Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(9):486-492

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

In-vitro antibacterial activity, phytochemical investigation and characterization of *Anisomeles malabarica* (Linn) leaves

V. Kavimani[1], A. Ramadevi[1], K. Kannan[2], S. Gnanavel[2] and G. Sivaperumal[2]

[1]Department of Chemistry, ACCET, Karaikudi, Tamil Nadu
[2]Department of Chemistry, Government College of Engineering, Salem, Tamil Nadu

---

ABSTRACT

The present study deals with the investigation of the In vitro antibacterial activity of the plant extract of Anisomeles Malabarica linn leaves. The preliminary phytochemical analysis of the solvents extracts in water, petroleum ether, ethyl acetate and methanol revealed the presence of alkaloids, flavonoids, tannins, terpenoids, carbohydrates, phytosterol and coumarin. The TLC technique was used to identify and separation of possible compounds present in the methanol extract. The FT-IR spectral data shows the possible functional groups of chemical compounds present in the methanol extract of A.Malabarica. The methanol extract only used for the biological activity. The crude methanol extract of the leaves was used to evaluate the antibacterial activity by Disc Diffusion Method. The present study was carried out to evaluate In vitro antibacterial activity of the leaves methanol extract of A.Malabarica against Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Pseudomonas Auregenosa, and Klebsiella pneumonia. It was found that the methanol extract exhibited a maximum antibacterial activity at 100 µl/ml and produced 21mm zone of inhibition against both E.coli and P. Auregenosa. The results provide justification for the use of A. Malabarica to treat various infectious diseases.

Keywords: *Anisomeles Malabarica* leaves, Phytochemical analysis, TLC, FT-IR and antibacterial activity.

INTRODUCTION

Nature is an extraordinary treasure-house of various valuable resources. There is no illness, for which nature does not offer a universal remedy. Humans have been dependent on natural resources, mostly plants for nutrition and medicinal needs, since time immemorial. The emergence of plant-based drugs in the modern era stands testimony to the fact that they are safe without side effects. The importance of botanical, chemical and pharmacological evaluation of plant-derived agents used in the treatment of human ailments has been increasingly recognized in the last decades. The general belief among the people is that the herbal based products and medicines will not have harmful side-effects, unlike synthetic chemical drugs. Medicinal plants have been used in traditional treatments for numerous human diseases for thousands of years, and they continue to be an important therapeutic aid for alleviating the ailments of humankind [1]. Secondary metabolites of plants play an important role in human health and also nutritionally important [2]. The phenolic compounds are one of the largest and most everywhere groups of plant secondary metabolites. Phytochemical screening of various plants revealed the presence of different secondary metabolites like alkaloids, flavonoids, steroids, phenols, glycosides, saponins, etc. [3, 4]. Medicinal plants are very good sources of antioxidants since they synthesize secondary metabolites like alkaloids, flavonoids, etc. [5]. A wide range of medicinal plant parts is used as raw drugs with varied medicinal properties. The different parts used include root, stem, flower, fruit, twigs exudates and modified plant organs. While some of these raw drugs are collected in smaller quantities by the local communities and folk healers for local use, many other raw drugs are collected in larger quantities and traded in the market as the raw material for many herbal industries [6]. Although hundreds of plant species have been tested for antimicrobial properties, the vast majority has not been adequately evaluated [7]. *Anisomeles Malabaric* (L.) (Malabar catmint) Cogn.Syn. *Nepeta Malabarica L.*, (Family: Lamiaceae) is a medicinal

plant used as a folkloric medicine to treat amentia, anorexia, fevers, swellings, rheumatism [8]**.** It is distributed throughout India. The herb is reported to possess anticancer, allergenic, anthelmintic, anti allergic, anti-anaphylactic, antibacterial, anticarcinogenic, anti ecdemic, antihistaminic, anti-inflammatory, antileukemic, antinociceptive, antiplasmodial, antiseptic and antibiotic properties [9-12]. Not much work has not been done for antimicrobial activity of this plant. Hence, the aim of this study was to evaluate the antimicrobial activity of *Anisomeles Malabarica* leaves.

EXPERIMENTAL SECTION

Collection of plant materials

Fresh leaves of *Anisomeles Malabarica* [L] free from disease was collected from near Government College of Engineering, Salem District, Tamil Nadu in the month of December 2014 as shown in Fig.1.

Fig.1. *Anisomeles Malabarica plant*

Preparation of plant extract

The leaves of *Anisomeles Malabarica* was shade-dried and then powdered with the help of a mechanical blender. About 40 gram of shade-dried powder filled and extracted in Soxhlet apparatus with water, petroleum ether, ethyl acetate and methanol as shown in Fig-2

.

Fig.2: Soxhlet apparatus for the A.Malabarica leaves Extraction

The solvent from the extract was recovered under reduced pressure using a rotary evaporator as shown in Fig. 3 and subjected to freeze drying and stored in an airtight bottle until further use.

Fig.3: Rotary Evaporator for Concentration

Preliminary Phytochemical screening
The extracts were subjected to preliminary Phytochemical testing to detect the presence of different chemical groups of compounds. The plant extracts was carried out qualitatively for the presence of Alkaloids, carbohydrates, fixed oils, fats, tannins, gum mucilage, flavonoids, saponins, terpenoids, lignin and sterols by using the standard method given by (Harborne, 1998) [20].

TLC studies
The aluminium plates precoated with 0.20 mm layers of silica gel $60F_{254}$ (E.Merck, # 1.05570). Additionally, 20x20 cm aluminium plates precoated with 0.20 mm layers of silica gel. The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters.

FT-IR Studies
FT-IR spectra were recorded on KBr medium on a Perkin Elmer Rx, spectrophotometer in wave number region 400-4000 cm^{-1}.

Microorganisms Used
The five microorganisms (Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Pseudomonas Auregenosa, and Klebsiella pneumonia,) were used in the entire investigation. The broth cultures of each test organism were prepared by inoculating a loop full of culture in a 5 ml of nutrient broth and incubated at 37°C for 14 to 16 hours.

Antibacterial assay
Agar well diffusion method was employed as per the modified method of Natarajan et al.[21] Disc diffusion method. A suspension of test organisms (0.1ml) was swabbed on the Muller Hinton Agar (MHA) by using sterile cotton swab. After that, a sterile cork borer (5 mm diameter) was used to made wells in the seeded Müller-Hinton agar. Then, 50µl of each extract was separately delivered into wells and allowed to diffuse at room temperature. Equal volumes of DMSO and 25µl of ciprofloxacin (0.1µg/µl) were served as negative and positive control. The plates were incubated at 37°C for 24 hours, and the zone of growth inhibition was measured in mm.

RESULTS AND DISCUSSION

The present investigation has been carried out to evaluate the antibacterial activities of the plant of A. Malabarica leaves for four different solvents extraction. Phytochemical analyses of the plant extract were carried out.

PHYTOCHEMICAL SCREENING
The phytochemical analysis revealed that the plants contain bioactive substances which are connected with the antibacterial properties of plants. The presence of alkaloids, tannins, flavonoids, phytosterol and coumarin were determined and indicated in Table 1.

Table 1 Preliminary phytochemical screening of *Anisomeles Malabarica* (L)

S.No	Test	Water	Petroleum ether	Ethyl acetate	Methanol
1.	Test for Alkaloids				
	a)Dragendraffs test	+	+	+	+
	b) Wagner test	+	+	+	+
	c)Hagers test	+	+	+	+
2.	Test for Flavonoids				
	a)Lead acetate test	+	+	-	-
	b) NaOH	+	+	-	-
3	Test for Phenols				
	a) $Fecl_3$	-	-	-	-
4.	Test for Tannins				
	a)$FeCl_3$	+	-	+	+
	b) $K_2Cr_2O_7$	+	-	+	+
	c)Lead acetate	+	-	+	+
5.	Test for Saponin				
	a)Foam test	-	-	-	-
6.	Test for Amino Acid				
	a)Xantho proteic test	+	-	-	-
	b) Biuret Test	+	-	-	-
7.	Test for Coumarin	+	+	-	-
8.	Test for Starch (Iodine test)	-	-	-	-
9.	Test for Quinone	-	-	-	-
10.	Test for Carbohydrates				
	a)Fehling test	+	-	-	-
	b) Benedict test	+	-	-	-
	c)Molishs test	+	-	-	-
11.	Test for Glycosides				
	a) Killer –Killani test	-	-	-	-
12.	Test for Terpenoids				
	a)Salkovaski test	-	-	-	+
	b) Lieberman's test	-	-	-	+
13.	Test for Phytosterol				
	a)Salkovaski test	-	+	-	+
	b) Lieberman's test	-	+	-	+
14.	Test for Anthraquinone				
	a)Extraction +NH_4OH	-	-	-	-
	b) Benzene Test	-	-	-	-

+ Present, - Absent

The test data available in Table .1 indicates that the water extract of A.Malabarica leaves contains alkaloids, flavonoids, tannins, carbohydrates and coumarin. The ethyl acetate extract indicates the presence of alkaloids and tannins only. The methanol extract indicates the presence of alkaloids, tannins, terpenoids and phytosterol. The petroleum ether extract indicates the presence of alkaloids, flavonoids amino acids and phytosterol. It is clearly evident from the table that the other phyto-constituents like phenols, saponins, carbohydrates, anthraquinone and glycosides were absent in all the four solvent extracts. These results suggest the presence of primary bioactive metabolite which acts as the precursors for the synthesis of secondary metabolites. These turns help in the development of new bioproducts for future.

TLC analysis

In TLC analysis of A.Malabarica leaves of methanol extracts was studied by thin layer chromatography (TLC) on analytical plates over silica gel of 0.2 mm thickness. The TLC was performed to find out the number of constituents in the respective extract. TLC is the method mainly uses to investigate the presence of chemical constituent qualitatively and quantitative in the plant extract. According to combination, it was found that TLC analysis of petroleum ether: ethyl acetate (7:3) and (9:1) system may be the best solvent system. The R_f values were calculated and results were 0.57, 0.64 and 0.78 and 0.14, 0.42 and 0.71 respectively. Three different separations of compounds have been identified in PE:EA (7:3 and 9:1) systam was preferable method for the isolation of the compounds and also all the three compunds are UV active in the methanol extract as shown in fig.4.

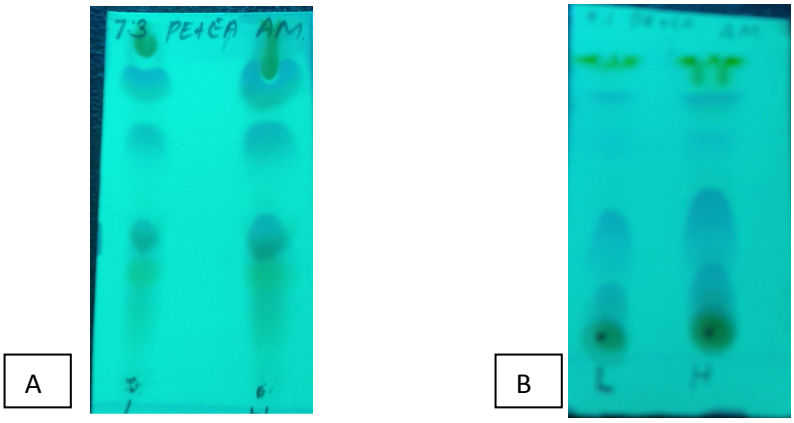

Fig.4. TLC analysis of A.Malabarica leaves methanol extract
Petroleum Ether: Ethyl Acetate A) 7:3 system and B) 9:1 system

FT-IR – analysis

The spectrum was recorded in the wavelength region between 400 cm^{-1} to 4000 cm^{-1} as shown in fig.5 The spectrum shows peaks at 3774 $cm^{-1}$and 3448 cm^{-1} for alcohol or phenolic compounds (strong O-H bonding) which indicate the presence of –O-H stretching of the carboxyl group. These peaks indicate the presence of bonded hydroxyl groups. Further, the peaks observed at 2924 cm^{-1} and 2855 cm^{-1} confirm and C-H stretching (alkanes) 2078 cm^{-1} represents the stretching bonds of alkynes. The peak observed at 1636 cm^{-1} represent the C=O stretching carbonyl compounds. The sharp peak at 1455 cm^{-1}.1384 cm^{-1} and 1251cm^{-1} are assigned to C-O starching (primary alcohol and ester).The peak observed at 602 cm^{-1} represents the presence of different functional groups of halogen derivatives.

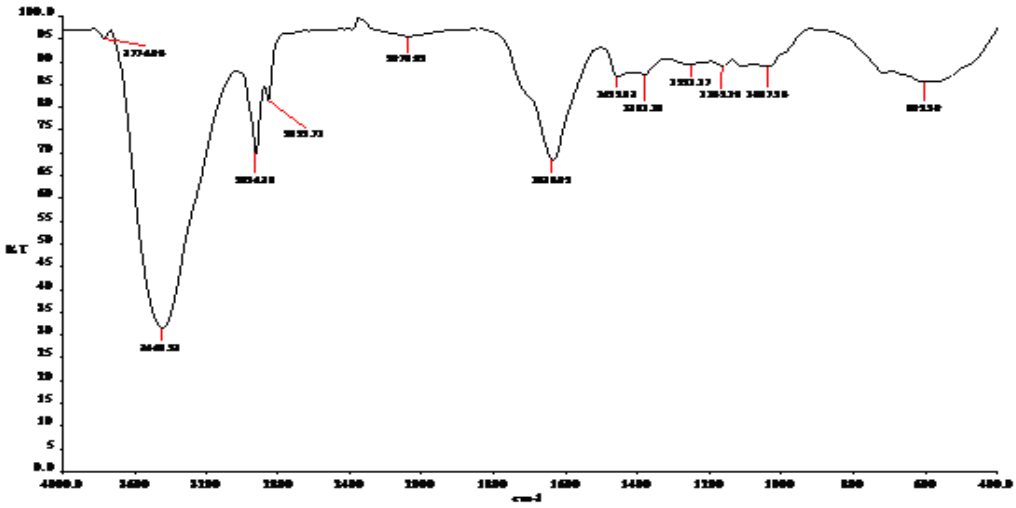

Fig. 5. FT-IR Spectrum of methanol extract of A.Malabarica leaves

Antibacterial Activity

The results of antibacterial activity of leaves extract of A.Malabarica were done by agar well diffusion and disc diffusion methods as shown in fig. 6. The results showed that agar well diffusion method is an ideal for assay of antibacterial tests than disc diffusion method. Anti-bacterial activity of A.Malabarica leaves methanol extract was carried out for five microorganisms such as E.Coli, Bacillus subtilis, Pseudomonas Auregenosa, Staphylococcus aureus and K. Pneumonia. The results for the antibacterial activity of A.Malabarica leaves extract showed a clear zone of inhibition as indicated in Table -2 against Staphylococcus aureus, Bacillus subtilis, Pseudomonas Auregenosa, Escherichia coli. The concentration of 50 µl/ml, 75 µl/ml and 100 µl/ml was used. The crude A.Malabarica leaves extract shows antibacterial activity against all the microorganisms for extensively good antibacterial activities.

Table- 2 Antibacterial activity of methanol extracts of A.Malabarica leaves

S.No	Microorganisms	Zone of Inhibition (mm) Concentrations (µl/ml)			Control* (µl/ml)
		50	75	100	
1.	*E.Coli*	7	13	21	22
2.	*Bacillus subtilis*	7	7	9	18
3.	*Pseudomonas Auregenosa*	17	19	21	20
4.	*Staphylococcus aureus*	17	18	20	20
5.	*K. Pneumonia*	7	10	12	20

**Antibiotics*

The A.Malabarica methanol extract was shown the maximum zone inhibition of 21mm for E.Coli and Pseudomonas Auregenosa. The result clearly shows that alkaloids, tannins and terpenoids which were abundantly found in methanol extract were found to be responsible for the antibacterial activity. The antibacterial activity of the extract might be attributed to the presence of the foresaid secondary metabolites in the extract.

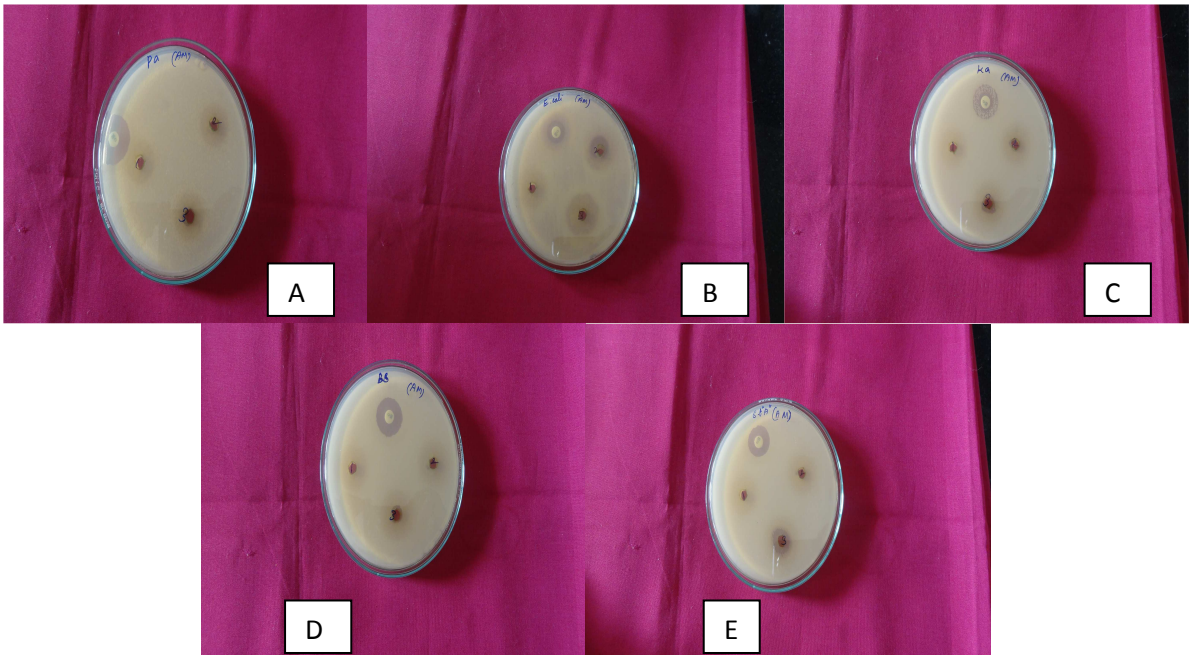

Fig.6. Zone of inhibition of A.Malabarica leaves using methanol extract against different microorganisms A) E.Coil, B) Bacillus subtilis C) Pseudomonas Auregenosa D) Staphylococcus aureus E) K. Pneumonia

CONCLUSION

It could be concluded that the method extract of A.Malabarica possesses significantly good anti-bacterial activity. It could be concluded that A.Malabarica leaves extract contain some pharmaceutically important phytochemical constituents like alkaloids, tannins. Phytosterols, terpenes, carbohydrates, flavonoids and amino acid. The TLC study of methanol extract indicates the presence of possible compounds. The results of FT-IR analysis from the methanol extract of A.Malabarica leaves shows that the peaks at 3774 $cm^{-1}$and 3448 cm^{-1} for alcohol or phenolic compounds (strong O-H bonding) which indicates the presence of –OH stretching of carboxyl group.The maximum zone of inhibition (21mm) was obtained against E.Coli and Pseudomonas Auregenosa at a concentration of 100 µl/ ml.

REFERENCES

[1] Momin RK and Kadam VB. *Journal of Phytology*,**2011**, 3:52–54.
[2] Jeeva S, Sheela JD, Shamila RMI, Lakshmi PJ, Brindha RJ; *Asian Journal of Plant Science Research* **2012**, 2: 41–44.
[3] Mojab F, Kamalinejad M, Ghaderi N, Vahidpour HR; *Iranian Journal of Pharmaceutical Research*,**2003**, 2: 77–82 .
[4] Parekh J, Chanda S, *Plant Archives*,**2008**, 8, 657–662.

[5] Hagerman AN, Rield KM, Jones GA, Sovik KN, Richard NI, Hartzfeld PW, Riechel TL; *Journal of Agricultural and Food Chemistry*,1998, 46, **1998**.
[6] Parekh J, Chanda S. *Afr J Biotechnol*,**2008**, 7(3), 4349-4353.
[7] Kaur R, Kaur H. *Arch Appl Sci Res*,**2010**, 2(1), 302-309.
[8] Uniyal SK, Singh KN, Jamwal P, Lal B. *Journal of Ethno biology Ethno medicine*,**2006**, 2:1-14.15.
[9] Balandrin MF, Klocke JA, Wurtele ES, Bollinger WH. *Sci*, **1985**,228:1154-1160.
[10] Chopra RN, Nayar SL, Chopra IC. "Glossary of Indian Medicinal Plants". Council of Scientific and Industrial Research, New Delhi, **1956**.
[11] Variers PS. "Indian Medicinal Plants, a Compendium of 500 species". Orient Longman Ltd., Madras, **1994**.
[12] Jayachandran R, Mahesh A. Cindrella L. *Int. J.Cancer Res*,**2007**, 3(4):174-179.
[13] Kavitha, Nelson, Thenmozhi, Priya Scholars Research Library *J. Microbiol. Biotech. Res*.,**2012**, 2 (1)-1-5.
[14] R Lavanya, S Uma Maheshwari, G Harish, J Bharath Raj, S Kamali, D Hema Malini, J Bharath Varma, C Umamaheswara Reddy, *RJPBCS* ,**2010**, 1(4) , 737.
[15] Vijayalakshmi R and Ranganathan, *RJPBCS*,**2012**, Volume 3 (1),43.
[16] Remya Mohanraj, Someshwar Nath, Pankaj Jha, *Journal of Biotechnology and Biotherapeutics* R.Br,**2012**,Vol 2: 9.
[17] Devi Kanyakumari, p. Selvakumar and v. loganathan In vitro antibacterial and antifungal activities of Morinda the tinctoria leaf in different solvents,**2013**, vol. -2 .
[18] Packialakshmi N and Nilofer Nisha HM, *The Pharma Innovation Journal* ,**2014**, 3(6), 77-80.
[19] Ajudhia N. Kalia, Ishpinder Singh, *International Journal of Green Pharmacy*, July-September **2010**.
[20] Harborne J B. Phytochemical Methods, Chapman & Hall, London;**1998**, 1-271,
[21] Natarajan D, John Britto S, Srinivasan K, Perumal G. *J Ethnopharmacol*,**2005**, 102(1): 123-126.