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Bioautography screening of *Anisomeles malabarica* leaves and boiled leaves

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ABSTRACT
The present study deals with the antibacterial activity of *Anisomeles malabarica.* Sequential extraction was carried out by using solvent such as ethanol, methanol, petroleum ether and aqueous extract from the leaf and boiled leaf of plant were investigated for preliminary antibacterial property against some pathogenic bacteria. In polar studies the maximum zone of inhibition were found in *Staphylococcus aureus.* In non-polar studies the maximum zone of inhibition were found in *Pseudomonas aeruginosa.* All these findings have confirmed the uses of this plant in a broad spectrum to treat several bacterial infections. The results showed good antibacterial activity and it could play an important role in herbal formulations for the treatment of infectious diseases.

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

1. Introduction
The plant is known as medicine because they contain active substances that cause certain reactions from relenting to the cure of diseases on the human organisms. Plants are the main sources of food. They are rich in nutrients. They are also rich in compounds which have pain relieving and healing abilities. In ancient times the plants were used for the treatment of disease without knowledge about the compounds present and their mode of actions. *Anisomeles malabarica* is a medicinal plant that has been used as a folkloric medicine to treat amentia, anorexia, fevers, swelling and rheumatism [1]. The herb is reported possess anticancer, allergenic, antihelminthic, antibacterial, antiplasmodial and antiperotic properties (2). *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.

2. Materials and Methods
2.1 Sample Collection
The plant sample were collected from the Theekalamalai near Vaiyampatti, dry rocky region of Trichy district, Tamil Nadu, India. The plant 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.

2.2 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 leaf and boiled leaf were extracted with solvents. Ethanol, methanol, aqueous and petroleum ether. The extracts are separately prepared and incubated at room temperature for 48 hours with stirring at regular interval. The extracts were filtered with the whatman filter paper and then dried by using rotary evaporator. The filtrate was stored in screw cap bottle at -20 ^{0}C for further use.

2.3 Antimicrobial activity
Detection of antimicrobial activity of *Anisomeles malabarica* by bioautography. Test microorganisms selected for antimicrobial activity are *Staphylococcus aureus, Streptococcus epidermis, Proteus vulgaris, Klebsiella pneumoniae* and *Escherichia coli.* The strains were obtained from MTCC, Chandigarh in India and maintained on agar slants.

2.4 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 so as to make lawn. The discs which had been impregnated with different extracts of leaf and boiled leaf 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.

2.5 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 susceptibility test.

3. Results

The result of the antimicrobial activity of the fresh leaf and boiled leaf of *Anisomeles malabarica* extracts is given in Table 1, 2, 3 and 4. The solvents were prepared as different concentrations compared with all the other concentrations µg/ml. Concentration gave the maximum zone of inhibition for all the extracts. Among the fresh leaf and boiled leaf of *Anisomeles malabarica* shows best antibacterial activity. Polar extracts like aqueous, ethanol and methanol to be the best solvent for extraction of antibacterial compounds from the *Anisomeles malabarica.*

Best zone of inhibition was produced by ethanol leaf extract *Staphylococcus aureus* (18 mm) and least was produced against *S. epidermis* (13 mm). The aqueous extract revealed the maximum zone of inhibition was produced *Pseudomonas aeruginosa* (17 mm) and least was produced against *S. epidermis* (13 mm). The maximum zone of inhibition was found in methanol leaf extract when compared with aqueous and ethanol leaf extracts. Best zone of inhibition was by methanol leaf *Staphylococcus aureus* (20 mm) and least was produced against *Pseudomonas aeruginosa* (13 mm). The petroleum ether shows maximum zone of inhibition was produced against *S. aureus, Bacillus sp* and *S. epidermis* (12 mm) and absences of zone in *Klebsiella sp.*

Best zone of inhibition was produced by ethanol boiled leaf extract *Pseudomonas aeruginosa* (23 mm), least was produced against *S. epidermis.* (10 mm). The aqueous extract revealed that maximum zone of inhibition was produced by *Pseudomonas aeruginosa* (16 mm) and least was produced against all other organisms, (15 mm). The methanol extract revealed that maximum zone of inhibition was produced against *Pseudomonas aeruginosa* (23 mm) and least zone was observed against *S. epidermis. (*8 mm).

The petroleum ether extract shows maximum zone of inhibition was produced at *Pseudomonas aeruginosa* (15 mm) and least was produced against *E. coli* and *Klebsiella sp*., (11 mm).

Overall, the antibacterial activity of the *Anisomeles malabarica* revealed that the best antibacterial activity was produced by leaf extract followed by boiled leaf extract. All the extract produce better zone of inhibition against *Pseudomonas* (23 mm) and *Staphylococcus aureus* (20 mm). In the present study to analyses the solvent extracts using both polar and non-polar extracts. The boiled leaf gave a maximum zone of inhibition in polar extracts methanol and the non-polar extracts (petroleum ether).

Table 1: Antibacterial activity of polar extract of *Anisomeles malabarica* leaf powder (zone of inhibition in mm)

S. no	Sample	µg/ml	SV	Bacterial strains	Aqueous (OV)	X2= [(O-E)2] /E	Ethanol	X2= [(O-E)2]	Methanol	X2= [(O-E)2]/E
1.	Leaf extract of Anisomeles malabarica	128µg	22	*E. coli*	14	2.909	17	1.136	18	0.727
2.			22	*Klebsiella sp*	14	2.909	16	1.636	16	1.636
3.			22	*P. vulgaris*	16	1.636	17	1.136	16	1.636
4.			22	*P. aeruginosa*	17	1.136	15	2.227	13	3.681
5.			22	*S. aureus*	16	1.636	18	0.727	20	0.181
6.			22	*S. epidermis*	13	3.681	13	3.681	16	1.636
7.			22	*Bacillus sp*	15	2.227	14	2.909	19	0.727

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

Table 2: Antibacterial activity of Non-polar extract of Anisomeles malabarica leaf powder (zone of inhibition in mm)

S.no	Sample	µg/ml	Standard value	Bacterial strains	Petroleum ether(OV)	X2= [(O-E)2] /E
1.	Leaf extract of Anisomeles malabarica	128 µg	22	*E.coli*	10	6.546
2.			22	*Klebsiella sp*	-	-
3.			22	*P. vulgaris*	11	5.5
4.			22	*P. aeruginosa*	12	4.545
5.			22	*S. aureus*	12	4.545
6.			22	*S. epidermis*	12	4.545
7.			22	*Bacillus sp*	12	4.545

Table value **$X^{2(0.05)=3.84}$, Chi - square value is not significance at 5% level**
OV- Observed value

Table 3: Antibactial activity of polar extract of Anisomeles malabarica boiled leaf powder (zone of inhibition in mm)

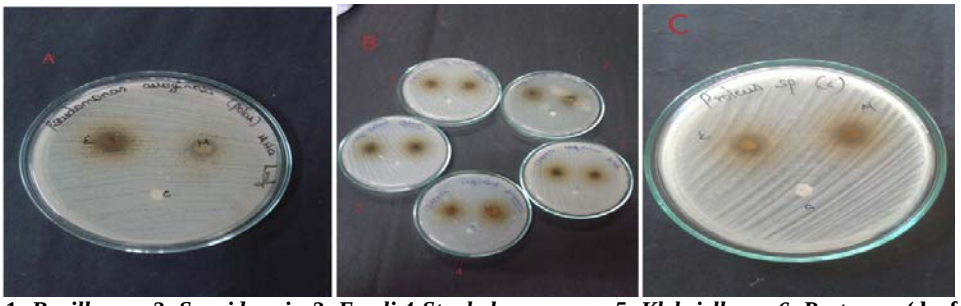
S.no	Sample	µg/ml	SV	Bacterial strains	Aqueous (OV)	X2=[(O-E)2]/E	Ethanol (OV)	X2=[(O-E)2]/E	Methanol (OV)	X2=[(O-E)2]/E
1.	Boiled leaf Extracts of Anisomeles malabarica	128µg	22	*E.coli*	15	2.227	14	2.909	13	3.681
2.			22	*Klebsiella sp*	15	2.227	14	2.909	12	4.545
3.			22	*P. vulgaris*	15	2.227	15	2.227	11	2.227
4.			22	*P. aeruginosa*	16	1.636	23	0.045	23	0.045
5.			22	*S. aureus*	15	2.227	20	0.181	20	0.181
6.			22	*S. epidermis*	15	2.227	10	6.546	8	8.909
7.			22	*Bacillus sp*	15	2.227	18	0.727	12	4.545

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

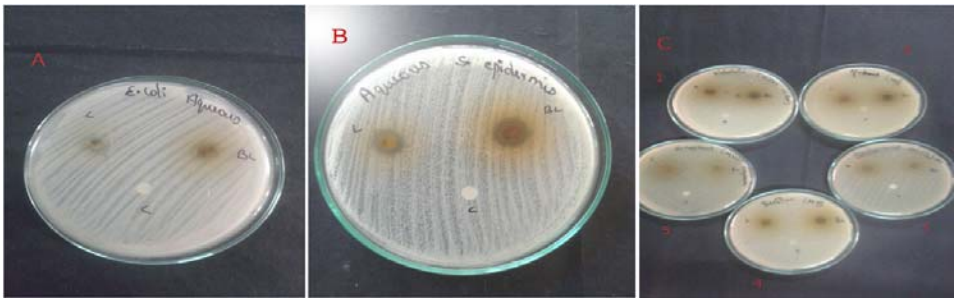
Table 4: Antibacterial activity of Non-polar extract of *Anisomeles malabarica* boiled leaf powder (zone of inhibition in mm)

S.no	Sample	µg/ml	Standard Value	Bacterial strains	Petroleum Ether Observed value	X2=[(O-E)2] /E
1.	Boiled leaf extract of Anisomeles malabarica	128 µg	22	*E.coli*	11	5.5
2.			22	*Klebsiella sp*	11	5.5
3.			22	*P.vulgaris*	13	3.681
4.			22	*P.aeruginosa*	15	2.227
5.			22	*S.aureus*	11	5.5
6.			22	*S.epidermis*	14	2.909
7.			22	*Bacillus sp*	11	5.5

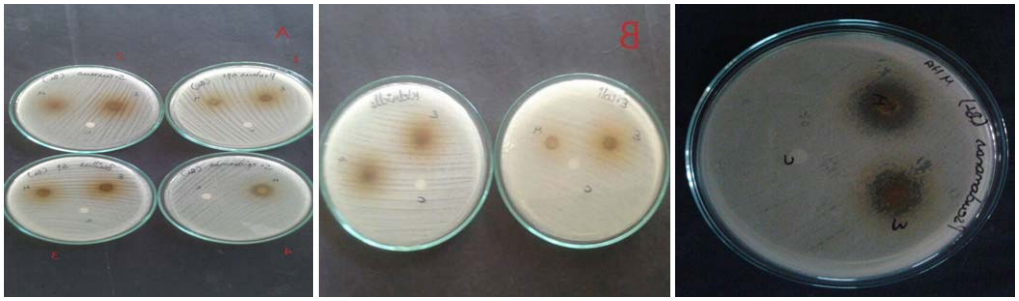
Table value $\mathbf{X^{2(0.05)=3.84}}$**, Chi - square value is not significance at 5% level**



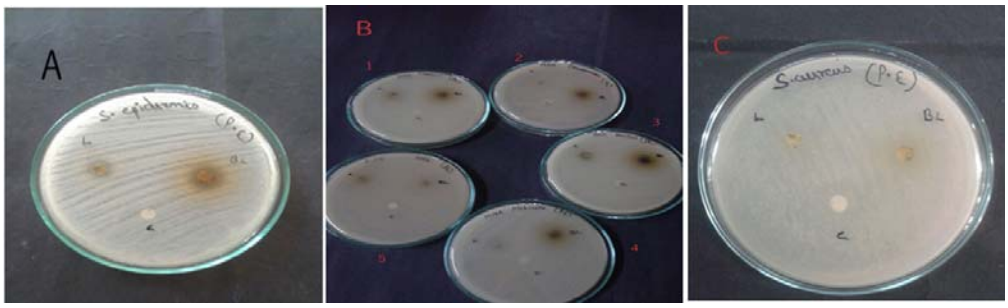
A- *Pseudomonas sp* ,B -1: *Bacillus sp, 2- S. epidermis, 3- E.coli,4-Staphylococcus sp, 5- Klebsiella sp, 6- Proteus sp*(leaf extract in ethaol and methanol solvent).



A- *E. coli,* B- *S. epidermis,* C- 1. *Klebsiella sp,* 2- *Proteus sp,* 3- *Pseudomonas sp,* 4- *Bacillus sp,* 5- *Staphylococcus sp (* leaf and boiled leaf extract in aqueous).



A- 1. *Proteus sp,* 2- *Staphylococcus sp,* 3- *Bacillus sp,* 4- *S. epidermis,* B- *E. coli, Klebsiella sp and* C- *Pseudomonas sp (*Boiled leaf extract in ethanol and methanol solvent).



A- *S. epidermis,* B-1. *Proteus sp,* 2- *Pseudomonas sp,* 3- *Bacillus sp,* 4- *Klebsiella sp,* 5- *E. coli* C - *S. aureus(* Leaf and boiled leaf extract in petroleum ether solvent).

Fig 1: Zone Inhibition formed by Polar and Non- polar extract of *Anisomeles malabarica* Leaf and Boiled leaf

4. Discussion

In earlier studies (3), reported that the whole plant of *Anisomeles malabarica* contains higher amount of flavonoids and phenolic compounds which correspond to greater antioxidant activity. In vitro assays indicate that this plant extracts is a better source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses.

In previous research (4), reported that the anti-proliferative and apoptosis-inducing properties of *Anisomelic* acid in both breast and cervical cancer cells. The *Anisomelic* acid offers potential for application in breast and cervical cancer therapy.

Followed by the researchers reported that the effects of *Anisomeles malabarica* methanolic extract on DMBA-induced HBP carcinogenesis. The medium and higher dose of AMME (250 mg/kg bw and 500 mg/kg bw) were found to be more effective in inhibiting HBP carcinogenesis compared to low dose (5).

In previous study reported that morphological and anatomical characteristic of the leaf along with the deterimnation of physio chemical constants, phytochemical screening and volatile oil content determination on the leaves of *Anisomeles malabarica (Lamiaceae)* to provide some pharmocognostical standards and serves a reference for the identification of *Anisomeles malabarica* (6). Followed by (7), the leaves of *Anisomeles malabarica* possess antioxidant properties and could serve as free radical inhibitor or scavenger.

In the present study to analyse the solvent extracts using both polar and non-polar extracts. The boiled leaf gave the maximum zone of inhibition in the non-polar extracts (petroleum ether) *Pseudomonas aeruginosa* (15 mm). When compared to leaf extracts *Bacillus subtilis* and *Pseudomonas aeruginosa* (12 mm).

In polar (methanol boiled leaf) extracts the maximum zone of inhibition was observed in *Pseudomonas aeruginosa* (23 mm). The ethanol extract maximum zone of inhibition was observed at boiled leaf extract against *Pseudomonas aeruginosa* (23 mm).The aqueous leaf extract gave the maximum zone of inhibition against *Pseudomonas* (17 mm).

5. Conclusion

The present study showed the antibacterial activity of the leaf and boiled leaf extracts from various solvents of *Anisomeles malabarica* against pathogenic organisms. Hence this plant can be used to cure the infection caused by the treated strains. Further studies are needed to isolate the pure compounds from this plant extract and to establish the mode of actions of the isolated compounds.

6. Acknowledgements

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7. References

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