Original Article

Gas chromatography–Mass spectrometry analysis and antibacterial activity of essential oil from aerial parts and roots of *Anisomeles indica* Linn

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The essential oil from the aerial part and roots of *Anisomeles indica* Linn. from the Toranmal forest, Maharashtra (India), was obtained by hydrodistillation after drying and grounding of the herbal material. A total of 27 components were resolved in the essential oil by GC-MS analysis, while 18 compounds were identified comparing the recorded mass spectra with the Nist 98/Nbs 75 K GC-MS library of mass spectra. The microbiological activity of the isolated essential oils was investigated as well. It was found that the essential oil shows microbiological activity on *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus pumilus* and *Staphylococcus aureus* with a range of minimum inhibitory concentration values extended from 31.25 to 250 μg/ml. The percentage inhibition values calculated also support the minimum inhibitory concentration findings. The MBCs also performed for the same pathogens. Limonene, pinene, isobornyl acetate and eugenol can be considered as the main antimicrobial constituents in the essential oils of plants.

Key words: *Anisomeles indica* Linn, essential oil, microbiological activity

INTRODUCTION

Anisomeles indica Linn. (Family: Labiatae) is found throughout tropical and subtropical region of India. The plant is used in folk medicine as a cure in gastric catarrh and intermittent fever and essential oil present in herb is used in uterine affection.[1,2] *A. indica* Linn. is reported to have antipyretic, analgesic, anti-inflammatory activity and it also acts as natural herbicide in wheat fields.[3,4] It is also reported that the leaves of *A. indica* consists of diterpenoids, ovatodiolide and its derivatives that are used as HIV inhibitors.[5] Roots contain stigmasterol, β-sitosterol, paraffins and fatty acids. In India, the plant is used as a carminative and as aromatics.[6] As a result of indiscriminate use of antimicrobial drugs in the treatment of infectious diseases, microorganisms have developed resistance to many antibiotics. There is need to develop alternative antibiotic drugs from plants. One approach is to screen local medicinal plants, which represent rich source of novel antimicrobial agents.

As far as our literature survey could ascertain, neither the antimicrobial activities nor the composition of *A. indica* essential oils have been studied before. The present study was carried out to investigate the antibacterial properties and gas chromatography–mass spectrometry (GC-MS) analysis of essential oil from *A. indica* aerial parts (AILEO) and roots (AIREO). Inhibitory effect by minimum inhibitory concentration (MIC) and minimum bactericide count (MBC) was also carried out in the present study.

MATERIALS AND METHODS

Plant Material

Whole plant of *A. indica* was collected from Toranmal forest, at an altitude of 1800 m (Satpuda valley) Maharashtra, India, in the month of Aug–Sep 2006. Botanical identification was carried out by Dr. D.A. Patil Taxonomist, SSVP'S Science College, Dept. of Botany, Dhule (MS), India. Voucher specimen of the plant material has been deposited at Institute level (RCPCOP/AI-06).

Essential Oil Extraction

Essential oil was extracted from air-dried aerial part (including leaves, stem and flowers) and roots of plant by hydrodistillation using Clevenger type apparatus for 3 h to yield essential oil, and then dried over anhydrous sodium sulfate.[7]

GC–MS Analysis

The GC (Perkin-Elmer™) system coupled to Perkin-Elmer Turbo Mass MS. Perkin Elmer™ 30 m × 0.25 mm ×

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Received: 15-04-2009; **Accepted:** 14-11-2009; **DOI**: 10.4103/0973-8258.63884

0.25 μm PE-1 methyl silicone column was used with helium as the carrier gas 1 ml/min. The oven program was kept at 60°C for 10 min, programmed to reach 180°C at a rate of 5°C/min, and 1 ml injection (split 1:10) at 280°C were made. Mass spectra were recorded at 70 eV. Mass range was *m/z* 40-250. 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.

The identification of the separated volatile organic compounds was achieved through retention indices and mass spectrometry by the comparing mass spectra of the unknown peaks with those stored in the Nist 98/ Nbs 75 K GC-MS Library.[8,9]

Antimicrobial Activity

Microbial strains

The essential oil from aerial part and roots of *A. indica* were individually tested against four pathogenic microorganisms: *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. The inoculum size of each test strain was 1 × 10^8 bacteria/ml for twofold dilution series method. Bacterial strains were kindly supplied by stock cultures from Dept. of Microbiology, R.C. Patel Arts and Science College (Shirpur, India).

MIC and MBC determination

Bacterial strain were grown in Mueller–Hinton (MH) agar broth and suspended in double strength (DS) MH broth for the finding of MIC.[10] The MIC values against bacterial strains were performed using twofold dilution series method.[11] The twofold dilution series of an essential oil or its major constituent dissolved in DMSO was prepared: 1000, 500, 250, 125, 62.5, 31.3, 15.6 and 7.8 μg/ml. The negative and positive control was used for the comparison. After inoculation at 37°C for 24 h the abovementioned concentration of essential oils were transferred to Microplate ELIZA reader (BIO-Tek Power wave™ XS, Model 96-well microplate) at 540 nm, to calculate % inhibition antimicrobial activity for obtaining a more precise measure of the MIC. The bacterial suspensions were tested aerobically and incubated for 24 h at 37°C. MIC value for gentamicin was also determined in MH broth using standard method.[12,13]

The MBC was carried out by transferring MH broth aliquots from tubes to the agar plates. The growth in tube was observed by turbidity and inhibition was determined by absence of growth. The MIC was defined the lowest concentration of sample that inhibits any visible bacterial growth and the MBC was defined as lowest concentration of test material that resulted in no recoverable bacteria or that killed the bacteria. The experiment was performed twice and each oil was tested in triplicate to obtain accurate and precise results.

Statistical Analysis

Data reported in Tables 1 and 2 as the mean ± SD of three measurements.

RESULT AND DISCUSSION

Water distillation of aerial parts of *A. indica* yielded 0.29% v/w of the scarlet red essential oil, while the roots was yielded 0.66% v/w (calculated on dry weight basis). About 18 constituents were identified by means of GC-MS analysis of the essential oils from *A. indica*. Mainly monoterpenes and sesquiterpenes compound such as isobornyl acetate (64.6% and 55.36%), isothujone (6.01% and 12.37%), nerolidol (3.17% and 7.19%), camphene (3.54% and 5.52%), decanal (2.29% and 1.61%) and eugenol (3.25% and 4.15%) represent the most abundant compounds in AILEO and AIREO, respectively. These compounds are listed in Table 1 along with their retention time, and relative content.

The microbial resistance is serious and growing problem in treatment of infectious diseases. The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutics.[14] It is well established that several secondary metabolites synthesized by plant play central role in plant defense against microbial attack.[15] Thus, plant secondary metabolites offer molecules with potential antimicrobial activity.

Table 1: Chemical composition of essential oil

Rt (min)	Compound	% Composition	
		AILEO	AIREO
3.247	α-Pinene	2.42	4.07
3.427	Camphene	3.54	5.52
3.687	β-Pinene	2.36	2.65
4.095	o-Cymene	tr	tr
4.096	Cymol	tr	tr
4.130	Limonene	tr	tr
4.130	Nerol	tr	tr
4.170	Thujene	tr	tr
4.170	Sabinene	tr	tr
5.834	Decanal	2.29	1.61
6.688	Iso-bornyl acetate	64.60	55.36
7.341	Eugenol	3.25	4.15
7.648	Decyl ester of acetic acid	2.28	1.33
7.761	Fristolane	-	0.24
7.968	Farnesene	0.55	0.65
8.608	Farnesol	tr	0.31
12.48	Nerolidol	3.17	7.19
12.916	Isothujone	6.01	12.37

Rt- retention time; tr- trace amount

A. indica essential oils shows antibacterial activity and overall roots are more active than leaves. The AILEO and AIREO show same MIC value for *E. coli* and *S. aureus*, whereas AIREO showed strong antimicrobial activity against *Bacillus pumilus* and *P. aeruginosa* as compared to AILEO. In particular, we found a remarkable activity against *P. aeruginosa*, which is responsible for several opportunistic infections and often resistant to conventional antibiotics. Results obtained from measurement of MIC indicated that, *B. pumilus* spore-forming bacterial species is the most sensitive microorganism tested with lowest MIC values (62.5 and 31.25 μg/ml) in the presence of AILEO and AIREO, respectively. *P. aeruginosa* was another test microorganism against the oils applied with lower MIC values 125 μg/ml for AILEO and 62.5 μg/ml for AIREO. The oils tested also exhibited antimicrobial activity against *S. aureus* and *E. coli* with a range of MIC values extended from the oil concentration 125-250 μg/ml.

The MIC values of AILEO and AIREO supported by the percentage inhibition value, which was calculated by using the formula $[Ab_{Sam} - Ab_{Cont}/Ab_{Cont} \times 100$ (Ab_{Sam}: absorbance of sample, Ab_{Cont}: absorbance of positive control). The percentage inhibition antimicrobial activity results are mentioned in Table 2 and antimicrobial activity pattern of AILEO and AIREO are represented in Figures 1 and 2.

It was an impressive finding that standard antimicrobial agent does not show MBC against *B. pumilus*, whereas AILEO and AIREO give MBC at 62.5 and 7.81 μg/ml, respectively. Against *P. aeruginosa* the AILEO gives MBC 62.5 μg/ml and AIREO gives 31.25 μg/ml. The MBC for *E. coli* and *S. aureus* was found to be in the range 125-250 μg/ml [Table 3].

The MIC and MBC values were compared with the standard antibiotic gentamicin on the basis of which we can conclude that both oils possess effective antimicrobial activity against wide range of pathogens.

Table 2: Percentage inhibition antimicrobial activity of AILEO and AIREO

Conc. (μg/ml)	*Escherichia coli* Mean ± SD		*Pseudomonas aeruginosa* Mean ± SD		*Bacillus pumilus* Mean ±SD		*Staphylococcus aureus* Mean ± SD	
	AILEO	AIREO	AILEO	AIREO	AILEO	AIREO	AILEO	AIREO
3.91	0.00±0.03	2.85±0.03	2.90±0.01	4.80±0.01	0.00±0.01	4.29±0.02	0.50±0.02	1.42±0.03
7.81	8.13±0.04	10.95±0.01	3.80±0.04	8.50±0.05	10.48±0.06	3.80±0.03	10.57±0.02	1.90±0.03
15.63	30.14±0.04	16.19±0.09	10.48±0.04	12.40±0.01	21.90±0.01	50.48±0.02	20.67±0.03	9.52±0.08
31.25	39.71±0.01	21.43±0.12	30.14±0.01	17.14±0.01	50.48±0.01	53.33±0.01	31.25±0.08	25.24±0.06
62.5	43.06±0.02	45.71±0.08	49.76±0.01	55.23±0.04	59.04±0.03	55.71±0.02	50.00±0.01	40.00±0.01
125	51.20±0.02	47.62±0.05	58.57±0.01	59.52±0.01	59.62±0.01	57.14±0.08	55.29±0.02	56.66±0.02
250	59.33±0.02	62.85±0.02	59.52±0.02	58.09±0.01	59.04±0.07	58.57±0.01	55.77±0.01	59.05±0.01
500	58.37±0.01	65.47±0.02	58.57±0.03	58.57±0.02	60.00±0.01	59.04±0.04	57.21±0.01	60.00±0.01
- ve control	57.90±0.01	64.76±0.03	58.09±0.08	57.20±0.06	58.09±0.04	57.62±0.04	57.14±0.03	60.48±0.01
Std. A	59.52±0.01	65.47±0.07	58.09±0.01	59.52±0.04	59.04±0.02	58.57±0.02	57.69±0.01	59.52±0.002

Std. A- Gentamicin; SD- Standard deviation

Table 3: MICs and MBCs of AILEO and AIREO (in μg/ml)

Microorganism	MIC			MBC		
	AILEO	AIREO	Std. A	AILEO	AIREO	Std. A
Escherichia coli NCIM 2109	250	250	150	250	250	150
Pseudomonas aeruginosa NCIM 2036	125	62.5	50	62.5	31.25	50
Bacillus pumilus NCIM 2327	62.5	31.25	-	62.5	31.25	-
Staphylococcus aureus NCIM 2079	125	125	75	125	125	75

MIC- minimum inhibitory concentration; MBC- minimum bactericidal count

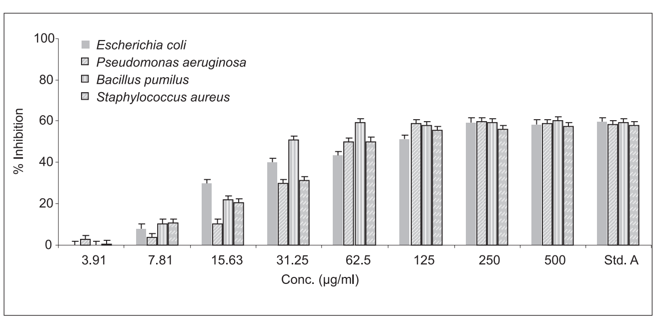

Figure 1: Percent inhibition antimicrobial activity pattern of AILEO

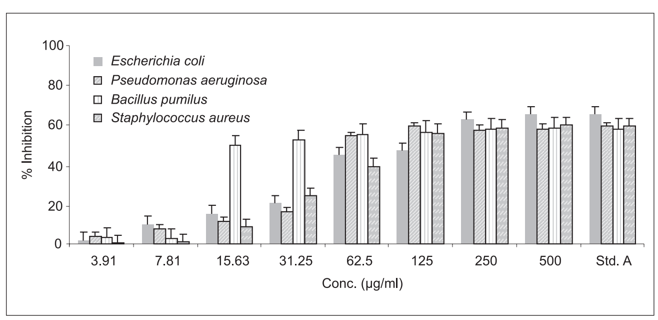

Figure 2: Percent inhibition antimicrobial activity pattern of AIREO

To the best of our knowledge, the antimicrobial activities of the essential oils of AIL and AIR have not been reported before. Literature survey revealed that an essential oil may have antimicrobial activities and responsible for bactericidal effect against several microorganisms. Some of main constituents identified in study such as limonene, pinene and eugenol are reported to have antibacterial property.[16,17] Therefore, antibacterial constituents from *A. indica* essential oils could hold promise for future application in therapy.

ACKNOWLEDGMENTS

The author(s) thank Dr. D. R. Patil, Principal, R.C. Patel Arts and Science College, Shirpur, Dist: Dhule, for providing facilities for this study.

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Source of Support: Nil, **Conflict of Interest:** None declared.