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CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF LEAF ESSENTIAL OIL OF *EUGENIA COTINIFOLIA* SSP. *CODYENSIS* (MUNRO EX WIGHT) ASHTON

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ABSTRACT: The study was carried out to investigate the essential oil composition and antibacterial effects of *Eugenia cotinifolia* ssp. codyensis leaves. The fresh leaf essential oil was extracted hydrodistillation process using Clevanger apparatus. The compounds of the essential oil were analyzed using Gas Chromatography Mass Spectrometry (GC-MS) technique identified a total of 84 numbers of chemical constituents and resulted 99.99%. The essential oil was characterized by high content of sesquiterpene compounds (79.23%) and the major constituent being Germacrene D (17.95%). The leaf oil exhibited higher antibacterial effects against gram negative bacteria compare to gram positive bacteria. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of the oil against the organism ranged between 0.25 – 0.75 mg/ml and 0.5 – 2.5 mg/ml respectively. The essential oil exhibited maximum inhibition at 0.25 mg/ml against *Proteus vulgaris* and the same inhibited *Bacillus subtilis* and *Staphylococcus aureus* at 0.75 mg/ml. The result of essential oil chemical constituents and antibacterial activity showed useful for preparation of commercial products.

INTRODUCTION: *Eugenia cotinifolia* ssp. *codyensis* is one of the species belonging to family Myrtaceae and has been categorized as an endangered tree species under the International Union for Conservation of Nature (IUCN) red list of threatened species[1]. This subspecies is known only from a single collection in the south of Karnataka. An additional collection, imprecisely located in the Nilgiris, and a record from the Agastyamalai hills has been reported. Moreover, the species is endemic to India [1].

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Eugenia species exhibits antidiabetic and antiulcer [2], anti-inflammatory [3], xanthine oxidase inhibitiory [4], antibacterial [5] activity activities and also reduces blood pressure [6].

Literature survey revealed that no study on essential oil of this species has been conducted thus far. In this context, the present effort is to evaluate the chemical composition and antibacterial activity of the leaf essential oil of *Eugenia cotinifolia* ssp. *codyensis*.

MATERIALS AND METHODS:

Collection of plant material: Fresh leaf materials were collected from 75 years old tree at Chembra, Wayanad District, Kerala and specimens collected and further confirmed by Dr P. Sujanapal, Kerala Forest Research Institute (KFRI), Peechi, Kerala

and the voucher specimens (KFRI 23374) deposited at the KFRI herbarium.

Extraction of essential oil:

Five hundred grams of fresh leaves coarsely chopped and subjected to hydrodistillation for 4 h using a Clevenger apparatus[7]. Distilled essential oil was dried over anhydrous sodium sulfate and stored at 4°C.

Gas Chromatography-Mass Spectrometry analysis:

GC-MS analyses were conducted using Agilent MSD (5975B-inert XL MSD) apparatus equipped with National Institute of Standards and Technology (NIST) reference libraries; column DB-5MS (J&W Scientific) cross-linked fused-silica capillary column (30 m × 0.25 mm × 0.25 µm thickness), coated with 5% phenyl-polymethylsiloxane; column temperature, 80°C for 0 min, rising to 150°C at 10°C/min, then 250°C at 5°C/min, then rising to 270°C at 20°C held for 6 min. injector temperature 270°C, injection mode, split; split ratio 1:20; volume injected, 2 µL of the oil. Helium was used as a carrier; interface temperature 270°C; acquisition mass range, m/z 55-550. The compounds of the oil were identified by comparing their retention indices (RI), with NIST library. Relative Retention Indices (RRI) of lower and higher homologue was obtained from standard hydrocarbon data calculated as:

$$\text{Number of carbon atoms} \times 100 + \frac{\text{Retention time of the compound} - \text{Retention time of lower homologue}}{\text{Retention time of the higher homologue} - \text{Retention time of lower homologue}}$$

Microbial isolates:

Four Gram negative (*Pseudomonas aeroginosa, Proteus vulgaris, Klebsiella pneumoniae* and *Escherishia coli*), two Gram positive (*Staphylococcus aureus* and *Bacillus subtilis*) bacterial strains were used in this study. The bacterial stock culture were maintained on nutrient agar medium stored at 4°C.

Antibacterial assay:

The oil was tested for their antibacterial activity using disc diffusion method. Bacterial species were sub-cultured on nutrient agar medium which were then incubated at 37°C for 24 h. Test solutions of essential oil at concentrations of 1000 µg, 500 µg, 250 µg, 100 µg/ml were impregnated on sterile discs. Ampicillin were used as positive control. The disc impregnated with Dimethyl sulfoxide (DMSO) was used as negative control. The discs were placed on the surface of the nutrient agar for bacteria and incubated at 37°C for 24 h. Inhibition zones were calculated as the difference between disc diameter (6 mm) and the diameters of inhibition [8]. Antibacterial activities were evaluated by determining MIC using micro broth dilution assay [9].

TABLE 1: ESSENTIAL OIL CHEMCIAL COMPOSITION OF LEAF OIL

S. No.	RRI*	Compound	Area (%)
1	919	Nonanal	0.01
2	950	β-Cyclocitral	0.01
3	961	3-Heptylacrolein	0.01
4	974	3,7-Dimethylocta-1,3,7-triene	0.03
5	979	Alloaromadendrene	2.13
6	985	*cis*-Thujopsene	0.37
7	987	3,3,7,7-Tetramethyl-5-(2-methyl-1-propenyl)tricyclo[4.1.0.0(2,4)]heptane	0.18
8	997	Germacrene D	17.95
9	1007	β-Caryophyllene	11.46
10	1026	β-Patchoulene	1.55
11	1029	Calarene	1.77
12	1030	*cis*-α-bisabolene	4.97
13	1035	1,2,3,5,6,7,8,8a-Octahydro-1-methyl-6-methylene-4-(1-methylethyl)- Naphthalene	1.04
14	1037	4,9-Cadinadiene	9.42
15	1044	γ-Muurolene	8.14
16	1048	Seychellene	1.73
17	1049	α-Gurjunene	0.82
18	1052	δ-Cadinene	6.82
19	1058	Epizonarene	1.21
20	1060	1,4-Cadinadiene	2.71

S. No.	RRI*	Compound	Area (%)
21	1064	Eudesma-3,7(11)-diene	0.53
22	1066	Eremophilene	0.75
23	1069	β-Farnesene	0.85
24	1069	2,3,5,6,7,8,9,9a-Octahydro-5,5,9-trimethyl-3-methylene- (9S-trans)- 1H-Benzocycloheptene	0.35
25	1075	Octahydro-4,8,8,9-tetramethyl-1,4-Methanoazulen-7(1H)-one	0.14
26	1078	Spathulenol	0.67
27	1079	3,4,4a,5,8,8a-Hexahydro-8a-methyl- trans-1(2H)-naphthalenone	0.57
28	1081	α-Ylangene	0.28
29	1082	3,4,4-Trimethyl-3-(3-oxo-but-1-enyl)-bicyclo[4.1.0]heptan-2-one	0.11
30	1085	1s,4R,7R,11R-8Hydroxy-1,3,4,7-tetramethyltricyclo[5.3.1.0(4,11)]undec-2-ene	0.50
31	1088	Aromadendrene	0.11
32	1089	Epiglobulol	0.16
33	1092	8,9-Dehydro-cycloisolongifolene	0.23
34	1105	T-Muurolol	2.39
35	1111	α-Cadinol	2.06
36	1116	4-Methylene-5-methyl-methyl ester 6-heptenoic acid	0.23
37	1122	4-Hydroxyindole-3-carboxylic acid	0.12
38	1124	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol	0.14
39	1127	1,2,3,4,4a,8a-Hexahydro-alpha, alpha, 4a,8-tetramethyl-[2R-(2.alpha, 4a. alpha, 8a. alpha)]-2-naphthalenemethanol	0.07
40	1130	Cycloisolongifolol	0.06
41	1134	2-Phenyl-1-pentene	0.25
42	1142	2,6-Dimethylpyridine N-oxide	0.03
43	1145	1,1,6-Trimethyltetralin	0.02
44	1147	Humulen-(v1)	0.02
45	1149	Tricycle[3.3.3.0(1,5)]undec-6-ene-2,3,6-tricarbonitrile	0.15
46	1157	9,10-Dehydro-isolongifolene	0.10
47	1167	Alloaromadendrene	0.03
48	1185	2-Benzoyl-N-(3,4-dichlorophenyl)-benzamide	13.38
49	1190	(3-Chloro-5,5-dimethyl-2-cyclohexen-1-ylidene)-(E)-acetonitrile	0.07
50	1192	1-Methyl-1-(2,4,6-trimethoxyphenyl)-2-propanone	0.12
51	1196	N,N'-1,2-Ethanediylidenebis[2,4-dimethyl-3-pentanamine	0.06
52	1200	5-Dodecyne	0.13
53	1204	*trans*-Nuciferol	0.06
54	1207	N-Methyl-N-methoxy-5,6,7,8,-tetrahydro-1-naphtamide	0.04
55	1216	δ-Selinene	0.02
56	1222	4-Aminostilbene	0.24
57	1235	2-Isopropyl-5-methyl-9-methylene-bicyclo[4.4.0]dec-1-ene	0.03
58	1237	1-Acetyl-tetrahydropyrrol[2,3-b]-1H-2,3-dihydroindole	0.02
59	1243	2-Methylhexadecanoic acid methyl ester	0.01
60	1281	1-(1,3a,4,5,6,7-Hexahydro-4-hydroxy-3,8-dimethylazulen-5-yl)ethanone	0.01
61	1293	Aspidinol	0.01
62	1318	Benzal-P-Toluidine	0.03
63	1470	14-Methyl-5α-cholest-8-ene-3β,6α-diol	0.01
64	1477	3,5-Dinitrobenzoyl chloride	0.11
65	1481	2-Amino-4-ethylthiomethyl-6-morpholino-1,3,5-triazine	0.05
66	1485	5.Beta-iodomethyl-1.beta-isopropenyl-4.alpha., 5.alpha.-dimethyl-6.beta.bicyclo[4.3.0]nonane	0.07
67	1488	Vinbarbital	0.05
68	1492	Cycloartenol Acetate	0.03
69	1493	1,4-Dimethyl-9H-carbazole	0.04
70	1497	4-Chloro-N-[2-phenyl-2-(phenylamino)ethyl]- Benzenesulfonamide	0.07
71	1505	Ledene	0.16
72	1509	4,5,5a,6,6a,6b-Hexahydro-4,4,6b-trimethyl-2-(1-methylethenyl)- 2H-Cyclopropa[g]benzofuran	0.18
73	1512	N,o-bis(3-cyclopentylpropionyl)-methyl ester1-serine	0.31
74	1514	4α,14-Dimethyl-9β,19-cyclo-5α-ergost-24(28)-en-3β-ol acetate	0.35
75	1521	Decahydro-3,3,4,7a-tetramethyl-1H-cyclopenta[a]pentalen-7-ol acetate	0.20
76	1526	1-Isopropenyl-4,5-dimethylbicyclo[4.3.0]nonan-5-ylmethyl phenyl sulfoxide	0.13

S. No.	RRI*	Compound	Area (%)
77	1529	3-(4-Aminophenyl)-2-(4-nitrophenyl)-2-propenenitrile	0.34
78	1536	2,3,3a,4-Tetrahydro-3,3a,6-trimethyl-1-isopropyl-1H-indene	0.17
79	1543	2,7-Dimethyloct-7-en-5yn-4-yl ester-2-furoic acid	0.04
80	1546	Valencene	0.04
81	1554	9-Octylhexacosane	0.06
82	1557	5-Bromo-4,6-dimethyl-2(1H)-Pyrimidinone	0.07
83	1585	3-(Hexahydro-1H-azepin-1-yl)-1,1-dioxide-1,2-benzisothiazole	0.02
84	1624	n-Octacosane	0.01

Monoterpene hydrocarbon	0.05
Sesquiterpene hydrocarbon	79.23
Alkanes hydrocarbon	0.09
Others	20.62

*RRI value calculated from DB-5MS column

Germacrene D, a sesquiterpenoid compound that has deterrent effects against herbivores and also reported possess insecticidal activity against mosquitoes [10] and repellant activity against aphids [11] and ticks [12]. The compound is also suggested to serve as biogenetic precursor to a number of different sequiterpenoid skeletons [13, 14]. Essential oil containing large concentration of Germacrene D is reported to be accompanied by the formation of cadinene and muurolene sequiterpenoids [15-19] which has also been proved from our study in *Eugenia cotinifolia* ssp. *codyensis*.

Antibacterial activity:

The major compounds dominant in the leaf oil are sequiterpenoid compounds that make up 79.23% of the total oil. Sequiterpenoid compounds are known to possess high medicinal properties [20-23] that may make this tree species a highly potent medicinal plant against various diseases. The leaf oil possesses significant antibacterial effects against the tested pathogenic organisms (**Table 2**).

TABLE 2: ANTIBACTERIAL ACTIVITY OF LEAF ESSENTIAL OIL

Microorganisms	Zone of inhibition (mm)			
	Leaf oil (mg/ml)			Ampicillin
	5	15	25	(10µg)
Escherichia coli	9	13	14	11.2
Pseudomonas aeruginosa	7	10	11	14.3
Klebsiella pneumonia	5	6	8	12.1
Proteus vulgaris	10	13	16	17.9
Staphylococcus aureus	3	4	7	10
Bacillus subtilis	3	5	6	16

Both gram positive and gram negative bacteria were found to be sensitive against the leaf oil. Among the screened micro organism, the oil showed higher activity against *Proteus vulgaris* and moderate activity against *Bacillus subtilis* and *Staphylococcus aureus*.

The oil exhibited MIC values against the organism in the range of 0.25 - 0.75 mg/ml and MBC values in the range of 0.5 - 2.5 mg/ml (**Table 3**). The essential oil showed maximum inhibition at 0.25 mg/ml against *Proteus vulgaris* whereas the same oil inhibits the growth of *Escherichia coli* at 0.3 mg/ml, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* at 0.5 mg/ml. It is also observed that gram positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) were inhibited at higher concentration of 0.75 mg/ml of the sample. The leaf oil is effective towards *P. aeruginosa*, *E. coli*, and *K. pneumoniae* compared to other *Syzygium* species like, *S. alternifolium* and *S. samarangense* [24].

TABLE 3: MIC AND MBC VALUE FOR LEAF ESSENTIAL OIL

Micro organisms	Leaf oil	
	MIC mg/ml	MBC mg/ml
Escherichia coli	0.3	0.75
Pseudomonas aeruginosa	0.5	1.25
Klebsiella pneumonia	0.5	1.5
Proteus vulgaris	0.25	0.5
Staphylococcus aureus	0.75	2.5
Bacillus subtilis	0.75	2

CONCLUSIONS: The leaf essential oil of *Eugenia cotinifolia* ssp. *Codyensis* has higher activity against gram negative bacteria and the essential oil can be utilized for such antibacterial formulations and applications.

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