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Research Article

ISOLATION AND CHARACTERIZATION OF PENTADECANOIC ACID ETHYL ESTER FROM THE METHANOLIC EXTRACT OF THE AERIAL PARTS OF *ANISOMELES MALABARICA* (L). R.BR.

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ABSTRACT

Anisomeles malabarica (L). R.Br., (Lamiaceae) is distributed in major parts of India, especially in South India it is known as a traditional medicinal plant reported to possess anti-spasmodic, anti-inflammatory properties and is used in Rheumatoid arthritis. The preliminary phytochemical investigation on the methanolic extract of the aerial parts of the plant revealed the presence of carbohydrates, phytosterols and triterpenoids. The aim of the current study was to isolate and characterize the bioactive compounds from the aerial parts of *Anisomeles malabarica* as the plant is reported to possess potent anti-inflammatory properties and is also used in the treatment of Rheumatism. For isolation purpose, the dried powder of was extracted with methanol using Soxhlet apparatus continuously for 16 hours. The extract was dried under reduced pressure to evaporate the solvent and the dried mass was taken for the isolation work. Pentadecanoic acid ethyl ester was isolated by column chromatography from the methanolic extract of aerial parts of *Anisomeles malabarica*. The structural elucidation of the isolated compound was on the basis of spectroscopic analysis.

KEYWORDS: *Anisomeles malabarica*; pentadecanoic acid ethyl ester; rheumatoid arthritis; ^{1}H-NMR; ^{13}C-NMR; LC-ESI-MS

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INTRODUCTION

Today, Ayurvedic, Homeo and Unani Physicians utilize numerous species of medicinal plants (Mujumdar AM *et al.*, 2000). Many compounds used in today's medicine have a complex structure and synthesizing these bioactive compounds chemically at a low price is not easy (Madhava C, 1998). The increasing awareness about side effects of drugs had made the western pharmaceutical industries to turn towards the plant based Indian and Chinese medicine (Balandrin MJ & Klocke JA 1988). *Anisolmeles malabarica* (L). R.Br., (Lamiaceae) is distributed in major parts of India and especially in South India as a traditional medicinal plant commonly known as *Peymarutti* (Tamil), *Gouzaban* (Hindi), *Chodhara* (Marathi), *Karithumbi* (Kannada) and Malabar catmint (English)(Kritikar KR & Basu BD, 1935). The herb is reported to possess anti-spasmodic, anti-periodic properties and used in Rheumatoid arthritis (Nadkarni KM, 2006). It is used for the traditional treatment of snakebite as antidote and plant leaves are used as carminative, astringent, stomachic, rheumatism and diaphoretic in Coimbatore district and also used as dentifrice to cure various problems (Kalyani K *et al.*, 1989). The methanolic extract of aerial parts of *Anisomeles malabarica* (L). R.Br., (AmA) produced significant anti-rheumatic activity in a dose-dependent manner (200 mg/Kg and 400 mg/Kg body weight) to that of standard drug indomethacin (10 mg/Kg). The extract exhibited inhibitory effect in carrageenan induced hind paw oedema in rats with all the doses used when compared to the control group. The data obtained indicate that the crude extracts of the aerial parts of the plant AmA possess potential anti-rheumatic activity by supporting the folkloric usage of the plant to treat various inflammatory conditions (Setty AR, 2005).

AmA extract was tested for cytotoxicity in RAW and L-929 cell lines and was found to be non-toxic. Based on the results, non-toxic doses of extracts were tested for their inhibitory activity against LPS induced TNF-α production. AmA showed better activity by reducing the LPS induced TNF-α production by 38.75 % (Ismail SM *et al.*, 2012). So based on the various *in-vivo* and *in-vitro* studies conducted, it can be concluded that the plant AmA possesses potent immuno-modulatory and anti-rheumatic properties. With the above findings, the present work was carried to isolate and characterize the bio-active phyto-constituents present in AmA.

MATERIALS AND METHODS

Collection of plant material

Fresh leaves of *Anisomeles malabarica* free from disease was collected from different regions in & around Bangalore and were authenticated by taxonomists & the Voucher specimen was deposited in the department for future reference.

a) Chemicals

Hexane, ethyl acetate, chloroform, methanol and silica gel of mesh size 60–120 and 200–400 was purchased from Sd fine chemicals, Mumbai, India. Column length was 100 cm and column diameter was 3 cm.

b) Extraction procedure

Dried powder of AmA was extracted with methanol using Soxhlet extraction unit for 18 hours as per standard procedure (Mukherjee PK, 2010) the extract was dried under reduced pressure to evaporate the solvent and dried mass (20 gm) was taken further for isolation work.

Isolation of phytochemicals

I. Column Chromatography Purification Of Methanolic Extract

a) Adsorption of sample on silica gel

The methanolic extract of AmA (dried mass ;15 gms) was adsorbed on dry silica and the adsorbed sample was kept for complete drying and later used for coloumn elution.

b) Loading of column (wet packing)

Column was packed with silica gel slurry of mesh size 60–120 with hexane. Column length was 100 cm and diameter was 3 cm. On top of silica bed activated sample was loaded and cotton was placed on top of it to avoid any disturbance to the sample bed.

c) Elution of the column

Initially Hexane solvent was eluted in small quantity for correct distribution of activated sample in the column and later eluted with two solvent combinations with increasing order of polarity.

Based on preliminary TLC observations, column elution was started with hexane, ethyl acetate and methanol combinations. Fractions were collected in 50 ml portions.

Pattern of column elution:

1. Hexane
2. Hexane: Ethyl acetate: 7:3
3. Ethyl acetate
4. Ethyl acetate : Methanol : : 5 : 5
5. Methanol

From the above elution process, the fractions were pooled as per their mobile phase. From the above fractions, Ethyl acetate: Methanol: 5:5 was further processed for purification through column chromatography. The other fractions were not used due to their low yield.

II. Column Chromatography Purification Of Ethyl Acetate: Methanol: : 5 : 5

a) Adsorption of sample on silica gel

The ethyl acetate : methanol : : 5: 5 fraction (dried mass; 5–8 gms, brown colour powder) was adsorbed on dry silica and the adsorbed sample was kept for complete drying and later used for column elution.

b) Loading of column (wet packing)

Column was packed with silica gel slurry of mesh size 200–400 with chloroform. On top of silica bed, activated sample was loaded and cotton was placed on top of it to avoid any disturbance to the sample bed.

c) Elution of the column

Initially chloroform was eluted in small quantity for correct distribution of activated sample in the column and later with two solvent combinations with increasing order of polarity.

Column elution was started with chloroform and methanol combinations. Fractions were collected in 15 ml portions.

d) Evaporation of fractions

Based on TLC profiles of the eluted fractions, they were pooled and evaporated. Chloroform: methanol : : 7 : 3 fractions were dried under reduced pressure and then subjected for preparative TLC for purification.

e) Preparative TLC of chloroform : methanol : : 7 : 3 fractions

The dried fraction was purified by preparing the TLC plate with silica gel G, a mobile phase of Chloroform : methanol : : 7.5 : 3 was used for TLC. After TLC separation, the plate was air dried and observed under UV light. A yellow fluorescent band was scrapped off and the band was eluted by mixing with methanol and later centrifuged. The solvent was collected, dried and later checked for purity by TLC and the compound (under investigation) was sent for spectral analysis i.e., IR, MASS, C^{13} NMR &^{1}H NMR for structural elucidation.

RESULTS

Spectral studies

The Compound in its ESI-MS (positive mode) spectrum exhibits a peak at m/z 279 for an ion $[M+Na]^{+}$ suggesting a molecular weight of 256.

In its ^{1}H-NMR spectrum (Figure 1–4) it showed peaks at δ 0.80 showing the presence of methyl groups in the compound. The large

singlet at δ 1.15 and the signals at δ 1.80 were due to the long chain methylene groups. The signal at δ 1.90 is due to a methylene adjacent to a carbonyl group. The signal at δ 3.40 may be due to the protons attached to oxygen function.

In the ^{13}C-NMR (Figure 5) the signals at δ 20.00 are due to methyl group, at δ 25, 28.00 to 31.00 are due to the methylene carbons. The signal at δ 70.00 is due to the carbon attached to the oxygen function. The signal at δ 172.00 confirms the presence of a carbonyl group. The results of LC-ESI-MS is depicted in Figure 6.

Based on the above data the structure of the compound is **Pentadecanoic Acid Ethyl Ester** (Figure 7).

Figure 1.: ^{1}H-NMR of bio-active compound from the aerial parts of *Anisomeles malabarica* R.Br. at 279 MHz

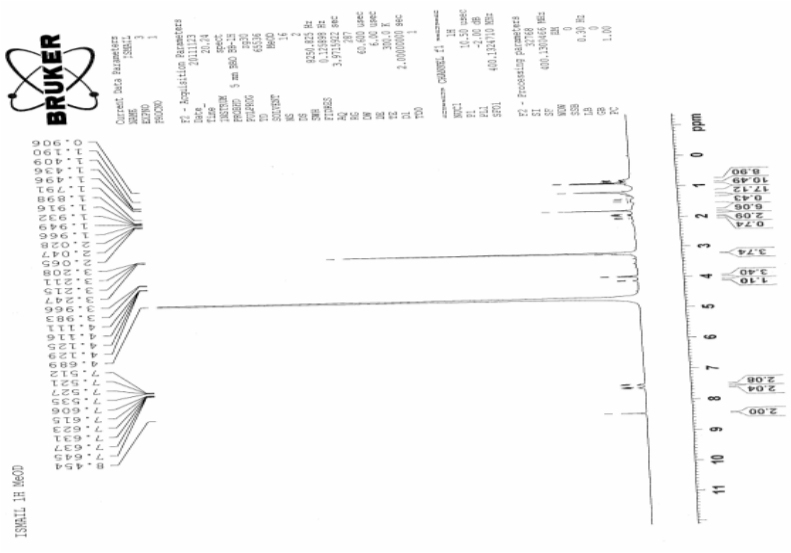


Figure 2.: ^{1}H-NMR of bio-active compound from the aerial parts of *Anisomeles malabarica* R.Br.

Figure 3: ^{1}H-NMR of bio-active compound from the aerial parts of *Anisomeles malabarica* R.Br.

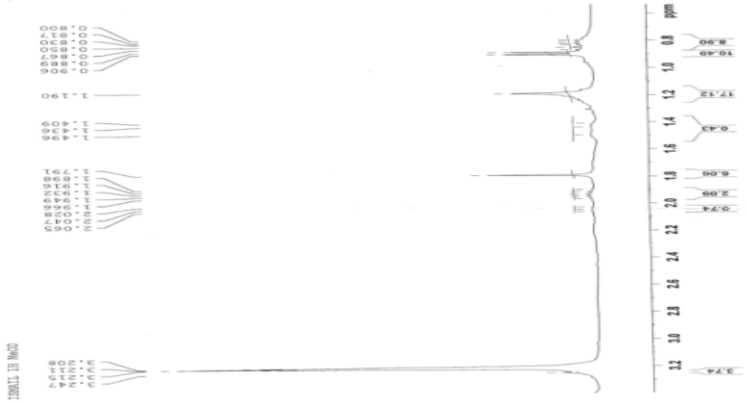


Figure 4.: ^{1}H-NMR of bio-active compound from the aerial parts of *Anisomeles malabarica* R.Br.

Figure 5.: ^{13}C-NMR of bio-active compound from the aerial parts of *Anisomeles malabarica* R.Br

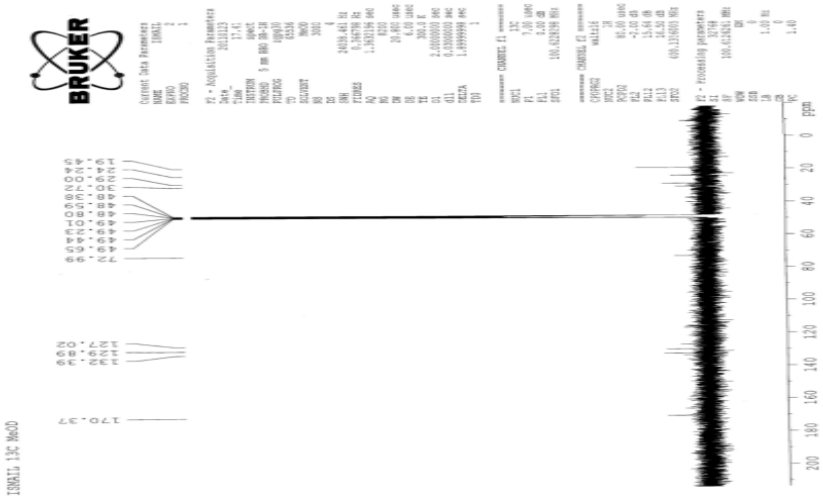


Figure 6.: LC-ESI-MS of bio-active compound from the aerial parts of *Anisomeles malabarica* R.Br.

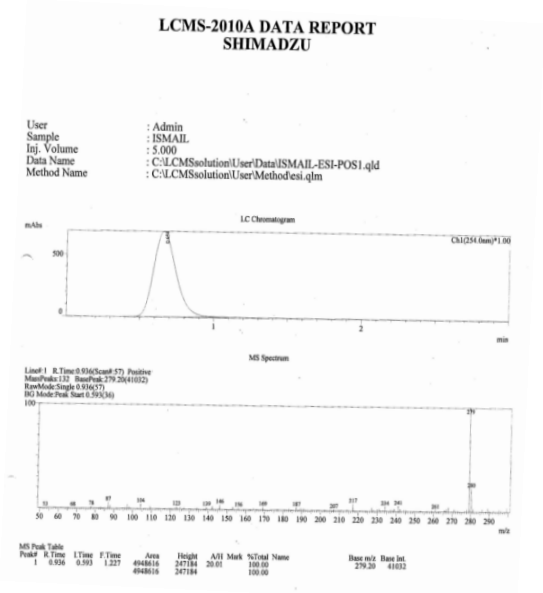
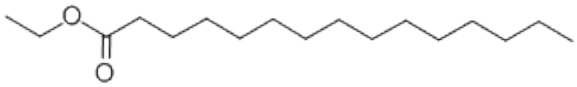


Fig.7. Pentadecanoic Acid Ethyl Ester

CH_3- $(CH_2)_{13}$- COO-CH_2- CH_3



Molecular formula: $C_{17}H_{34}O_2$
Synonym: Ethyl N-Pentadecanoate

DISCUSSION

Anisomeles indica L., and *Anisomeles malabarica* R. Br. Ex Sims, is found growing wild in India. The chemical composition and antibacterial activity of the essential oils from *Anisomeles indica* L and *A. malabarica* were investigated together. 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 forty 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 (Ushir Y & Patel K, 2011).

Seven fatty acids were identified from the methanolic extract of *Anisomeles indica* L., and *Anisomeles malabarica* L. R. Br. Ex Sims aerial parts. The extracted fatty acids were methyl-esterified and then analyzed by GC-MS. The relative contents of the fatty acids were calculated with Area normalization.

Seven fatty acids amounting to 77.778% in *A. indica* and 68.027% in *A. malabarica* of the total contents were detected. The major fatty acids found in *A. indica* were palmitic acid (23.334%), stearic acid (22.749%), lignoceric acid (21.54%) and, in *A. malabarica*, palmitic acid (35.252%), stearic acid (21.43%). The results the content of fatty acids was abundant in *Anisomeles* species, and it had a great range of potential utilities and a prospect of development in foods, medical and health care (Ushir Y *et al.,* 2011).

Based on the above findings by (Ushir Y *et al.,* 2011) it can be concluded that *Anisomeles malabarica* R.Br. possesses potent bio-active compounds, both reported in literature and yet to be reported. So it was investigated to evaluate the in vivo and *in vitro* activity of the isolated compound namely, Pentadecanoic Acid Ethyl Ester from the aerial parts of AmA.

CONCLUSION

The current investigation from the methanolic extract of aerial parts of the plant *Anisomelos malabarica* has revealed the presence of Pentadecanoic Acid Ethyl Ester. Also the plant is reported to posses' inhibition of *invitro* TNF-α production and possesses anti-rheumatic and immuno-modulatory properties.

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