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Original Article

Isolation and Identification of Compounds from the Leaf Extract of *Melaleuca alternifolia*

Gagan Shah[1]*, Dhandeep Singh[2], Uttam Singh Baghel[3]

ABSTRACT

Introduction: *Melaleuca alternifolia* also known as Tea tree oil belonging to family Myrtaceae. This plant has diverse and therapeutic uses in traditional herbal medicine for treating Skin care, First Aid, Household Cleaning, Hair care, Aromatherapy, Feminine care, Chronic illness, and Dental care in Australia. **Method:** The methanolic extract of *Melaleuca alternifolia* family Myrtaceae was subjected for Soxhlet extraction in round bottomed flask with petroleum ether. The petroleum ether extracted leaf powder was dried and once again subjected to Soxhlet extraction successively with different solvents viz, chloroform, ethyl acetate and methanol. **Result:** The IR spectra showed characteristic absorption bands at 3421 cm^{-1} indicating the presence of a OH group, at 1691cm^{-1} for C=O group, 2848 cm^{-1}. The ^{1}HNMR spectra showed a triplet signal at δ 10.44, 9.48, 13.21 showing the presence of OH group and Carboxylic acid. The ^{13}C NMR spectra showed signals at δ 16.28 for a methyl carbon, δ 29.89 for a methylene carbon, The ESI-negative mode mass spectrum showed pseudo molecular ions at m/z 515 for [M+] ion. **Conclusion:** Based on spectral analysis and comparison of the spectral data with literature values, the compounds were identified as 3,3'dimethylellagic acid and its aglycone portion with some little impurity.

Key words: Methalonic, Myrtaceae, *Melaleuca alternifolia,* 3, 3'dimethylellagic acid, Aromatherapy,Chloroform.

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INTRODUCTION

Melaleuca alternifolia is likewise called tea tree oil (TTO), the unstable fundamental oil got principally from the Australian local plant. Utilized for its antimicrobial properties, TTO is utilized as the dynamic fixing in numerous plans used to treat cutaneous contaminations. *Melaleuca alternifolia* accessible over the counter in Australia, Europe, and North America and is promoted as a solution for different sicknesses.[1] This plant is essential for therapeutic uses and herbal products. The leaves of "tea trees" were utilized for the treatment of hack or were spread on wounds,[2] and tea tree leaves were drenched to make a mixture for treatment of sore throats or skin illnesses.[3]

Tea Tree oil is an active ingredient of many topical preparations for the treatment of cutaneous infections including wound infections [4] fungal dermatoses,[5] otitis media,[6] and acne.[7] Although several clinical studies suggest that tea tree oil possesses antimicrobial activity.

Melaleuca alternifolia is consisting of terpene hydrocarbons, which contain monoterpenes, sesquiterpenes, and their associated alcohols. Brophy and colleagues [8] examined over 800 Tea Tree oil samples by gas chromatography and GC-MS and report approximate 100 components and their Percentage composition in Table 1.

The bark and stem of *Melaleuca alternifolia*(Myrtaceae) led to the isolation and identification of 3,3' dimethylellagicacid and five pentacyclic triterpenes: 23 trihydroxyolean12en28oic acid (arjunolic acid) 3 βhydroxylup20 (29) en27,28dioic acid (melaleucic acid), Betulinic acid, betuline, 3 β-*the*-acetylurs12en28oicacid and the mixture of fatty acids and esters, and several hydrocarbons.[9]

MATERIAL AND METHOD

Collection and authentication of plant material

The leaves of *Melaleuca alternifolia* (Myrtaceae) were collected from the hills of the Nilgiris district of Tamil Nadu, India in January 2016 from the healthy plants. The herbarium was prepared by using plant material and Dr. K. Madhava Chetty (Assistant Professor, Department of Botany, Sri Venkateshwara University, Tirupati, Andhra Pradesh, India) authenticated the plant under voucher specimen no.1241, dated May 28, 2016. A voucher specimen of plant material has been retained in the Department of Pharmacognosy and Phyto chemistry, IKG Punjab Technical University, Kapurthala, Punjab, India.

Extraction and isolation procedure

a. Crude Extraction

The plant *Melaleuca alternifolia* Leaf powder (1600 g) was subjected for Soxhlet extraction in round bot-

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Table 1: **Composition of *M. alternifolia* (tea tree) oil.**

Component	Typical Composition (%)
Terpinen-4-ol	40.1
γ Terpinene	23.0
α -Terpinene	10.4
1,8-Cineole	5.1
Terpinolene	3.1
p-Cymene	2.9
α -pinene	2.6
α -Terpineol	2.4
Aromadendrene	1.5
δ-Cadinene	1.3
Limonene	1.0
β-phellandrene	0.9
Globulol	0.8
myrcene	0.8
α -thujene	0.8
β-pinene	0.6
sabinene	0.4
α -phellandrene	0.4
Viridiflorol	0.3

tomed flask with methanol (12.5 lts) for 12h. The extract was concentrated under reduced pressure at 50-60˚C till complete drying. The dried crude methanolic extract of the plant (yield 347 g, 21.68 %) was stored in a closed vessel at 4˚C.

b. Successive extraction

The methanolic extract (347 g) was subjected for Soxhlet extraction in round bottomed flask with petroleum ether (2.0 lts) for 12h. The extract was concentrated under reduced pressure at 50-60˚C till complete drying. The dried successive petroleum ether extract (yield 20 g, 5.76%) was stored in a closed vessel at 4˚C in a refrigerator till further use.

The petroleum ether extracted leaf powder was dried and once again subjected to Soxhlet extraction successively with different solvents viz, chloroform, ethyl acetate and methanol (2.0 lts each). The extracts were concentrated and stored as described above. Yields, successive chloroform extract, 38 g, 10.95%, successive ethyl acetate extract, 28 g, 8.06% and successive methanol extraction 54 g, 15.54%. The yields were calculated in percentage with reference to the air-dried drug. The successive extracts were stored in closed vessels at 4˚C in a refrigerator till further use.

Isolation of medicinal compound from Chloroform Fraction

I. Adsorbing the chloroform extract to Silica gel

1. Add about 5g of silica gel (for column chromatography 60-120 mesh) to the crude extract.
2. Gently heat the mixture using a hair dryer until the silica gel becomes free flowing.

II. Packing the Column for Chromatography

1. Take a cylindrical glass column and plug in a small piece of cotton.
2. Mount the column on the stand.
3. Take 25g of fresh silica gel (for column chromatography 60-120 mesh) in a 250-ml beaker.
4. Pour 100mL of petroleum ether into the beaker and stir well using a glass rod to make slurry of the silica.
5. Pour the slurry into the column
6. Place a conical flask below the mounted column and drain out the excess solvent (petroleum ether).
7. Close the stop cock when the level of the solvent reaches just above the settled silica gel.

III. Loading the Crude material on to the Column

1. Total 38 gm of Chloroform fraction was loaded in column.
2. Column runs with different solvents systems to collect fraction of that solvent with ratio of petroleum ether and chloroform (100, 75:25, 50:50 and 25:75).
3. Further continuation with ratio of Chloroform and Ethyl Acetate (100, 75:25, 50:50 and 25:75).
4. Further continuation with ratio of Ethyl Acetate and Acetone (100, 75:25, 50:50 and 25:75).
5. Further continuation with ratio of Acetone and Methanol (100, 75:25, 50:50 and 25:75).
6. Ratio of Chloroform and Ethyl Acetate fraction that is (100, 75:25, 50:50 and 25:75).
7. Elution was collected in a different beaker than dried and weighed.

IV. Washing of dried elute

1. The solid obtained in Chloroform and Ethyl Acetate (50:50) ratio washed with hexane to remove minor compounds.
2. Hexane insoluble compound was treated with charcoal to remove colour part.
3. Precipitation then filtered by using Whatman filter paper. The obtained solid dried compound was weighed (30 mg) and stored.

TLC Studies

Extract: 1mg/ml in Ethyl acetate

The sample was dissolved in Ethyl acetate and filtered before spotting the sample.

RESULTS AND DISCUSSION

Spectral report for the compound

The IR spectra showed characteristic absorption bands at 3421 cm^{-1} indicating the presence of a OH group, at 1691cm^{-1} for C=O group, 2848cm^{-1}

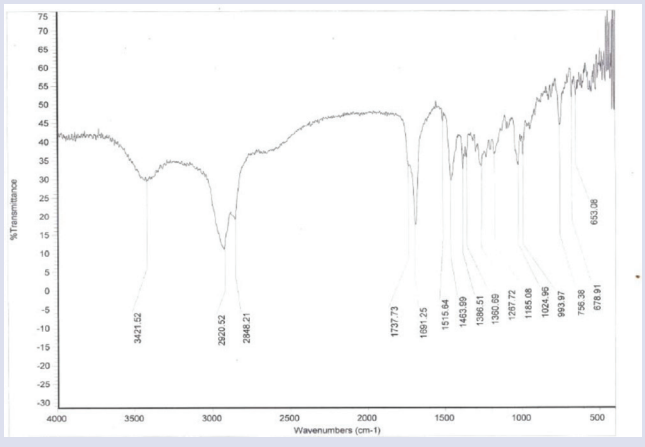

Figure 1: IR SPECTRA.

(stretching frequency) and at 1463 and at 1386cm-1 for C-H group (bending frequency) and at 1024 cm^{-1} for C-O group Figure 1.

The ^{1}H NMR spectra showed a triplet signal at δ 10.44, 9.48, 13.21 showing the presence of OH group and Carboxylic acid. The signal at δ 7.27 shows the presence of 2 Aromatic ring and signal at 3.71 shows the presence of 2 CH group Figure 2.

The ^{13}C NMR spectra showed signals at δ 16.28 for a methyl carbon, δ 29.89 for a methylene carbon, δ 49.27 for a methylene carbon under nitrogen function, a bunch of signals between 115.40 and 145.31 for aromatic carbon atoms.

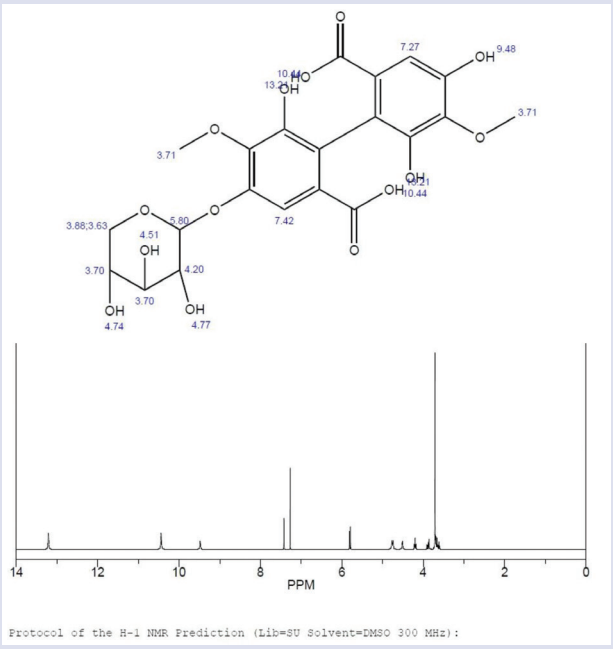

Figure 2: ChemNMR 1h Estimation.

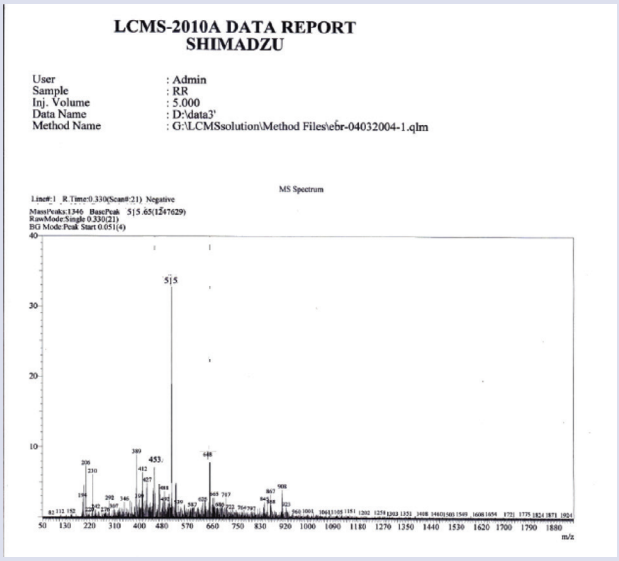

Figure 3: LC-MS SPECTRA.

The ESI-negative mode mass spectrum showed pseudo molecular ions at m/z 515 for [M+] ion. The ESI-positive mode mass spectrum showed pseudo molecular ions at m/z 515[M+Na] + ion suggesting a molecular weight of 515 Figure 3.

IR-3421 cm^{-1}, 1691cm^{-1}, 1024 cm^{-1}.

^{1}H NMR (300 MHz, DMSO-d6): δ = 10.44, 9.48, 13.21 (6OH, j=18,22,16,17.15,21), δ =7.27 (2CH J=12, 3), δ=3.71(2 CH3, J=24,26)

^{13}C NMR (126 MHz, DMSO-d6) δ = 114.3 (C-1), 140.9 (C-2), 141.8 (C-3), 151.4 (C-4), 111.9 (C-5), 112.8 (C-6), 158.4 (C-7), 111.8 (C-1), 141.6 (C-2), 140.4 (C-3), 153.0 (C-4), 111.8 (C-5), 110.6 (C-6), 158.5 (C-7), 61.6 (3-OCH3), 60.9 (3-OCH3), 101.4 (C-1"), 73.3 (C-2"), 76.5 (C-3"), 69.5 (C-4"), 77.3 (C-5"), 60.6 (C-6"). Đ ESI-IT-MS (positive ion mode, 40 V): m/z = 515.0805 [M+Na] + (100), calcd. 515.0801. Compound was identified as 3,3-di-O-methylellagic acid-4-O--d-glucopyranoside.

The structure of the isolated compound shown in Figure 4.

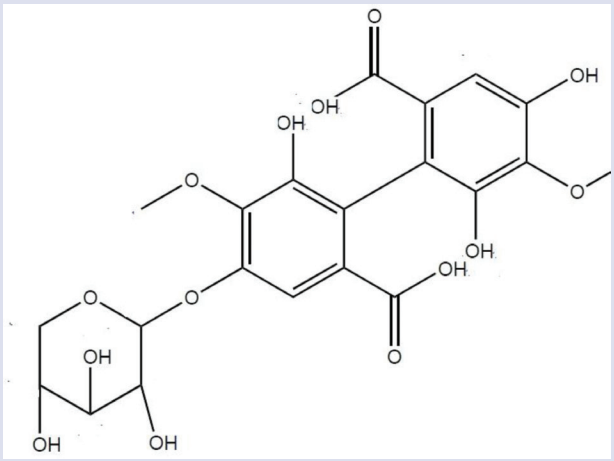

Figure 4: 3,3-di-O-methylellagic acid-4-O--d-glucopyranoside.

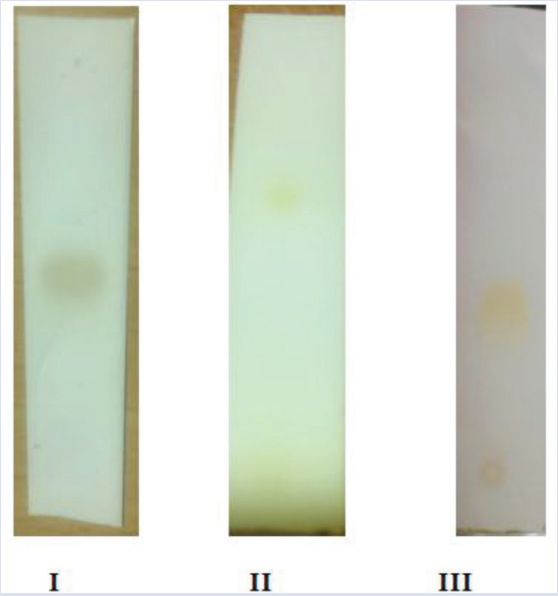

Figure 5: TLC Plates.

Tlc studies

The sample was dissolved in Ethyl acetate and filtered before spotting the sample. Figure 5.

Chamber Saturation time: 30 min.

Rf Value

Distance travelled by solute

Distance travelled by Solvent

Sl.No.	Solvent mobile phase	Ratio in ml	Rf Value
I	Chloroform: Acetone	4:1.5	0.48
II	Chloroform: Ethyl acetate	3.5:1.5	0.69
III	DiChloroform: Acetone	4.0:1.0	0.51

CONCLUSION

Based on spectral analysis the compounds were identified as 3,3-di-O-methylellagic acid-4-O--d-glucopyranoside portion with some little impurity.

CONFLICTS OF INTEREST

The authors are declared that there is no conflict of interest.

ABBREVIATIONS USED

NMR-Nuclear Magnetic Resonance Spectroscopy**, IR**-Infrared Spectroscopy**, LC-MS**-Liquid Chromatography-Mass Spectrometry.

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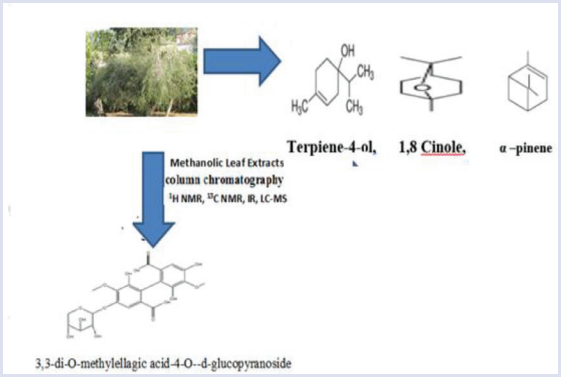
GRAPHICAL ABSTRACT



SUMMARY

The IR spectra showed characteristic absorption bands at 3421 cm-1 indicating the presence of a OH group, at 1691cm-1 for C=O group, 2848cm-1 (stretching frequency) and at 1463 and at 1386cm-1 for C-H group (bending frequency) and at 1024 cm-1 for C-O group. The 1H NMR spectra showed a triplet signal at δ 10.44, 9.48, 13.21 showing the presence of OH group and Carboxylic acid. The signal at δ 7.27 shows the presence of 2 Aromatic ring and signal at 3.71 shows the presence of 2 CH group. The ^{13}C NMR spectra showed signals at δ 16.28 for a methyl carbon, δ 29.89 for a methylene carbon, δ 49.27 for a methylene carbon under nitrogen function, a bunch of signals between 115.40 and 145.31 for aromatic carbon atoms. The ESI-negative mode mass spectrum showed pseudo molecular ions at m/z 515 for [M+] ion. The ESI-positive mode mass spectrum showed pseudo molecular ions at m/z 515[M+Na] + ion suggesting a molecular weight of 515. Based on spectral analysis the compounds were identified as 3,3-di-O-methylellagic acid-4-O--d-glucopyranoside portion with some little impurity.

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