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Antimicrobial activity and Phytochemical analysis of *Anisomeles malabarica* R. Br.

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Abstract

The present study was aimed to investigate antimicrobial activity and phytochemical analysis of *Anisomeles malabarica* R. Br., leaves extracted with alcohol and water. Regarding the antibacterial activity both aqueous and alcohol extracts exhibited better activity against gram positive bacteria such as *Bacillus subtilis*, *Bacillus megaterium*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus faecalis* than gram negative bacteria such as *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Vibrio cholera*. In fungal strains namely *Fusarium oxysporum*, *Aspergillus niger*, *Aspergillus flavus*, *Candida albicans* and *Trichoderma viride* the antifungal activity was found to be good in leaves extracted with aqueous than alcohol. Phytochemical analysis of leaf of both extracts of *A. malabarica* showed positive results for alkaloids, carbohydrates and tannins.

Key Words: *Anisomeles malabarica*, Gram positive and negative bacteria and phytochemical screening

Introduction

Anisomeles malabarica R. Br., (Lamiaceae) is distributed in major parts of India and especially in South India. It is a traditional medicinal plant commonly known as Peymarutti (Tamil). It is commonly found in Western Ghats from Maharashtra to Karnataka, Andhra Pradesh, Kerala and Tamil Nadu in India (Gupta and Tandon, 2004). It is used for the traditional treatment of snakebite as Antidote (Perumalsamy, 2008). From the earlier studies the alcohol and aqueous leaf extract of *A. malabrica* reported to have a significant spermicidal activity (Setty, 1976). The plants also have reported for the presence of antisomelic acid, ovatodiolide, anisomelyl acetate (diterpene) and betulinic acid triterpene (Devi, 1978).

Plant have been known to synthesize active secondary metabolites such as tannins, steroids, phenolic compounds etc, found in essential oils with established potent antimicrobial activities, which indeed has formed the basis for their applications in some pharmaceuticals, alternative medicines and natural therapies (Hammer and Carson, 1999). The antimicrobial compound from plant may inhibit bacterial growth by different mechanisms than those presently used (Eloff, 1988). Ethnobotanically, the leaves of *A. malabarica* are used against convulsions, in dyspepsia, intermittent fever, colic, boils, tetanus (Reddy, 1991). The herb is reported to possess antibacterial, antiallergic, anti-inflammatory, antiseptic, and antinociceptive properties (Jeyachandran *et al.*,2007). Analysis of anti-bacterial and phytochemical screening by using different *A. malabarica* samples was reported (Nisha Nilofer and Packialakshmi, 2014).

Multiple drug resistance has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases (Service, 1995), making it a global growing-problem. In recent years' multiple drug resistance in human pathogenic microorganisms has developed due to indiscriminative use of commercial antimicrobial drugs commonly used in the treatment of diseases. The present study was to investigate the antimicrobial activity and phytochemical screening of leaf extracts of *A. malabarica* against some selected bacterial and fungal pathogens.

Materials and Methods

Collection of plant materials

Fresh, young plants of *A. malabarica* R. Br., were collected from Alwarkurichi in Tirunelveli, District, Tamilnadu, India and authenticated in Institute of Herbal Botany, Plant Anatomy Research Centre, SPK, Alwarkurichi. The leaves were separated from stem and flower, cleaned in water and dried at room temperature. The dried plant leaves were milled to a fine powder, the wood was debarked and chipping was done with chipper. Both leaf powder and wood chip were stored in the dark at room temperature in closed containers until required.

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Preparation of extracts

The collected plants were washed with distilled water to remove adhering materials. Then it was dried at room temperature not exceeding 50ºC. The dried plant material sliced into small pieces and pulverized by mechanical grinders. The powdered materials extracted with aqueous and alcohol.

Phytochemical studies

Phytochemical analysis of aqueous, alcoholic extracts of *A. malabrica* was carried out by the standard methods provided for the presence and absence of metabolites such as alkaloids, glycosides, flavonoid, tannins, saponins. The preliminary phytochemical analysis was performed as per the method (Kokate, 1999).

Microbial strains used

Different microbial strains were used to evaluate the antimicrobial effect of *A. malabrica*. Five gram positive bacterial strains (i.e) *S. aureus, S. epidermis, B. substilis, S. epidermidis, S. faecalis,* and four gram negative bacterial strains (i.e) *K. pneumoniae, P. aeruginosa, S. typhi and V. cholerae* and five fungal strains (i.e) *F. oxysporum, A. niger, A. flavus, C. albicans* and *T. viride* were used to find the antimicrobial efficiency of A. *malabrica*. The strains were obtained from MTCC, Chandigarh in India and maintained on agar slants.

Antibacterial Agar Well Method

The extracts were screened for their antibacterial activity *in vitro* by agar well cut method using *B. subtilis, B. megaterium, S. aureus, S. epidermidis, S. faecalis, K. pneumoniae, P. aeruginosa, S. typhi* and *V. cholera*. Colonies were selected and transferred to 5 ml broth with a loop and the broth cultures were incubated at 37° C for 24. After the incubation was over the suspension was checked for 0.7 McFarland value and was used as the seeding culture. For screening the activity, Muller-Hinton agar was prepared and seeded with respective bacterial pathogens. Then the wells were made by using a sterile cork borer and was added with different volumes (25µl, 50µl and 75µl) of the crude extract of *A. malabarica* samples (1g/10ml Double distilled water) and kept for incubation at 37 °C for 24 hours. After incubation at 24h and 48h the results were recorded for the formation of zone of inhibition. Streptomycin (10µg/5ml) was used as bacterial standard.

Antifungal Agar Well Method

The extracts were screened for their antifungal activity in vitro by agar well cut method using *F. oxysporum, A. niger, A. flavus, C. albicans* and *T. viride.* For Screening the activity potato dextrose agar was prepared and seeded with respective fungal. Then the wells were made by using a sterile cork borer and was added with different volumes (25 µl 50µl ,75µl) of the crude extract of *A. malabarica* samples (1g/10ml D.W) and kept for incubation at 280 C for 24 hours. After incubation at 24h and 48h the results were recorded for the formation of zone of inhibition. Fluconazole (10µg/5ml) was used as fungal standard.

Results and Discussion

Aqueous and alcoholic extracts of *A. malabarica* R. Br., were subjected to suitable chemical tests to confirm the presence and absence of various phytoconstituents. Aqueous extracts of leaves of *A. malabarica* showed the presence of alkaloids, carbohydrate, tannins, protein, xantho protein and aromatic acid. Terpenoids, steroids, flavonoids, saponins, reducing sugar and phenolic compound were found to be absent in leaves extracted with aqueous. Alcoholic extracts of leaves of *A. malabarica* exhibited the presence of compounds such as alkaloids, carbohydrate, steroids, saponins, tannins, reducing sugar and absence of terpenoids, flavonoids, protein, xantho protein, aminoacids, phenolic compound and aromatic acid and were reported in Table-1.

Table -1: Preliminary photochemical screening of *Anisomeles malabarica* R. Br.

S.No	Phytochemicals	Aqueous Extract	Alcoholic extract
1	Alkaloids	+	+
2	Carbohydrate	+	+
3	Terpenoids	-	-
4	Steroids	-	+
5	Flavonoids	-	-
6	Saponins	-	+
7	Tannins	+	+
8	Protein	+	-
9	Xantho protein	+	-
10	Reducing sugar	-	+
11	Aminoacids	+	-
12	Phenolic compound	-	-
13	Aromatic acid	+	-

(+) Presence of chemicals ; (-) Absence of chemicals

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Table -2 Antibacterial activity of aqueous extract of *Anisomeles malabarica* R. Br., gram positive bacterial species

S.No	Test organisms	Zone of inhibition(mm) in different concentration				
		DMSO Control	25µg/ml	50 µg/ml	75 µg/ml	Standard Streptomycin (5mg/ml)
1	*Bacillus subtilis*	C	-	-	-	12±1.00
2	*Bacillus megaterium*	C	10±0.50^{a}	12±1.00^{a}	14±1.32^{a}	14±1.15^{ab}
3	*Staphylococcus aureus*	C	11±0.5^{a}	15±1.15^{b}	19±2.00^{c}	15±1.32^{b}
4	*Staphylococcus epidermidis*	C	10±0.5^{a}	10±0.5^{a}	11±0.62^{a}	14±1.15^{ab}
5	*Streptococcus faecalis*	C	12±0.76^{a}	13±1.00^{a}	13±1.00^{a}	16±1.60^{b}

Experiments were conducted three times. Values are mean ± standard deviation. Mean value superscript with same letter(s) along each column indicate no significant difference at P<0.05 as determined by DMRT.

Table -3: Antibacterial activity of alcoholic extract of *Anisomeles malabarica* R. Br., gram positive bacterial species

S.No	Test organisms	Zone of inhibition(mm) in different concentration				
		DMSO Control	25µg/ml	50 µg/ml	75 µg/ml	Standard Streptomycin (5mg/ml)
1	*Bacillus subtilis*	C	10±0.5^{a}	11±0.76^{ab}	11±0.72^{a}	12±1.04^{b}
2	*Bacillus megaterium*	C	13±1.5^{a}	15±1.25^{b}	18±1.73^{c}	14±1.00 ^{ab}
3	*Staphylococcus aureus*	C	11±0.86^{a}	13±1.00 ^{b}	15±1.15 ^{c}	13±1.00 ^{b}
4	*Staphylococcus epidermidis*	C	15±1.25^{ab}	17±1.60 ^{bc}	19±2.00^{c}	14±1.34 ^{a}
5	*Streptococcus faecalis*	C	13±0.97 ^{b}	14±1.32 ^{b}	15±1.41^{b}	10±0.50 ^{a}

Experiments were conducted three times. Values are mean ± standard deviation. Mean value superscript with same letter(s) along each column indicate no significant difference at P<0.05 as determined by DMRT.

Table -4: Antibacterial activity of aqueous extract of *Anisomeles malabarica* R. Br., gram negative bacterial species

S.No	Test organisms	Zone of inhibition(mm) in different concentration				
		DMSO Control	25µg/ml	50 µg/ml	75 µg/ml	Standard Streptomycin (5mg/ml)
1	*Klebsiella pneumoniae*	C	10±0.28 ^{a}	11±0.5 ^{ab}	12±1.00 ^{bc}	14±1.32 ^{c}
2	*Pseudomonas aeruginosa*	C	10±0.28 ^{a}	12±1.00^{ab}	13±1.04 ^{b}	14±1.15 ^{b}
3	*Salmonella typhi*	C	-	10±0.05 ^{a}	13±1.04 ^{b}	12±1.00 ^{ab}
4	*Vibrio cholera*	C	10±0.05 ^{a}	10±0.05 ^{a}	11±0.07 ^{a}	13±1.00 ^{b}

Experiments were conducted three times. Values are mean ± standard deviation. Mean value superscript with same letter(s) along each column indicate no significant difference at P<0.05 as determined by DMRT.

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Table – 5: Antibacterial activity of alcoholic extract of *Anisomeles malabarica* R. Br., gram negative bacterial species

S.No	Test organisms	Zone of inhibition(mm) in different concentration				
		DMSO Control	25µg/ml	50 µg/ml	75 µg/ml	Standard Streptomycin (5mg/ml)
1	*Klebsiella pneumoniae*	C	13±1.00 [a]	15±1.25[b]	15±1.27 [b]	16±1.60[b]
2	*Pseudomonas aeruginosa*	C	-	-	-	-
3	*Salmonella typhi*	C	-	15±1.25[a]	18±1.73 [c]	16±1.61[b]
4	*Vibrio cholera*	C	-	10±0.50[a]	12±0.86 [b]	13±1.00 [b]

Experiments were conducted three times. Values are mean ± standard deviation. Mean value superscript with same letter(s) along each column indicate no significant difference at P<0.05 as determined by DMRT.

Table – 6: Antifungal activity of aqueous extract of *Anisomeles malabarica* R. Br.

S.No	Test organisms	Zone of inhibition(mm) in different concentration				
		DMSO Control	25µg/ml	50 µg/ml	75 µg/ml	Standard Fluconazole (5mg/ml)
1	*Fusarium oxysporum*	C	16±1.60 [a]	17±1.72 [ab]	19±2.00 [bc]	20±2.25 [c]
2	*Aspergillus niger*	C	11±0.5 [a]	13±1.04 [ab]	14±1.15 [b]	17±1.72 [c]
3	*Aspergillus flavus*	C	10±0.28 [a]	12±1.00 [ab]	14±1.15 [bc]	16±1.44 [c]
4	*Candida albicans*	C	12±1.00 [a]	13±1.04 [ab]	16±1.60 [b]	18±1.73 [c]
5	*Trichoderma viride*	C	16±1.61 [b]	15±1.25 [ab]	14±1.15 [a]	15±1.25 [ab]

Experiments were conducted three times. Values are mean ± standard deviation. Mean value superscript with same letter(s) along each column indicate no significant difference at P<0.05 as determined by DMRT.

Table -7: Antifungal activity of alcoholic extract of *Anisomeles malabarica* R. Br.,

S.No	Test organisms	Zone of inhibition(mm) in different concentration				
		DMSO Control	25µg/ml	50 µg/ml	75 µg/ml	Standard Fluconazole (5mg/ml)
1	*Fusarium oxysporum*	C	8±0.05 [a]	10±0.28 [b]	12±1.00 [c]	15±1.25 [d]
2	*Aspergillus niger*	C	10±0.28 [a]	11±0.05 [ab]	12±1.00 [bc]	14±1.15 [c]
3	*Aspergillus flavus*	C	10±0.28 [a]	12±1.00 [ab]	14±1.15 [bc]	16±1.60 [c]
4	*Candida albicans*	C	-	-	-	15±1.25
5	*Trichoderma viride*	C	10±0.28 [a]	12±1.00 [ab]	14±1.15 [b]	17±1.72 [c]

Experiments were conducted three times. Values are mean ± standard deviation. Mean value superscript with same letter(s) along each column indicate no significant difference at P<0.05 as determined by DMRT.

Antibacterial activity was assayed in vitro by good diffusion method against nine bacterial strains. At the concentration of 75µg/ml of aqueous extract produced good antibacterial activity for both gram positive and gram negative bacteria. The antibacterial activity of the aqueous extract of leaf of *A. malabarica* was more effective against *S. aureus, B. subtilis, B. megaterium, S. epidermidis* and *S. faecalis* with varying inhibitory zones. The aqueous extracts showed maximum zone of inhibition against *S. aureus* (19±2.00 mm) and was14 ± 1.32 mm against *B. megaterium*. Zone of inhibition

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observed against *S. faecalis* and *S. epidermidis* was found to be 13 ± 1.00 mm and 11 ± 0.62 mm respectively. No bacterial activity was noticed against *B. subtilis* (Table-2).

The alcoholic extract showed 19 ± 2.00 mm zone of inhibition against *S. epidermidis* and 18± 1.73 mm zone of inhibition against *B. megaterium* and less effective with 10±0.05 mm zone of inhibition against *B. subtilis* (Table-3). The antibacterial activity of the aqueous leaf extracts of *A. malabarica* was not much effective against gram negative bacteria. The aqueous extracts showed a 13 ± 1.04 mm zone of inhibition against *P. aeruginosa* and 13±1.04 mm zone of inhibition against *S. typhi.* At the concentration of 25µg/ml no antibacterial activity was observed against *Salmonella typhi*. Zone of inhibition with 12± 1.00 mm and 11± 0.07mm against *K. pneumoniae* and *V. cholera* was observed respectively at 50 µg/ml of aqueous extract (Table-4). At 75µg/ml the alcoholic extracts showed 18±1.73 mm zone of inhibition against *S. typhi*, whereas no bacterial activity was observed against *P. aeruginosa* (Table-5).

The antifungal activity of the aqueous and alcoholic extracts of leaves of *A. malabarica* was more effective against *F. oxysporum*, *A. niger*, *A. flavus*, *C.albicans* and *T. viride* and showed varying inhibitory effects. The aqueous extract produced good antifungal activity at 75µg/ml. The aqueous extracts showed a 19 ± 2.00 mm zone of inhibition against *F. oxysporum* and 16± 1.60 mm zone of inhibition against *C. albicans*. The alcoholic extracts showed a similar inhibitory effect with a zone of 14 ± 1.15 mm against both the *A. flavus* and *T. viride*, whereas no antifungal activity was found against *C. albicans*. The result of antifungal activity of aqueous and alcoholic extracts of *A.malabarica* was illustrated in table-6 and 7 respectively.

Kavitha et al., 2012 studied antibacterial activity of ethanol and diethyl ether extracts of A. *malabarica* against gram positive and gram negative bacteria such *as, S. aureus, B.subtilis, B.megaterium and P.aeroginosa, K.pneumoniae* and emphasized the inhibitory effects of extracts were directly proportional to the increasing concentration of both extracts with varying degree of zone of inhibition. Similar antibacterial activity was observed against the tested gram positive and gram negative bacteria due to alcohol and aqueous extracts of *A. malabarica.* Ethanolic and methanolic extract of *A. malabarica* showed less activity compared to that of aqueous extract (Kavitha, et al., 2012) coincided the result of the present study.

Remya Mohanraj (*et al*., 2012) indicated the methanol extract of leaf *of A. malabarica* has good antibacterial, while that of hexane ethyl acetate extract have less antibacterial activity against four pathogenic bacteria *K. pneumoniae, S. aureus, V. cholerae* and *Aeroginosa*. The preliminary phytochemical screening of *A. malabarica* alcoholic leaf extract revealed the presence of phenolic in high amount followed by protein and carbohydrate in trace. The presence of such phytochemicals may be correlated with the facts that aqueous extracts showed maximum activity against the bacterial strains. The active constituents of plants usually interfere with growth and metabolism of microorganisms in a negative manner.

When comparing the antimicrobial activity by using one-way ANOVA - DMRT test, results were significant to one another ($p< 0.05$). *A. malabarica extracted* with aqueous and alcoholic extracts has more or less similar impact of antimicrobial activity. The aqueous extracts of 19± 2.00 mm zone of inhibition against *S. aureus*, alcoholic extracts of 19± 2.00 mm zone of inhibition against *S. epidermidis* and aqueous extracts of 19 ± 2.25 mm zone of inhibition against *F. Oxysporum* were observed. Statistically the results obtained due to the extracts of present study were compared to the standard, as indicated by one way AONVA and Duncan's multiple range test (Tables 2-7).

Conclusion

Aqueous and alcoholic extracts of *A. malabarica* was tested for antimicrobial efficacy against gram positive and gram negative bacterial and fungal organisms. The aqueous extract of *A. malabarica* was exhibited maximum antibacterial activity when compared than alcoholic extracts. Hence it can be concluded that the aqueous extract of *A. malabarica* possess a significant antimicrobial activity. Further study was needed to isolate pure compounds from this plant extracts and to establish the mode of action of the isolated compound

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