GC-MS Analysis of Phyto-Constituents of the Essential Oil from the Leaves of *Melaleuca citrina* (Curtis) Dum.Cours.

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Abstract *Melaleuca citrina* (Curtis) Dum. Cours. commonly known as ‘red bottle brush’, is one of those medicinal plants with great medicinal importance. It is a beautiful evergreen tree belonging to the family Myrtaceae. It is indigenous to Queensland and New South Wales and cultivated throughout India in gardens. The plant is known in folk medicine for its anticough, antibronchitis, insecticidal effects and its volatile oil has been used as antimicrobial and antifungal agents. Moreover, aerial parts of *Melaleuca citrina* are practiced traditionally in ethnic tribal communities and very little are known about its importance on scientific grounds. The present study conducted to explore the Phyto-Constituents of the essential oil from the leaves of *Melaleuca citrina*. In the present analysis, the phyto-constituents of the leaves of essential oil of *Melaleuca citrina* by GC-MS analysis clearly showed the presence of 19 compounds. The results revealed that Eucalyptol (55.14%), Octadien-3-ol, 3,7-dimethyl- (11.48%), 1,5-Dimethyl-1-vinyl-4-hexenyl butyrate (11.48%), L-.alpha.-Terpineol (16.25%) and .alpha.-Terpineol (16.25%) were reported as 5 major components in the essential oil leaves of *Melaleuca citrina*. Eucalyptol is an ingredient in many brands of mouthwash and cough suppressant. Eucalyptol is an effective treatment for nonpurulent rhinosinusitis. Eucalyptol reduces inflammation and pain when applied topically. It kills leukaemia cells. The essential oils from leaves of *Melaleuca citrina* may serve as potent natural anti-cancer compounds with important roles in human health.

Keywords Phyto-Constituents, Leaves, Essential oil, Eucalyptol, Leukaemia

1. Introduction

Plants have been an important source of medicine for thousands of years. Use of plants for the treatment of many diseases dated back to prehistory and people of all continents have this old tradition. Every culture on earth has relied on the vast variety of natural chemistries’ found in plants for their therapeutic properties. Plants are known to produce a variety of compounds to protect themselves against a variety of pathogens. Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body [1].

Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases. A vast knowledge of how to use the plants against different illnesses may be expected to have accumulated in areas where the use of plants is still of great importance [2]. Plant synthesizes a wide variety of chemical compounds, which can be sorted by their chemical class, biosynthetic origin and functional groups into primary and secondary metabolites. Primary metabolites make up the physical integrity of the plant cell and are involved with the primary metabolite process of building and maintaining of living cells [3].

*Melaleuca citrina* (Curtis) Dum. Cours. commonly known as ‘red bottle brush’, is one of those medicinal plants with great medicinal importance. The name of the plant, Callistemon, is derived from Greek kalos meaning beautiful and stemon meaning stamens and citrinus from Latin citrinus meaning lemon, referring to the scent of the leaves. It is a beautiful evergreen tree belonging to the family Myrtaceae. It is indigenous to Queensland and New South Wales and cultivated throughout India in gardens.
The plant is commonly named as bottle brush because the cylindrical brush like flowers resembles traditional bottle brush. Different parts of the plant are used by rural people of India. The plant is known in folk medicine for its anticough, antibronchitis, insecticidal effects and its volatile oil has been used as antimicrobial and antifungal agents. Moreover, aerial parts of Melaleuca citrina are practiced traditionally in ethnic tribal communities and very little are known about its importance on scientific grounds.

The leaves of Melaleuca citrina were evergreen, aromatic, alternate, lanceolate with entire margin and anomocytic stomata. Stem was grey in colour. Phytochemical study on leaves reported the presence of flavonoids, alkaloids, terpenoids and steroids. As there is no detailed record of research work available on GC-MS analysis of Phyto-constituents of the essential oil from the leaves of M.citrina. This is the first report on the chemical composition of essential oil of leaves from this species. The aim of the present study is to analyse the bioactive compounds of the essential oil from the leaves of Melaleuca citrina.

2. Materials and Methods

2.1. Collection of Plant Materials

The fresh leaves of Melaleuca citrina were collected from the campus of Sri Kaliswari College (Autonomous), Sivakasi. The collected specimens were properly identified with the standard literature and authenticated with valid voucher specimens. The voucher specimens were deposited in the herbarium of Department of Botany, SKC.

2.2. Extraction of Essential Oil through Hydro-distillation Method

The chopped fresh leaves of Melaleuca citrina were subjected into Clevenger type apparatus by using hydrodistillation for about 2-3 hrs. The essential oils were extracted then, the oils were collected separately and dehydrated over anhydrous sodium sulfate and stored in air tight vials at 4°C for further study.

2.4. Phytochemical Analysis by GC-MS

GC-MS analysis of extracted essential oils were performed by using a Shimadzu GC-MS QP2010 Ultra model and Gas Chromatograph interfaced to a mass spectrometer (GC-MS) equipped with a Rxi-5Sil MS, fused silica capillary column (30 ml × 0.25 mm ID × 1× df, composed of 100% Dimethyl polysiloxane1,4-bis(dimethylsiloxy) phenylene dimethyl polysiloxane). For GC/MS detection, an electron ionization system with an ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1 ml/min and an injection volume of 1 µl was employed (Split ratio of 50:1) injector temperature 250°C; ionsource temperature 280°C. The oven temperature was programmed from 100°C (isothermal for 2 min) with an increase of 5°C/min to 200°C, then 10°C/min to 280°C, ending with a 2 min isothermal at 280°C. Mass spectra were taken at 70eV; a scan interval of 0.3 seconds and fragments from 40 to 800 Da total GC running time was 32 minutes and the software adopted to handle mass spectra and chromatograms was a Lab Solutions.

2.5. Identification of Phytochemical Components

The identity of the components in the extracts was assigned by the comparison of their retention indices and mass spectra fragmentation patterns with those stored on the computer library and also with published literatures. NIST08s.LIB, WILEY8. LIB library sources were used for matching the identified components from the essential oil.

3. Results and Discussion

Medicinal plant possesses many bioactive compounds including phenolic and polyphenolic compounds which play key function in detoxification of stress induced by free radicles and exhibit antimicrobial activities [4]. Plants act as important source for all treatments in traditional medical system of the world. The Herbal plants have been used for medical treatment since ancient times [5]. The plants are traditionally used to treat thorn or glass puncture wounds, abscesses [6], Scrofulosis, carbuncles, dysentery, Rheumatism, isthmus and crural aches, fractures, superficial infections, fingernail inflammation, tumor, difficult labour and pueral pain [7]

The GC-MS analysis of Phyto-constituents of the essential oil from the leaves of Melaleuca citrina is shown in the table 1. The GC-MS identification of the phyto-constituents was based on comparison of their mass spectra with NIST and WILEY libraries. Structures were defined by percentage similarity values. The identification of the chemical compounds was confirmed based on the peak area, retention time and mass spectrum. In this present analysis, the phyto-constituents of the leaves of essential oil of Melaleuca citrina by GC-MS analysis clearly showed the presence of 19 compounds. In GC-MS analysis, Melaleuca citrina showed 5 major and 14 minor compounds (Figure 1.).
Figure 1. GC-MS Chromatogram of Phyto-Constituents identified from essential oil of *Melaleuca citrina*
Table 1. List of Phyto-Constituents identified from essential oil of *Melaleuca citrina* through GC-MS analysis

| S.No. | RT  | Name of the Compounds                                      | Area % | Mass Spectrum |
|-------|-----|------------------------------------------------------------|--------|---------------|
| 1.    | 2.125| .beta.-Pinene                                              | 3.30   |               |
| 2.    | 2.125| Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-  | 3.30   |               |
| 3.    | 2.408| Eucalyptol                                                 | 55.14  |               |
| 4.    | 2.815| Octadien-3-ol, 3,7-dimethyl-                               | 11.48  |               |
Table 1 Continued

|   |   |                                                                 |     |
|---|---|------------------------------------------------------------------|-----|
| 5. | 2.815 | 1,5-Dimethyl-1-vinyl-4-hexenyl butyrate                           | 11.48 |
| 6. | 3.515 | Terpinen-4-ol                                                    | 3.39 |
| 7. | 3.515 | 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-              | 3.39 |
| 8. | 3.657 | L-α-Terpineol                                                    | 16.25 |
Table 1 Continued

|   |  |   |   |
|---|---|---|---|
| 9 | 3.657 | alpha-Terpineol | 16.25 |
| 10 | 7.467 | Benzene, 1,2,3-trimethoxy-5-(2-propenyl)- | 1.66 |
| 11 | 7.959 | (-)-Globulol | 3.56 |
| 12 | 7.959 | Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethyldene)-, (4aR-trans)- | 3.56 |
|   |   |                                                                                                      |   |
|---|---|-----------------------------------------------------------------------------------------------------|---|
|13.| 7.959 | Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1R-(1.alpha.,3a.beta.,4.alpha.,7.beta] | 3.56 |
|14.| 8.053 | 1H-Cycloprop[e]azulen-4-ol, decahydro-1,1,4,7-tetramethyl-[1aR (1a.alpha., 4.beta., 4a.beta., 7.alpha., 7a.beta)] | 2.55 |
|15.| 8.053 | Epiglobulol                                                                                         | 2.55 |
|16.| 8.053 | 1-Cycloheptene, 1,4-dimethyl-3-(2-methyl-1-propene-1-yl)-4-vinyl-                                   | 2.55 |
| RT  | RT   | Compounds                                                                 |
|-----|------|---------------------------------------------------------------------------|
| 8.365 | 2.66 | 1H-Indene, 1-ethylideneoctahydro-7a-methyl-,(1Z,3a,alpha.,7a.beta.)-         |
| 8.365 | 2.66 | 2-Naphthalenemethanol, decahydro-alpha..alpha..alpha..4a-trimethyl-8-methylene-, [2R-(2.alpha.,4a.alpha.,8a.beta.)- |
| 8.365 | 2.66 | 2-Naphthalenemethanol, 2,3,4,4a,5,6,7,8-octahydro-alpha..alpha..alpha..4a,8-tetramethyl-, [2R-(2.alpha.,4a.beta.)- |

RT - Retention Time
The results revealed that Eucalyptol (55.14%), Octadecane (7.80%), Palmitic acid (5.38%) and butyl hydroxy toluene (4.11%) and fokienol (3.37%), while for steam distillation were: 12-carboxyuedesma-3,11 (13)-dien (56.81%); 2,3-didehydrocosic acid (3.25%); butyl hydroxy toluene (2.63%) and pentacosane (2.31%). Seed oil of Garcinia xanthochymus composed of nine major fatty acids including myristic acid (0.11%), palmitic acid (32.96%), oleic acid (45.87%), stearic acid (0.96%), palmitoleic acid (17.65%), linoleic acid (1.93%), linolenic acid (0.34%), arachidic acid (0.07%) and behenic acid (0.07%). The oil also demonstrated for antimicrobial activity against gram positive bacteria [10].

[3] Manikandan et al., 2017 reported that the leaves of Petroleum ether extract of Millettia pegaunus by GC-MS analysis clearly showed the presence of 10 compounds. The results revealed that Pentadecane (32.73%), Tetradecane (29.79%) and Octadecane (22.77%) was reported as 3 major components in the Petroleum ether leaves extract of the Milletia pegaunus. The seven minor compounds such as Eicosane (7.23%), Undecane, 5-methyl- (2.13%), 9-methylheptadecane (1.40%), Sulfurous acid, dodecyl hexyl ester (1.13%), Heptadecane, 2,6,10-tetramethyl-(0.99%), 2-Bromodecane (0.96%) and Henecisone (0.87%) was also reported from leaves.

In this present study, High amount (55.14%) of Eucalyptol compound present in leaf essential oil in the Melaleuca citrina. Eucalyptol, 1,8 cineole, is an essential oil present in large amounts in a variety of plants which is frequently used in the manufacture of cosmetics, to increase percutaneous penetration of drugs, as a nasal decongestant and anticough agent, in aromatherapy, and in dentistry [11-14]. Eucalyptol is naturally produced cyclic ether and monoterpenoid. Eucalyptol is an ingredient in many brands of mouthwash and cough suppressant. It controls airway mucus hyper secretion and asthma via anti-inflammatory cytokine inhibition. Eucalyptol is an effective treatment for nonpurulent rhinosinusitis. Eucalyptol reduces inflammation and pain when applied topically. It kills leukaemia cells in vitro. The uses of identified phyto-constituents of leaf essential oil of the Melaleuca citrina are shown in table 2.
The growth of certain microorganisms and as antioxidant. This directed the study to think about the broad spectrum of microorganisms and antifungal activity are potent natural occurring antimicrobial agent against broad spectrum of microorganisms and antifungal activity and it has nematocidal activity and have good antioxidant activities. This directed the study to think about the influence of the volatile oil on the growth of certain microorganisms and as antioxidant.

[38] Manikandan et al., 2019 reported that, the GC-MS analysis has showed the different chemical constituents in the methanolic tuber extract of Morinda cymbalaria. A total of 23 chemical constituents were identified in the methanolic tuber extract of M. cymbalaria. At (16.98) retention time 2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethyl-terpineol, (–)-Globulol. Previous studies [15-33] on the composition of the oil from leaves of other Callistemon species showed that 1,8-cineole is the main component in those species between (45-80%), while B-pinene represents the major constituent in case of C. polandii, methyl eugenol in case of C. viridiflorus. [34-37] other authors observed that 1,8-cineole (53.03%) is the main component in Cc followed by eugenol (12.1%), methyl eugenol (8.3%), α-terpineol (4.3%) then carvone (3.4%) in Callistemon. It was interesting to know that Cc produces high amount of cineole, eugenol and methyl-eugenol that those compounds are potent naturally occurring antimicrobial agent against broad spectrum of microorganisms and antifungal activity and it has nematocidal activity and have good antioxidant activities. This directed the study to think about the influence of the volatile oil on the growth of certain microorganisms and as antioxidant.

Table 2. Uses of Phyto-Constituents identified from essential oil of Melaleuca citrina

| S.No. | Name of the Compounds | Uses |
|-------|-----------------------|------|
| 1.    | β-Pinene              | Anti-depressant, Antibacterial & Antimicrobial activity. |
| 2.    | Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene- (1S)- | Odor agents |
| 3.    | Eucalyptol            | Cosmetics, Treatment of Alopecia with Monoterpenoids |
| 4.    | Octadien-3-ol, 3,7-dimethyl- | Flavoring agents |
| 5.    | 1,5-Dimethyl-1-vinyl-4-hexenyl butyrate | Anti-parasitic, Anti-bacterial & Antimicrobial activities. |
| 6.    | Terpinen-4-ol         | Antimicrobial and antioxidant activities. |
| 7.    | 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)- | Cough suppressant, Anti-Infective Agents &Protective Agents |
| 8.    | L-α-alpha-Terpineol   | Disinfectants, antioxidants medicines and constituent of flavourings. |
| 9.    | α-alcohol-Terpineol   | Antiulcer, anticancer & antibronchitis activity. |
| 10.   | Benzene, 1,2,3-trimethoxy-5-(2-propenyl)- | Antioxidant effects, anti-inflammatory and antithrombotic effect. |
| 11.   | (–)-Globulol          | Antimicrobial and Anti-inflammatory. |
| 12.   | Naphthalene, decahydro-4α-methyl-1-methylene-7-(1-methylhexylidene)-, (4αR-trans) | Antimicrobial, Antioxidant and medicine of skin disease. |
| 13.   | Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylhexyl)-, [1R-(1a.alpha, 3a.beta, 4α.beta., 7𝛽.beta] - | Anti-inflammamatory and Anti-cancer. |
| 14.   | 1H-Cyclopren[ε]azulen-4-ol, decahydro-1,1,4,7-tetramethyl-[1αR (1α.alpha., 4β.beta., 4α.beta., 7α.beta., 7α.beta] - | Anti-proliferative activity and Antimicrobial activity. |
| 15.   | Epiglobulol           | Anti-perspirants or body deodorants. |
| 16.   | 1-Cycloheptene, 1,4-dimethyl-3-(2-methyl-1-propene-1-yl)-4-vinyl- | Colourings, flavours, etc added to food for human consumption. |
| 17.   | 1H-Indene, 1-ethylideneoctahydro-7α-methyl-1, (1Z,3a.alpha, 7α.beta) - | Antimicrobial and Antioxidant. |
| 18.   | 2-Naphthalenemethanol, decahydro-α-allo, alpha, 4α-trimethyl-8-methylene-, [2R-(2α.alph, 4α.alph, 8α.be] - | Anti-inflammamatory and medicine of skin disease. |
| 19.   | 2-Naphthalenemethanol, 2,3,4,4α,5,6,7,8-octahydro-α-allo, alpha, 4α,8-tetramethyl,-, [2R-(2α.alph, 4α.bet, 4α.alph, 8α.be] | Antimicrobial, ulcers and gastritis |
The current study concludes that the highly active phyto-constituents of Eucalyptol; Octadien-3-ol, 3,7-dimethyl-1,5-Dimethyl-1-vinyl-4-hexenyl butyrate; L-\(\text{\textalpha}\)-Terpineol and .alpha.-Terpineol present in the essential oil from leaves of *Melaleuca citrina*. It may be served as potent natural anti-cancer and anti-microbial compounds with important roles in human health and these compounds might be applicable in nature medicines.

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