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CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF THE ESSENTIAL OIL FROM THE ROOTS OF *COLEUS VETTIVEROIDES K.C. JACOB*

A.Saraswathy*[1] and Mercy Lavanya S[2]

[1] Director, [2] SRF, Department of Microbiology, Captain Srinivasa Murti Drug Research Institute for Ayurveda, Anna Hospital Campus, Arumbakkam, Chennai- 600 106.

***Corresponding Author Email: csmdria@gmail.com**

ABSTRACT

The root of the plant Coleus vettiveroides Linn. (Lamiaceae), known as kuruver in Tamil, is used in Ayurveda and Siddha systems of medicine. The plant is cultivated at Kollidam, a village at Nagai District. The oil from the root of the plant was investigated to determine the possible chemical constituents present through GC-MS analysis. This led to the identification of thirty six compounds. The analysis also revealed that the plant contains mainly androstan-17-one, 3-ethyl-3-hydroxy-, (5α)- (25%) and -(-) spathulenol (9%). The other compounds were found to be α- bisabolol (7%), Z-valerenyl acetate (7%), megastigma-4,6(E),8(Z)-triene (6%), 1H-cycloprop(E)azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene- (5%), myrtenol (2%), 1-naphthalenol (2%), caryophyllene oxide (2%), abieta-9(11),8(14),12-trien-12-ol (2%). All other compounds were found to be less than 2%. The oil was also estimated for antibacterial activity against 8 ATCC strains and 5 clinical isolates and found to inhibit 4 ATCC cultures and 3 clinical isolates 400µg/well concentration.

KEY WORDS

Essential oil, Clevenger apparatus, GC-MS, Antibacterial activity, MIC

INTRODUCTION

Medicinal plants have become the focus of intense study and traditional medicine based on plants has played a key role in the health care systems of many countries like India and China[1]. The family Lamiaceae has over 3000 species to its credit[2], most of which are used for treating ailments. Flavonoids, glycosides, phenolic compounds and volatile constituents have been reported as the major phytoconstituents of the *Coleus* species. There are a number of reports available on *C. cromaticus* and *C. forskohlii* while sporadic reports are there on the other species, *C. vettiveroides*[3]. The plant *C.vettiveroides* is a small succulent herb 45-53 cm high with a procumbent stem and thick, purplish, pubescent leaves. The plant bears long (35-50 cm) fibrous roots which are straw coloured and strongly aromatic when fresh. The plant is grown under cultivation and is propagated by vegetative cuttings. The roots are harvested when the plants are 4 months old. The fresh fragrant roots are used for decorating temples and as hair dressings[4]. The plant is bitter, cooling, diuretic, trichogenous and antipyretic. It is useful in hyperdipsia, vitiated conditions of pitta burning sensation, strangury, leprosy, skin diseases, leucoderma, fever, vomiting, diarrhea and ulcers[5]. The world wide interest on medicinal plants makes the validation of traditional claims regarding health care mandatory and the development of microbial resistance to the available antibiotics further makes the search for newer antibiotics from medicinal plants a necessity. The essential oil from the roots of this plant was subjected to chemical analysis through Gas chromatography – mass Spectroscopy and the antibacterial property of the oil was also studied against both ATCC cultures and clinical isolates.

MATERIALS AND METHODS

(i) Plant Material

The roots of *Coleus vettiveroides* were collected from Kollidam, Tamil Nadu, and identified by the botanist of Captain Srinivasa Murti Drug Research Institute for Ayurveda. The plant was washed to remove all debris and was shade dried. The dried plant material was made to a coarse powder.

(ii) Extraction of Oil

The dried root sample of *C.Vettiveroides* (100 g) was subjected to hydrodistillation using Clevenger apparatus[6] for 4 hr. The oil was dried over anhydrous sodium sulphate and weighed. The procedure was performed in triplicate and the mean value for the yield was recorded.

(iii) Identification of the compounds

Essential oil components were identified by gas chromatography – mass spectrometry via peak matching and by utilizing their retention indices on VF-5ms column. GC was run at column oven temperature 70˚C; injector temperature 240˚C; injection mode split; split ratio 10; flow control mode linear velocity; column flow 1.51 ml/min; carrier gas helium 99.9995% purity; injection volume 1 microlitre. Computer matching against commercial libraries (NIST08, WILEY8) were utilized in the characterization of the constituents.

(iv) Antibacterial assay

The oil was tested against 13 strains of bacteria, 8 typed strains and 5 clinical isolates. The typed strains *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* NCIM 2106, *Bacillus subtilis* ATCC 6633, *Klebsiella pneumoniae* NCIM 2957, *Proteus vulgaris* ATCC 9484 and *Proteus mirabilis* NCIM 2388 were procured from National Chemical Laboratory, Pune and maintained by serial subculturing on to Nutrient agar slants. The clinical isolates *Escherichia coli*, *Staphylococcus aureus*, *Proteus mirabilis*, methicillin sensitive *Staphylococcus aureus* and methicillin resistant *Staphylococcus aureus* were obtained from a clinical laboratory. Well cut method[7] was employed. Norfloxacin 10 mcg was used as standard for Gram negative organisms and gentamicin 10 mcg was used as standard for Gram positive organisms. Dimethyl sulphoxide (DMSO) was used as vehicle. 40 mg of oil was mixed with 1000 µl of 10% DMSO. The organisms were inoculated as lawn culture on Mueller Hinton agar plates and well of 6 mm was cut equidistant with sterile plunger. 10 µl, and 20 µl of oil in DMSO were transferred into the wells. 10µl of 10% DMSO was added to one well as negative control. The plates were incubated at 37°C for 24 hrs and the zone of inhibition was measured in mm. MIC was determined for the organisms that were found to be sensitive to the oil. Minimal Inhibitory Concentration was determined using broth dilution technique[8]. To 4 tubes containing 2 ml of the broth, 10 µl of 18 hour old culture of the test organism was added. The oil was added in four different concentrations 8 µl (320µg), 4 µl (160µg), 2 µl(80µg) and 1 µl(40µg) into the tubes. The tubes were incubated at 37°C for 24 hrs. Turbidity was checked and compared with the control tube that contained only the broth and the oil. Sub-culturing was also made from the different concentrations on to sterile Mueller Hinton agar plates. The plate was incubated at 37°C for 24 hrs and observed for growth if any. The lowest concentration that had no growth was recorded[9].

RESULTS

The volatile oil constituents along with their retention time, percentage area and super impossability data obtained from GC-MS analyzer are given in **Table 1**. The results of the antibacterial study are given in **Table 2** and **3**. The chromatogram obtained is given as **Figure 1**.

Figure 1: GC of the essential oil from the roots of *C.Vettiveroides*

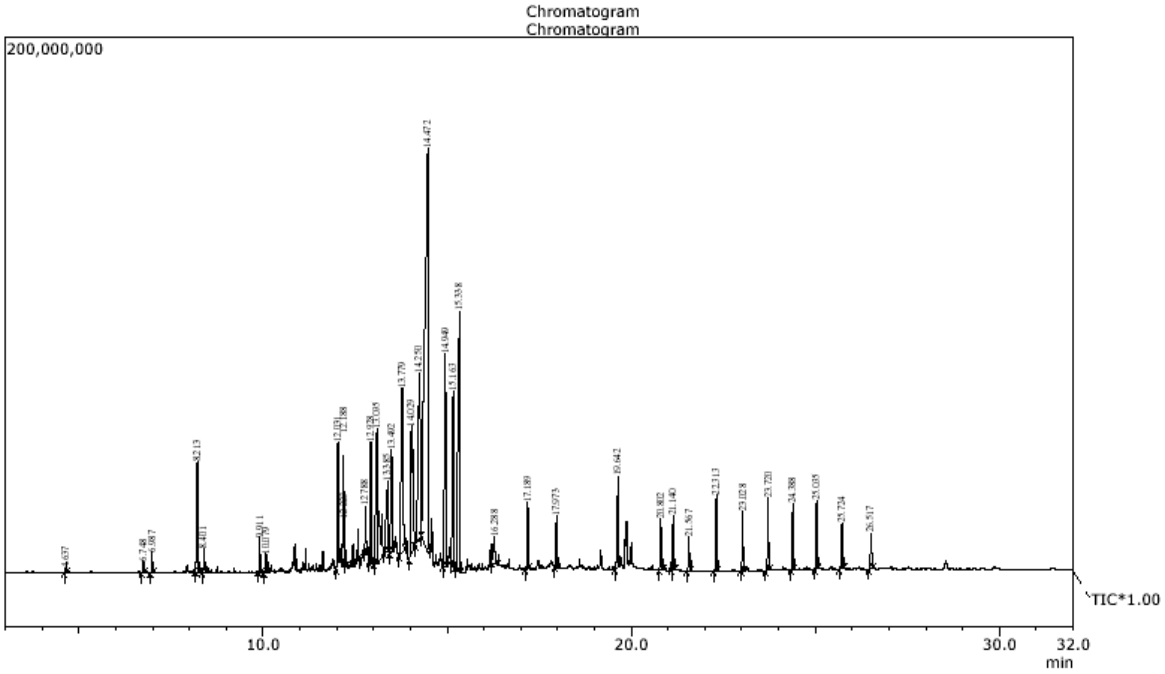

Table 1: GC-MS data of the essential oil from the roots of *C.Vettiveroides*

S.No.	Compounds	Retention Time (min)	Area %	SI %
1.	6-Ethyl-2-methyloctane	4.637	0.07	94
2.	(4-Isopropylphenyl)methanol	6.748	0.17	97
3.	Myrtenol	6.987	0.32	96
4.	Bicyclo[3.1.1.]Hept-2-ene-2-methanol	8.213	2.16	91
5.	Cuminic alcohol	8.401	0.37	98
6.	Myrtenyl acetate	9.911	0.51	91
7.	Trans(*β*)-caryophyllene	10.079	0.38	97
8.	1-Naphthalenol	12.031	2.19	88
9.	Caryophyllene oxide	12.188	2.00	95
10.	(-)-Globulol	12.223	0.57	95
11.	(-)-*δ*-Cadinol	12.788	0.77	79
12.	1,7,7-Trimethy,-acetate	12.928	2.27	75
13.	3-cyclohexane-1-methanol	13.095	4.44	82
14.	*α*– Bisabolol	13.385	2.49	95
15.	Azulene	13.492	2.64	84
16.	Megastigma-4,6(*E*),8(*Z*)-triene;	13.779	5.84	78
17.	1H-Cycloprop(*E*)azulene,decahydro-1,1,7-trimethyl-4-methylene-	14.029	5.97	88
18.	*Z*-Valerenyl acetate	14.250	7.20	84
19.	Androstan-17-one, 3-ethyl-3-hydroxy- (5α)-	14.472	24.69	86
20.	1H-cycloprop(e)azulen-7-ol	14.949	5.20	79
21.	3-Isopropyltricyclo undec-3-en-10-ol	15.163	9.03	72
22.	(-)-Spathulenol	15.338	9.03	81
23.	*n*-Hexadecanoicacid	16.288	0.98	93

24.	7-Isopropyl-1,1,4a-trimethyl-1,2,3,4,4a,9,10,10a-octahydrophenenthrene	17.189	1.19	90
25.	8.*β* –podocarpan-8-ol	17.973	0.88	76
26.	Abieta-9(11),8(14),12-trien-12-ol	19.642	2.26	89
27.	Celidoniol	20.802	0.99	95
28.	1,2-Benzenedicarboxylic acid	21.140	0.95	96
29.	Nonacosane	21.567	0.61	95
30.	Tetracosane	22.313	1.43	96
31.	*n*-Hexatriacontane	23.028	1.10	96
32.	Octacosane	23.720	1.34	96
33.	Tetratiacontane	24.388	1.25	95
34.	Tetrapentacontane	25.035	1.29	95
35.	Tetrateracontane	25.724	0.97	95
36.	*n*-Tritriacontane	26.517	0.83	95

Table 2: Antibacterial activity of the oil of *C.Vettiveroides* roots

S.No.	Organism	Zone of Inhibition		
		Std	10µl	20 µl
Typed Cultures				
1.	*Bacillus subtilis* ATCC 6633	25	-	-
2.	*Bacillus cereus* NCIM 2106	34	-	-
3.	*Escherichia coli* ATCC 25922	27	-	-
4.	*Proteus vulgaris* ATCC 9484	35	-	-
5.	*Proteus mirabilis* NCIM 2388	32	-	15
6.	*Klebsiella pneumonia* NCIM 2957	25	16	20
7.	*Staphylococcus aureus* ATCC 25923	26	17	20
8.	*Pseudomonas aeruginosa* ATCC 27853	27	14	17
Clinical Isolates				
9.	*Staphylococcus aureus*	38	25	26
10.	*Escherichia coli*	23	-	-
11.	*Proteus mirabilis*	16	-	-
12.	MSSA	22	15	20
13.	MRSA	26	16	18

Table 3: Minimal Inhibitory Concentration of oil of *C.Vettiveroides*

S.No.	Organism	Minimal Inhibitory Concentration			
		4 µl/ml	2 µl/ml	1 µl/ml	0.5 µl/ml
1.	*Klebsiella pneumonia* NCIM 2957	-	+	+	+
2.	*Staphylococcus aureus* ATCC 25923	-	-	-	+
3.	*Pseudomonas aeruginosa* ATCC 27853	-	+	+	+
4.	*Staphylococcus aureus*	-	-	-	-
5.	MSSA	-	-	+	+
6.	MRSA	+	+	+	+

+ Growth; - No growth

DISCUSSIONS

The essential oil obtained from root of *Coleus vettiveroides* (1.9%) was found to be brightly coloured (orange red) and was highly viscous with a pleasant odour. Thirty six compounds were characterized in the oil through GC-MS analysis. The oil was rich in oxygenated hydrocarbons and saturated hydrocarbons, including aldehydes, ketones and hydroxy groups. The major compound was found to be Androstan-17-one, 3-ethyl-3-hydroxy-, (5α) - (25%) followed by - (-) spathulenol (9%). Androstan-17-one, 3-ethyl-3-hydroxy-, (5α)- has been reported to be neuroactive, analgesic and anesthetic and the major constituent of the ethanolic extract of the leaf of *Andrographis paniculata* (Burm.f.) [10]. Spathulenol has been reported to possess immunomodulatory activity[11]. The other compounds were found to be α-bisabolol (7%), *Z*-valerenyl acetate (7%), megastigma -4, 6(*E*),8(*Z*)-triene (6%), 1H-cycloprop(*E*)azulen-7-ol, decahydro - 1, 1, 7 - trimethyl- 4 - methylene - (5%), myrtenol (2%), 1-naphathalenol (2%), caryophyllene oxide (2%), abieta-9 (11), 8(14),12-trien-12-ol (2%). All other compounds were found to be less than 2%. The plants belonging to the genus has been earlier studied for their antimicrobial activity. The root and leaves of *Coleus amboinicus* has been reported to posses antibacterial activity against both Gram positive and Gram negative bacteria[12]. The ethanolic extract of the roots of *Coleus forskohlii* has been reported to possess both antibacterial and antifungal activity[13]. The alcohol and water extracts of the leaves of *Coleus aromaticus* also have been reported to possess an appreciable amount of antibacterial activity against mainly gram negative bacteria[14]. The antibacterial activity of the essential oil against 8 typed cultures viz., *Bacillus subtilis* ATCC 6633, *Bacillus cereus* NCIM 2106, *Escherichia coli* ATCC 25922, *Proteus vulgaris* ATCC 9484, *Proteus mirabilis* NCIM 2388, *Klebsiella pneumonia* NCIM 2957, *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853 and against 5 clinical isolates viz., *Staphylococcus aureus* from sputum, *Escherichia coli* from semen, *Proteus mirabilis* from pus, MSSA from pus, MRSA from sputum showed that the oil was found to be active against 4 ATCC cultures and 3 clinical isolates viz., *Proteus mirabilis* NCIM 2388, *Klebsiella pneumonia* NCIM 2957, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus*, MSSA, MRSA. The oil demonstrated profound activity against all the strains of *S. aureus* tested including methicillin resistant *S.aureus*. The oil also showed appreciable inhibition on the growth of *Pseudomonas aeruginosa*. Further *K.pneumoniae* was also found to be inhibited. There existed a dose dependant response, the inhibition increased with increase in concentration of the oil tested. MIC study carried out using four varying concentrations against *Klebsiella pneumonia* NCIM 2957 and *Pseudomonas aeruginosa* ATCC 27853 revealed that the oil was active concentration was found to be 320µg/ml against both the organisms. The MIC of MSSA, *Staphylococcus aureus* ATCC 25923 and *Staphylococcus aureus* was found to be 160 µg/ml, 80 µg/ml and 40 µg/ml respectively. The MIC for MRSA tested was found to be more than 320 µg/ml. The plant possesses biologically active chemical constituents and potent antibacterial property.

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***Corresponding Author:**
Dr. A. Saraswathy,
Director,
CSMDRIA
Chennai – 600 106