*	22	13	3.681
2			Klebsiella sp	22	14	2.909
3			Staphylococcus sp	22	14	2.909
4			Bacillus sp	22	13	3.681
5			Proteus sp	22	16	1.636

Table value $X^{2(0.05)=3.84}$, Chi - square value significance at 5% level

SV-Standard value and OV-Observed value

Table 5- INFRA RED SPECTRUM ANALYSIS BY *ANISOMELES MALABARICA* FLOWER POWDER

S.no	Peak value	Stretching	Interpretation
1	422.41	C-I str	Halogen
2	576.72	C-Br str	Halogen
3	669.30	C-Cl str	Halogen
4	1116.78	C-C str	Alkanes
5	1323.17	C-F str	Halogen
6	1382.96	C-F str	Halogen
7	1402.25	C-H def	Alkanes
8	1442.75	C-H def	Alkanes
9	1525.69	N-H def	Alkanes
10	1564.27	N=O str	Nitro compounds
11	1633.71	C=O str	Ketones
12	1654.92	C=O str	Amides
13	1683.86	C=O str	Ketones
14	1743.65	C=O str	Esters
15	1805.37	C=O str	Acid anhydrides
16	1853.59	C=O str	Acid anhydrides
17	1878.67	C=O str	Ketones
18	2270.22	N-H str	Amino acids
19	2339.65	N-H str	Amino acids
20	2374.37	N-H str	Amino acids
21	2854.65	C-H str	Alkanes
22	2924.09	C-H str	Alkanes
23	3421.72	O-H str	Alcohols
24	3759.26	O-H str	Alcohols

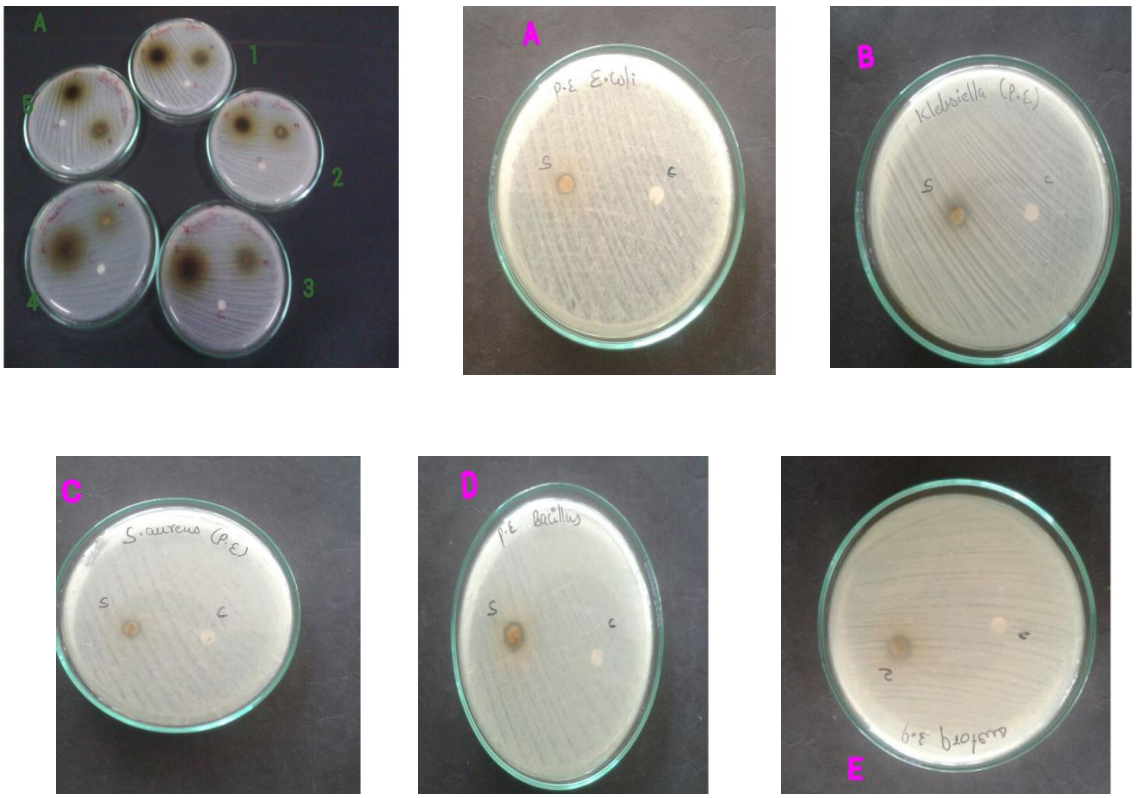
Table-6 INFRA RED SPECTRUM ANALYSIS BY *ANISOMELES MALABARICA* STEM POWDER

S.NO	PEAK VALUE	STRETCHING	INTERPRETATION
1	530.42	C-Br str	Halogens
2	617.22	C-Cl str	Halogens
3	667.37	C-Cl str	Halogens
4	777.31	C-Cl str	Halogens
5	1029.99	C-C str	Alkanes
6	1109.07	C-C str	Alkanes
7	1155.36	C-F str	Halogens
8	1246.02	C-F str	Halogens
9	1325.10	C-O str	Alcohols
10	1377.17	C-H def	Alkanes
11	1402.25	C-H def	Alkanes
12	1440.83	C-H def	Alkanes
13	1521.84	N=O str	Nitro compounds
14	1631.78	C=C str	Alkanes
15	1656.85	C=O str	Esters

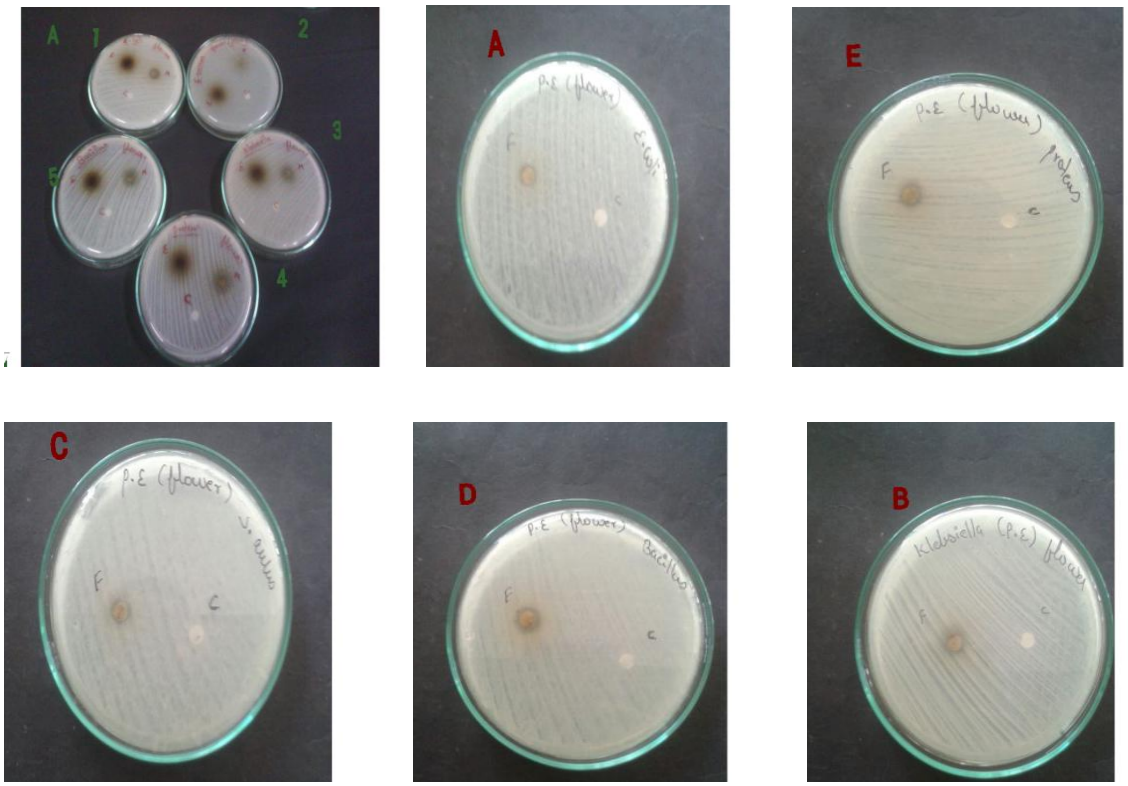
16	1739.79	C=O str	Aldehydes
17	1803.44	C=O str	Acid anhydrides
18	2374.37	N-H str	Amino acids
19	2852.72	C-H str	Alkanes
20	2922.16	C-H str	Alkanes
21	3423.65	N-H str	Amines
22	3759.26	N-H str	Amides

Figure1- Zone of inhibition formed by polar and Non-polar extract of *Anisomeles Malabarica* of Stem and Flower

(i) Stem



A-1. *Proteus sp, 2- E.coli, 3- Klebsiella sp, 4- Staphylococcus sp, 5- Bacillus sp* (stem in ethanol and methanol). A- *E.coli, B- Klebsiella sp, C- Staphylococcus sp, D- Bacillus sp, E- Proteus sp (stem in petroleum ether solvent).*



(ii) Flower :A-1. ***Proteus sp, 2- E.coli, 3- Klebsiella sp, 4- Staphylococcus sp, 5- Bacillus sp*** **Flower in ethanol and methanol). A-** ***E.coli, B- Klebsiella sp, C- Staphylococcus sp, D-Bacillus sp, E- Proteus sp (Flower in petroleum ether solvent)***

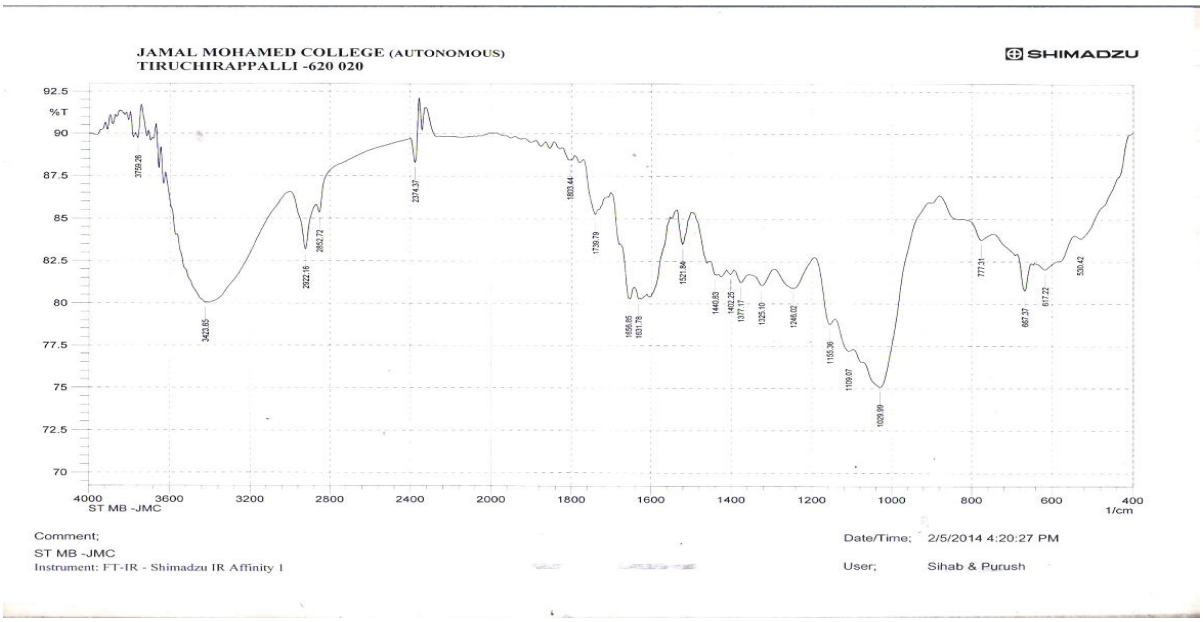


Fig.2 Infrared spectrum analysis of *Anisomeles malabarica* of Stem

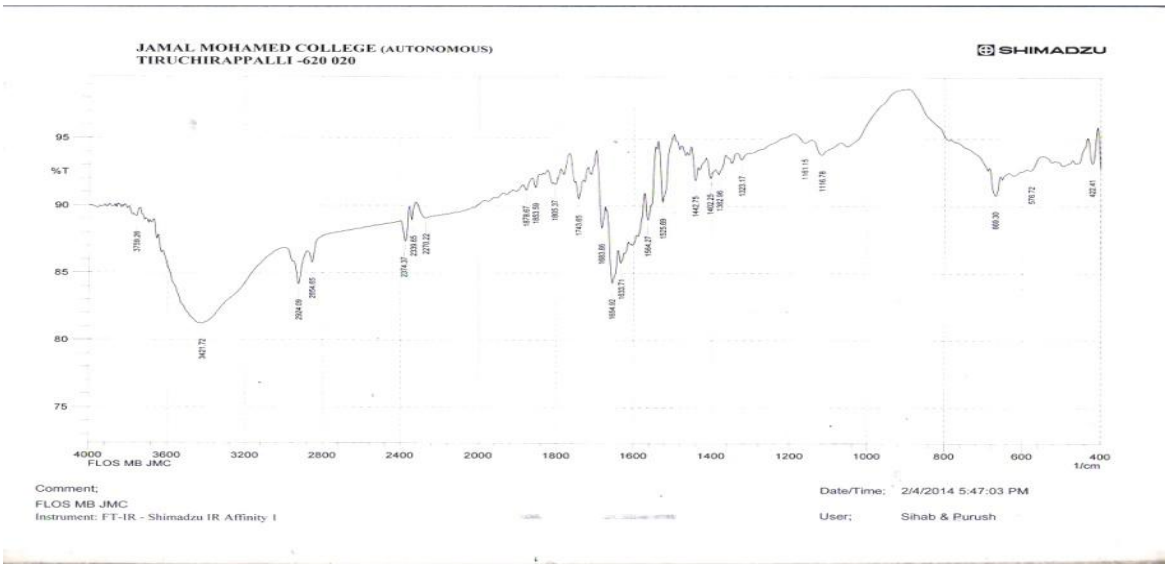


Fig 3. Infrared Spectrum analysis of *Anisomeles malabarica Flower.*

DISCUSSION

In earlier studies[4] reported that *Anisomeles malabarica* useful in halitosis, epilepsy, hysteria, amentia, anoxeria, dyspepsia, colic, flatulence, intestinal worms, fever, etc. A substances that stops the formation of intestinal gas and helps expel gas that has already formed.

Followed by [5], reported the antibacterial activity of aqueous extract of leaves of *Anisomeles malabarica (L.)* Sims were tested against six soil borne bacteria. *Bacillus subtilis and Staphylococcus aureus* showed a maximum inhibition of 30 mm at 50 µl concentration followed by *P.fluorescens* which was recorded 28 mm inhibition, *E.tracheiphila* recorded 22 mm inhibition at 50 µl concentration. Moderate activity was observed in 20 and 30 µl concentration in all test bacterial species. Compared to synthetic antibiotics Gentamicin and tetracycline, highly significant activity was observed in *B.subtilis, S.aureus and P.fluorescens.*

In previous research [6] investigated the preliminary bioactive phytochemicals present in the leaves extracts obtained by analytical standard hexane and ethanol solvents. The phytochemical were analysed such as alkaloids, saponins, tannins and terpenoids from both the leave extracts. The study evaluated the antimicrobial activity of *Anisomeles malabarica* leaves extracts. The In-vitro antimicrobial activity was performed by agar well discussion method against the clinically important multi drug resistant bacterial strains *Staphylococcus*

aureus (NCIM 2492), Bacillus subtilis (NCIM 2439) and *Klebsiella pneumoniae (NCIM 2719)* with the concentration extracts ranged from 25 to 75 µl. That the study shows the powerful antimicrobial activity against *Staphylococcus aureus* and *Klebsiella pneumoniae* bacterial strains with maximum inhibitory zone compared with standard antibiotic drug tetracycline.

Followed by[7], the leaves of *Anisomeles malabarica* consist of diterpenoids, ovatodiolide and its derivative are used as HIV inhibitors.

In the present study analyse the solvent extracts using both polar and non-polar extracts.The stem gave the maximum zone of inhibition in the polar and non-polar extracts(ethanol ,methanol and petroleum ether). The ethanol stem extract gave maximum zone of inhibition against *Staphylococcus aureus* (21 mm). The methanol extract of stem gave maximum zone against *Staphylococcus aureus* (20 mm) and the petroleum ether stem extract of gave maximum zone of inhibition at *Proteus vulgaris* (16 mm). The significant changes observed in flower and stem of *Anisomeles malabarica* offer the scope of research in bio prospection of this plant. The present work identify the functional groups. These groups do the wonderful role against pathogens.

CONCLUSION

The present study showed the antibacterial activity of flower and stem extracts from various solvents of *Anisomeles malabarica* against pathogenic organisms. FTIR spectroscopy technique showed that the presence of functional groups which can be isolated and further screened for different kind of biological activities depending their therapeutic uses. Hence this plant can be used to cure the infection caused by the treated strains. Further research will be needed to find out the structural analysis of compound by use of different analytical method such as NMR and Mass spectrophotometer.

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