Chemical Composition and Antibacterial Activity of Essential Oil from *Anisomeles* Species grown in India

Ushir Yogesh*[1] and Patel Krishnakant[2]

[1]Department of Pharmacognosy and Phytochemistry, Shree H. N. Shukla Institute of Pharmaceutical Education and Research, Rajkot-360001 (GS), India. [2]Department of Pharmacognosy and Phytochemistry, Sal Institute of Pharmacy, Bhadaj, Ahmadabad-380060 (GS), India.

ABSTRACT

Anisomeles indica L., and *Anisomeles malabarica* L. R. Br. Ex Sims, growing wild in India. These shrubs become gives biological effect because of chemical composition of essential oil. Now it is interesting to know available chemicals in it, which also support the claim biological activities still, by the researchers. The chemical composition and antibacterial activity of the essential oils from *A. indica* and *A. malabarica* were investigated together here for the first time. The aerial parts (Stem, leaves, flowers and fruit) of hydrodistilled essential oils were analyzed by gas chromatography-mass spectrometry (GC-MS), and antibacterial activity was individually evaluated against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus pumilus* using a paper disc diffusion method. Collectively more than fourty compounds were identified in *A. indica* and *A. malabarica*, representing 98.29–97.88% of the total essential oil, respectively. The major constituents of essential oils obtained from the *A. indica*, were linalyl acetate (15.3%), and α-thujone (11.9%). The most abundant compounds in essential oils of *A. malabarica*, were - α-thujone (17.6%), terpenyl acetate (16.45%) and, *δ*-cadinene (11.5). All tested G+ ve & G-ve were inhibited by essential oil samples. The GC-MS results of both plants indicated the essential oil is rich in monoterpenes and terpenoids, which have been implicated antibacterial activity, comparable to gentamycin, it was used as a positive probe. The current findings also help to differentiate the valuable *Anisomeles* species for phyto-pharmaceuticals.

Key words: *Anisomeles indica* L., *Anisomeles malabarica* L.R.Br., antibacterial activity, GC-MS

INTRODUCTION

Even from ancient times, herbs and spices have been added to different types of food to improve the flavor and organoleptic properties.[1] Especially popular today is the concept of food that combines nutritional and medicinal benefits. Many natural compounds isolated from plants have demonstrated a wide spectrum of biological activities. Among these various kinds of natural substances, essential oils from aromatic and medicinal plants receive particular attention as potential natural agents for food preservation.[2] Moreover, essential oils are proven to have various pharmacological effects, such as spasmolytic, carminative, hepatoprotective, antiviral and anticarcinogenic effects.[3]

Address for correspondence:
Ushir Yogesh,
Department of Pharmacognosy and Phytochemistry,
Shree H. N. Shukla Institute of Pharmaceutical Education and Research, Rajkot-360001 (GS), India.
Email: ushir29@gmail.com

DOI: ****

The genus *Anisomeles* L. R. Br. belongs to the Lamiaceae family, and comprises over 20 species whose centre of distribution is located in the tropical Asia and Australia. These are annual or perennial semi-bushy aromatic plants that inhabit arid, sunny, stony and rocky regions. Many members of this genus are well known for their aromatic and medicinal character.[4] Three species occur in India yet, *Anisomeles indica, Anisomeles malabarica* and *Anisomeles heyneana*.[5] Out of these *A. indica* and *A. malabarica* were investigated for their Pharmacognostical and various biological activities yet.[6-7]

Anisomeles indica are used in folk medicine all over the India. It is popularly known as 'Jirnya' in northeastern part of India, where it receives widespread used as folk medicine, predominantly in the treatment of intestinal disorders and intermittent fever. *Anisomeles indica* have anti-microbial, astringent, carminative, ethanolic extract (50%) of the herb showed hypothermic activity and when burn acts as a mosquito repellant. The essential oil present in the herb is useful in uterine affections. And, *Anisomeles malabarica* useful in halitosis, epilepsy, hysteria, amentia, anorexia, dyspepsia, colic, flatulence, intestinal worms, fever arising from teething

children, intermittent fever, gout, swelling and diarrhea. Recently the valued plant investigated for its herbaceous activity.[8]

A literature survey reveals a reports on the GC-MS study of essential oil of *A. indica*, and none for *A. malabarica*.[9]

In the present work, we investigated the essential oil chemical composition of *A. indica* and *A. malabarica*. In addition, the aim of this study was to determine physical constant and to perform antibacterial activity of the isolated essential oils, which have been not reported still date.

MATERIALS AND METHODS

Plant material and reagents

Plant samples of the 2 species were collected from their type localities Toranmal (Maharashtra), and Dindigul (Tamilnadu); India. The collection was carried out two times, mansoon & autumn season, to accurately reflect the chemical composition of the respective plants. The identity of the plant material was verified by Prof. (Dr.) H.B Singh, Head, Raw Materials Herbarium and Museum, NISCAIR, New Delhi, India. Voucher specimen number HNSIPER/Herb-03 of *A. indica* & HNSIPER/Herb-04 of *A. malabarica* was deposited at the Institute level.

All applied reagents were of the highest purity available and purchased from the Sigma–Aldrich Chemical Company.

Isolation of essential oil

The powder of aerial part (flower, leaves, and stem) of *Anisomeles indica* (AIA) and *Anisomeles malabarica* (AMA) was prepared by passing through sieve # 44, and kept in tightly closed polyethylene bags. Air-dried plant material of each was subjected to hydro- distillation for 2 h with a Clevenger-type apparatus, and then dried over anhydrous sodium sulphate. The oil was stored at 4 °C in a sealed brown vial until analysis.

Microbial strains

The essential oils from AIA and AMA were individually tested against four pathogenic bacterias: *Escherichia coli* NCIM 2109, *Pseudomonas aeruginosa* NCIM 2036, *Bacillus pumilus* NCIM 2327, and *Staphylococcus aureus* NCIM 2079. All the bacterial strains were grown and maintained on nutrient agar slants. Bacterial strains were kindly supplied by stock cultures from Dept. of Biosciences, Saurastra University (Rajkot, India).

Gas chromatography/mass spectrometry analysis of essential oil

The GC (Shimadzu GCMS Q.P. 2010^{TM}) system coupled to Shimadzu Turbo Mass MS. Shimadzu GCMS Q.P. 2010^{TM} 30 m x 0.25 mm x 0.25 µm BPX-5 (SGE) column was used with helium as the carrier gas 1ml/min. The oven program was kept at 50 ^{0}c for 10 min, programmed to reach 325^0 C at a rate of 5 ^{0}C / min, and 1 µl injection (split 1:10) at 280 ^{0}C were made. Mass spectra were recorded at 70 eV. Mass range was m/z 40 to 250.

The essential oil diluted with chloroform and then injected in column. The quantification of the components was performed on the basis of their GC peak areas on the column.[10] Identification of the oil components was based on their retention indices determined by reference to a homologous series of *n*-alkanes, and by comparison of their mass spectral fragmentation patterns with those reported in the literature, and stored on the MS library [NIST database 98/ NBS 75K]. The percentages of each component are reported as raw percentages based on total ion current without standardization.

Physical evaluations

The volatile oils individually evaluated to determine their, organoleptic character, percentage volatile oil content, density and, Refractive index.[11]

Antibacterial screening

Antibacterial activity of essential oils was tested by the paper disc diffusion method according to the slightly modified National Committee for Clinical Laboratory Standards Guidelines (NCCLS, 2001) using 100 µl of suspension of the tested microorganisms, containing 2.0x10^6 colony forming units (cfu/ml). Mueller-Hinton agar (15 ml), sterilized in a flask and cooled to 45–50 ^{o}C, was distributed to sterilized Petri dishes with a diameter of 9 cm. The filter paper discs (6 mm in diameter, Whatman No. 1) were individually impregnated with 10 µl of the sample dissolved in dimethylsulfoxide (DMSO), which was subsequently placed on the surface of the inoculated Petri dishes. The essential oils concentrations in DMSO were adjusted to 3.0 mg/ml. The Petri dishes were kept at 4 ^{o}C for 2 h, and then incubated at 37 ^{o}C for 24 h. The diameters of the inhibition zones were measured in millimeters. Controls were set up with equivalent quantities of DMSO. Studies were performed in triplicate, and the developing inhibition zones were compared with those of reference discs. Antibiotic gentamycin (30 µg) was used as reference.

RESULTS

GC-MS analysis

The essential oils of *A. indica* and *A. malabarica* were subjected to detailed GC-MS analysis in order to determine their volatile constituents. Exactly 41 compounds were identified in two samples are given in Table 1.

In AIAEO (Essential oil of *A. indica*), 36 compounds were identified, representing 98.29% of the total essential oil. The most abundant components were oxygenated monoterpene (26.10%) and oxygenated sesquiterpenes (44.86%). The high percentages of linalyl acetate (15.3%) and, α-thujone (11.9%) proved that this essential oil clearly belongs to the mixed chemotype. In contrast, the essential oil obtained from plant material of *A. malabarica* (AMAEO),

Table 1: The percentage concentration of compounds found in essential oils obtained from A. indica and A. malabarica

		Content (%) in	
RI[a]	Compound	AIAEO	AMAEO
931	α-pinene	1.2	2.4
946	Camphene	1.5	0.7
975	β-pinene	3.35	tr[b]
995	3-Octanol	-	0.6
1030	1,8-Cineole	tr	0.1
1066	cis-sabinene hydrate	0.1	1.7
1098	Linalool	0.1	1.1
1121	*cis-p*-menth-2,8-en-1-ol	0.2	0.2
1147	Camphor	6.5	4.7
1168	Borneol	2.9	2.9
1198	Myrtenol	tr	tr
1205	α-thujone	11.9	17.6
1210	Linalyl acetate	15.3	-
1229	Nerol	tr	tr
1235	Methyl ether Thymol	tr	tr
1258	Geraniol	0.1	0.1
1266	Geranial	tr	tr
1292	Thymol	1.8	3.8
1320	Bornyl acetate	-	0.37
1321	Isobornyl formate	5.7	1.45
1328	Terpenyl acetate	-	16.45
1335	Anisole	1.38	0.5
1337	2-Isopropylbenzaldehyde	1.38	0.5
1360	Eugenol	1.05	3.55
1488	*n*-Nonanyl acetate	tr	1.1
1536	δ-cadinene	-	11.5
1551	Caryophyllene	2.32	0.92
1553	Isocaryophyllene	-	0.31
1819	Caryophyllene oxide	2.86	7.81
1837	Epiglobulol	tr	1.25
1838	Globulol	tr	tr
1864	trans - Naphthalene	tr	tr
1864	Trifluoroacetyl - isomenthol	0.1	-
1947	Nerolidyl acetate	0.63	0.2
2122	Farnesyl acetone	4.89	9.98

Table 1: *continued*

RI[a]	Compound	Content (%) in AIAEO	Content (%) in AMAEO
2390	α-Bisabolol	5.85	2.75
2463	*trans*-Phytol	7.1	3.23
2464	Citronellol	1.1	1.35
2465	1,3 trans Menthol	tr	2.45
2465	Isomenthol	tr	tr
2646	Azulene	-	1.63
	Monoterpene hydrocarbons	14.40	19.47
	Oxygenated monoterpenes	26.10	23.44
	Sesquiterpene hydrocarbons	5.84	8.46
	Oxygenated sesquiterpenes	44.86	38.46
	Diterpene hydrocarbon	07.09	08.05
	Total identified	**98.29**	**97.88**

[a]Temperature program Kovat's retention index. [b]tr: Trace amount (< 0.1%).

was characterized by a high content of oxygenated sesquiterpenes (38.46%) and oxygenated monoterpenes (23.44%), with α-thujone (17.6%), terpenyl acetate (16.45%) and, *δ*-cadinene (11.5) as the main constituents. Thirty nine compounds were identified, representing the 97.88% of the total essential oil content.

Physical evaluations

The results of physical evaluation of essential oils are dipected in Table 2.

Antimicrobial screening

The antimicrobial activities of *A. indica* and *A. malabarica* essential oils were evaluated by a paper disc diffusion method against G+ve, and G-ve bacteria. Essential oils exhibited antibacterial activity against the tested strains, but in variable degree. Results are comparable to the antibiotic gentamycin, used as a positive probe (Table 3).

Table 2: Physical evaluation of volatile oil from A. indica and A. malabarica

Parameter	*A. indica*	*A. malabarica*
Organoleptic Character		
Color	Pale yellow	Pale yellow
Odor	Warm & Woody	Warm & terpeny
Taste	Slightly Pungent then spicy	Pleasant with spicy
Density	0.9666	0.9910
Refractive Index	1.4939	1.4897
% Volatile oil (w/w)	0.06	0.05

Table 3: Inhibition zones (mm) of the A. indica and A. malabarica essential oils

Micro-organisms	*A. indica** (10µl/disc) Mean ± SD‡	*A. malabarica †* (10µl/disc) Mean ± SD‡	Gentamycin (10µl/disc) Mean ± SD‡
Staphylococcus aureus NCIM 2079	14 ± 0.023	10 ± 0.028	41 ± 0.026
Bacillus pumilus NCIM 2327	13+0.013	11 ± 0.013	33 ± 0.018
Escherichia coli NCIM 2109	10 ± 0.027	08 ± 0.023	16 ± 0.005
Pseudomonas aeruginosa NCIM 2036	10 ± 0.019	10 ± 0.003	13 ± 0.006

*A. indica - collection at Toranmal (mansoon & autumn); †A. malabarica - collection at Dindigul (mansoon & autumn); ‡Standard deviation of three readings

CONCLUSION

The GC-MS results of both plants indicated the essential oil is rich in terpenes and terpenoids which have been implicated in plant's pharmacological activities. The results found in antibacterial activity, is because of monoterpenes & terpenoids presenet in essential oils. Linalyl acetate, α-thujone *δ*-cadinene, and terpenyl acetate are responsible for bacterial sensitivity. The data indicated that Gram-positive *B. pumilus* was the most sensitive strain tested to the oils of *A. indica* and *A. malbarica*. Gram-negative *P. aeruginosa* is known to have a high level of intrinsic resistance to virtually all known antimicrobials and antibiotics, due to a very restrictive outer membrane barrier, highly resistant even to synthetic drugs.[12] However, *A. indica* inhibit growth of this bacterium. Confirming previous reports, it was found that the strength and spectrum of activity varied between

investigated *Anisomeles* species and Gram-positive bacteria were generally more sensitive to the effects of the oils.

Although essential oils of *A. indica* and *A. malabarica* have significant differences in their chemical compositions, showed very effective antibacterial activities. The results of this study suggest the possibility of using the essential oil of these two *Anisomeles* species as natural food preservatives, and potential sources of antibacterial ingredients for the food and pharmaceutical industry. Our results suggest that the essential oils of those species may warrant further investigation for their potential therapeutic efficacy.

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