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Volatile chemical profiling and antimicrobial activity of leaf essential oil in *Eugenia mooniana* Wight

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Abstract
Eugenia mooniana Wight. Is an aromatic plant with restricted distribution and native to India and Sri Lanka? The present work reports the volatile chemical composition of *E. mooniana* leaves and their antimicrobial activity against standard strain. The leaf essential oil was isolated by hydrodistillation method (oil yield 0.2%v/w), and analysed by GC-MS (Shimadzu Nexis GC 2030). Fourty four compounds were identified from the oil. The major class of compounds were sesquiterpenoids followed by monoterpenoids. Major compounds identified in the leaf oil were the monoterpenoids myrcene (2.8-%), β-ocimene (9.9%), and sesquiterpenoids caryophyllene (5.5%), δ-cadinene (12.1%), epi α-cadinol (7.5 %) and α-cadinol (12.9%). The antimicrobial activity oil was tested against bacteria and fungus strain by agar well diffusion method. The oil showed inhibitory activity against fungus *Candida albicans* and gram negative bacteria *Proteus vulgaris*, *Salmonella typhii*, *Staphylococcus aureus* and *Vibrio fluvialis*.

Keywords: *Eugenia mooniana*, Myrtaceae, GC-MS analysis, agar well diffusion method

1. Introduction
The genus *Eugenia* L., the second largest genus in Myrtaceae family, consist of about 1,100 species [1], which are mainly distributed in Brazil, Africa, South-East Asia and Malaysia [2, 3]. Duthie [4] documented 131 species for former British India covering the geographical and political boundaries of India, Pakistan, Afghanistan, Nepal, Tibet, Myanmar, Bangladesh, Malayan peninsula and Sri Lanka. In India, the genus is represented by 28 taxa (25 species and two varieties) and all are represented in the Western Ghats, of which 21 are endemics to the region [5]. Kerala, the southwestern state of Peninsular India, houses 18 taxa, of which *E. annamaleinsis* E.S.S.Kumar *et al*., *E. argentea* Bedd., *E.shettayana* Murugan & Gopalan and *E.terpnophylla* var. *keralensis* Shareef *et al*. and *E. kalamii* Shareef,*.et al***,** are strictly endemics to the state [6, 7, 8]. This genus closely allied to Syzygium and their generic delimitation, under long debate until Schmid [1972] differentiated them convincingly providing adequate morphological and anatomical characters [9].
Essential oil are typically liquid, clearly and unusually colored, complex and the present compounds are volatile, characterized by a strong odor and synthesized by aromatic plants during secondary metabolites, which act to protect the plant against microorganism and insects. They can be synthesized in several plant organs such as buds, flowers, leaves, stems, branches, seeds, berries, roots, wood or bark, being stored in secretory cells, cavities, epidermal cells or trichomes [10].
Many species of Eugenia have been reported antimicrobial activity in their essential oil [11, 12]. Eugenia species were characterized by abundance monoterpene and sesquiterpene [13, 14, 15, 16, 17]. α-Pinene and limonene have been reported as the major compound in the leaf oil of *Eugenia speciosa* from South Brazil [18]. Leaves of *E.uniflora* are used in folk medicine to lower blood glucose levels [19] and as an anti-febrile, anti-rheumatic, anti-inflammatory and against stomach diseases [20]. Essential oil analysis of *Eugenia bracteata* characterized by the sesquiterpene as the major compounds [21].
The aim of the study was to investigate volatile chemical constituent and antimicrobial activity of leaves essential oil of *E. mooniana* Wight. The oil of *E. mooniana* has not been subjected to previous study so this was the first report of gas chromatography mass spectrometry (GC-MS) analysis and antimicrobial activity in essential oil. *E. mooniana* is shrub to small tree, 5m; branchlets is puberulent and native to India and Sri Lanka [6].

2. Materials and methods

2.1 Plant material

Fresh leaves of *E.mooniana* (Fig .1) were collected from Ponmudi hills, Thiruvanathapuram, Kerala, India on February 2020. The plant material was identified by SM Shareef, Technical officer, Tropical Botanical Garden and research institute and voucher specimen (Neethu SS & Sivu 96704) was deposited at the herbarium of JNTBGRI (TBGT).

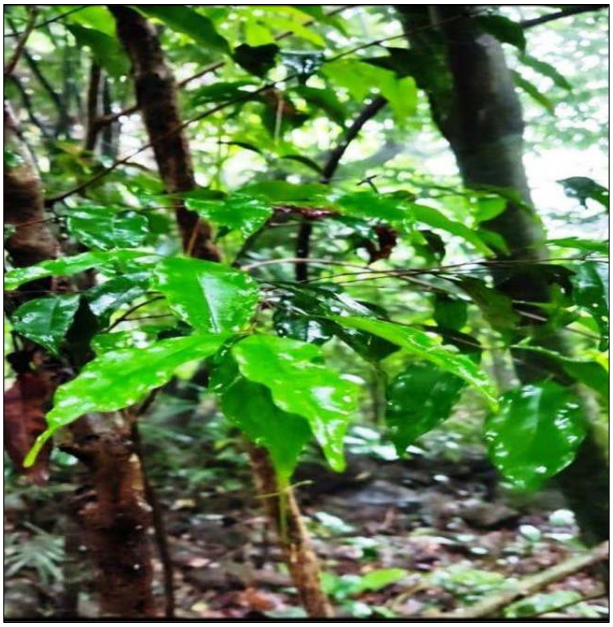

Fig 1: Habit of *Eugenia mooniana* Wight.

2.2 Essential oil isolation

The fresh leaves (300g) were cut in to small pieces and hydro distillation for 4 h using Clevenger type apparatus. The distillate was then dried over anhydrous sodium sulphate and kept in refrigerator at 4 °C until further analysis.

2.3 Essential oil analysis

The oils were analysed by Shimadzu Gas Chromatograph Mass Spectrometer (QP2020C NX) fitted with a Cross bond 1,4-bis (dimethylsiloxy) phenylene dimethyl polysiloxane Rxi-5 Sil MS capillary column (30 m x 0.32 mm, film thickness 0.25 µm) coupled with a single quadrupole 8030 series mass selective detector. Method- Injector temperature 240 °C in split mode, oven temperature 60-250 °C at a rate of 3 °C/minute. Mass detector-Ion source temp-240 °C, interphase temp-260 °C, solvent cut time 2.5 minute. The individual constituents were identified by MS library search (NIIST 17, Wiley 275) and by comparison of relative retention times and mass spectra of constituent published in literature reference [22]. Relative retention indices (RRI) of constituents were determined using n-alkanes as standards [23].

2.4 Antimicrobial activity

The anti-microbial activity was determined using the paper disc susceptibility test. Isolated endophytic bacterial strains were grown in 100ml nutrient broth (peptone: 0.5g; yeast extract: 0.5g and NaCl: 0.5g in 100ml distilled water) and incubated for five days in rotary shaker at 120 rpm. The culture media were centrifuged at 10,000rpm for 15 minutes. The supernatant of probiotic isolates were monitored for antibacterial activity against human pathogenic bacteria inoculated on nutrient agar. A total of 20µl of cell free supernatant was applied on 6mm diameter cellulosic disc on to the lawn of 200µl of indicator bacteria swabbed on nutrient agar along with Streptomycin as the standard antibiotic and control as nutrient broth. Six bacteria such as *Proteus vulgaris*, *Salmonella typhii*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Vibrio fluvialis* and fungus *Candida albicans* were used as indicator organisms. They are cultured in 10 ml nutrient broth and incubated for 18 hrs at 37 °C. The diameter of the inhibition clear zone was measured after 48 hrs of incubation at 30 °C.

3. Results and Discussion

3.1 GC-MS analysis of essential oil

Hydrodistilation of the fresh leaves of *Eugenia mooniana* afforded pale yellow colored oil and oil yiled 0.25% (v/w). Chromatogram of *Eugenia mooniana* is given below (Fig.2). The compositional profile of the essential oil isolated from the leaves of *Eugenia mooniana* resulted in the identification various type chemical constituents and the major compounds were terpenes. Leaf oil contained mainly monoterpene hydrocarbons, sesquiterpene hydrocarbon and oxygentated sesquiterpenes.

Using GC-MS system 44 components out of 50 were identified as listed in Table1.Essential oil analysis *E.mooniana* revealed the occurrence of sesquiterpene as the major compounds followed by monoterpene from the identified compounds. The major sesquiterpene were (above 5%) α- cadinol (18%), δ-cadinene (11%), Epi-α-cadinol (9%), α-Muurolene (6.9%), Caryophyllene (5%). These sesquiterpenes were grouped in accordance with the following biosynthetic pathways: cadinane, muurolane and caryophyllanae. Major Monoterpene hydrocarbon was β-Ocimene (9.1%). Occurence of these classes compounds have been reported from the essential oil of other Eugenia species also. Leaf oil *E.beaurepaireana* and *Eugenia pyriformis* were characterized by cadinane type family especially δ-cadinene and α-cadinol [24]. Myrcene, β- ocimene and α-phellandrene were reported from the leaf essential oil of *Eugenia rottleriana* [25]. Bicyclogermacrene and caryophyllene abundant in *E.florida* leaf oil [26].

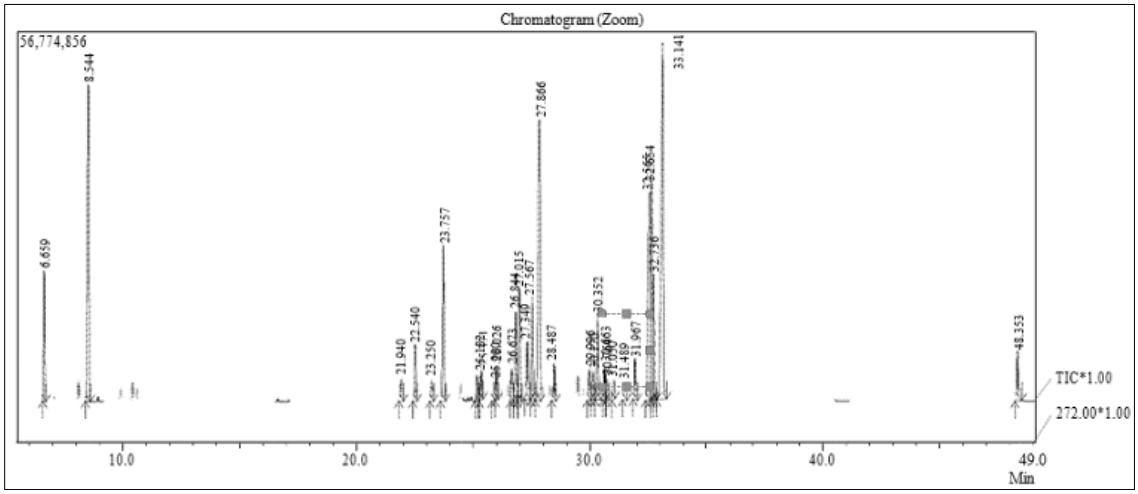

Fig 2: Chromatogram of GC-MS analysis.

Table 1: Chemical composition (%) of leaf oil of *Eugenia mooniana* Wight.

Sl. No.	RRI Cal.	RRI Lit.	Compound	Percentage
1	987	990	Myrcene	2.6
2	1002	1004	(3Z)-Hexenyl acetate	0.2
3	1006	1002	α –phellandrene	0.1
4	1027	1029	Limonene	0.1
5	1032	1037	β-ocimene	9.1
6	1055	1055	γ-terpinene	0.1
7	1084	1088	Terpinolene	0.3
8	1096	1098	Linalool	0.4
9	1372	1376	α-copane	0.6
10	1386	1390	β-elemene	1.5
11	1403	1409	α- gurjunene	0.6
12	1415	1419	E-Caryophyllene	5.6
13	1425	1433	β -gurjunene	0.2
14	1434	1441	Aromadendrene	0.5
15	1440	1444	6,9-guaiediene	0.1
16	1444	1450	Cis –Murrolene 1,5 diene	0.1
17	1450	1454	α-humulene	0.8
18	1454	1460	allo-Aromadendrene	0.9
19	1467	1475	γ -gurjunene	0.6
20	1470	1478	γ -muurolene	1.2
21	1476	1479	γ -amorphene	1.2
22	1479	1481	Amorpha-4,7(11)-diene	1.9
23	1483	1493	Cis- β-Guaiene	0.5
24	1490	1500	Bicyclogermacrene	0.5
25	1494	1500	α-murrolene	2
26	1502	1505	E-α-farnesene	3
27	1508	1513	γ-cadinene	2
28	1516	1523	δ-cadinene	3
29	1527	1529	Zonarene	11
30	1527	1534	trans-Cadina-1,4-diene	0.4
31	1531	1538	α -Cadinene	1
32	1557	1563	E-Nerolidol	0.9
33	1563	1568	Palustrol	0.9
34	1574	1583	Caryophyllene oxide	1
35	1579	1590	Globulol	3
36	1587	1592	viridiflorol	1
37	1590	1595	Cubeban-11-ol	0.6
38	1597	1602	Ledol	0.7
39	1599	1600	Rosifoliol	0.4
40	1608	1619	1,10-diepi-Cubenol	0.7
41	1627	1628	1-epi-Cubenol	1
42	1637	1640	epi –α- Cadinol	9
43	1641	1646	α-muurolol	3
44	1652	1652	α-cadinol	18

Caryophyllene and its derivative one of the most abundant compound in *E.mooniana* and it was reported as the common compound in leaf oil of many Eugenia species [27].

Analysis also revealed presence of terpenes like caryophyllene and α-humulene and it have anti- inflamattory, antibacterial, antioxident and natural wound healing effect were reported [28]. Some of the GC-MS peaks remained unidentified because lack of authentic sample and library data corresponding to compounds. The compounds with known medical properties were found in extract of leaf were caryophyllene, copane, cadinol, zonarene and β-Elemene [29, 30]. The abundance of linalool, globulol and virdiflorol in the essential oil is noteworthy and marked deviation from previous essential oil analysis of another species were reported.

3.2 Antimicrobial activity

Antibiotic commonly used for therapeutic purposes as well as antibiotic added to animal feedstuff for increasing animal flesh production, contribute to extensive spread of resistance. Many plants showed antimicrobial activity.

The study was made against five strains of Gram negative bacteria, one gram positive bacteria and a fungus strain. Preliminary antimicrobial activity of six endophytic bacteria estimated based on the clear zone production reveals that oil isolated from the leaves against tested bacteria was found to be positive except *Escherichia coli.* and *Enterococcus faecalis.* The antibiotic susceptibility profile of all strains is shown in table 2.

Table 2: Antimicrobial activity of oil

		Zone of inhibition (mm)		
		Streptomycin (+ve control)	**Diethyl ether (-ve control)**	**Oil sample**
Gram –ve Bacteria	*Proteus vulgaris*	18	NA	8
	Salmonella typhii	20	NA	8
	Staphylococcus aureus	15	NA	13
	Escherichia coli	8	NA	NA
	Vibrio fluvialis	12	NA	8
Gram +ve Bacteria	*Enterococcus faecalis*	10	NA	NA
Fungus	*Candida albicans*	NA	NA	16

Escherichia coli and *Enterococcus faecalis* showed high resistant capacity against oil as compared to another strains. *E. bracteata* have antimicrobial activity against *Enterococcus faecalis* has been repoted [4]. The presence zone of inhibition clearly revealed that the essential oil of *Eugenia mooniana* highly inhibited to gram negative bacteria except *E.coli* but antimicrobial activity *E.rottleriana* against *E.coli.* Has been reported [25].

Thus *Staphylococcus aureus* showed high susceptibility to leaves oil, which again confirm from the literature [31, 32]. So these study is very important for the treatment of infections caused by these bacteria; *S. aureus* is described as major causative of skin diseases and sometime pneumonia and its virulence and ability to acquire antimicrobial resistance results in a serious problem throughout the world for hospital and health professionals [33].

Proteus vulgaris is a Gram negative bacteria and it cause urinary tract, wound infection and diahhrea [34]. *Proteus vulgaris, Staphylococcus typhii, and Vibrio fluvialis* are clinically important strain and they are showed sensitivity to the leaf oil.

Candida albicans is the most prevalent fungal species of the human microbiota; this species asymptomatically colonizes many areas of the body, particularly the gastro intestinial and genitourinary tracts of healthy individuals [35]. According to this study we could find that *Candida albicans* have the great zone of inhibition (16 mm) as compared to other strains to be studied.

Essential oil contain complex mixture of components and thus have multiple antimicrobial properties; most of this action appears to derive from oxygenated Terpenoids particularly phenolic terpene, phenylpropanoids and alcohols; others constituents e.g. hydrocarbon that typically showed low activities, can be used in combination to increases their bioactivities [36].

As a typical lipophilic compound, essential oil cross the cell wall and cytoplasmic membrane and cytotoxic activity appears to be linked to disruption of the structures of the different layers of polysaccharides, fatty acids and phospholipids, due to mechanism of action that hits multiple targets at the same time [31]. Permeability, composition and charge of the outer structures of the microorganism mainly determined these difference; the lipophilic character of terpene is associated with the antimicrobial mechanism [37]. The antimicrobial mechanism involved with linalool is related to its high water solubility and to its ability to penetrate the cell wall [38]. One hypothesis is that linalool has the potential act as either a protein denaturing agent or as a solvent dehydrating agent, which may also contribute to its antimicrobial activity [39]. Antimicrobial effect of an essential depends on all of its chemical components [40].

4. Conclusion

Extensive documentation on the antimicrobial properties of essential oils and their constituents has been carried out by several workers. Although the mechanism of action of few essential oil components has been elucidated in many pioneering works in the past, detailed knowledge of most of the compounds and their mechanism of action is still lacking. This study important for the determination of the effect of essential oil of *E.mooniana* on different organism, how they work in combination with other antimicrobial compound and how it will used as medicine in future life. Thus we concluded as *Escherichia coli* and *Enterococcus faecalis* were highly resistant on essential oil, while *Staphylococcus aureus and candida albicans* strains were highly sensitive. According to this study essential oil of *Eugenia mooniana* have the ability to inhibit the growth of both bacteria and fungus. This type of GC-MS analysis and anti microbial study is the first step towards understanding the nature of active compound present in it and it will be helpful for further detailed study. Further investigations in to the pharmacological importance of *Eugenia mooniana* will add as new knowledge to the information in medical system.

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