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Antimicrobial efficacy and phytochemical constituents of essential oil on Coleus zeylanicus (benth.) L.H.Cramer - a valuable medicinal plant

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ABSTRACT

Coleus zeylanicus belongs to the family Lamiaceae and it is medicinally important aromatic herb. The genus Coleus contains a number of medicinally important compounds such as volatile oils, alkaloids, flavonoids, saponins and fatty acids. The present study describes the antimicrobial activity of Coleus zeylanicus against various pathogenic bacteria and fungi. The essential oil from 100g of leaves of the plant was extracted by hydro distillation using Clevenger type apparatus for 2-3 hours. The essential oil thus extracted was analyzed by GC-MS analysis for the first time. The essential oil was subjected to antibacterial tests, using disc diffusion method. In the GC-MS analysis totally sixty two components were identified of which γ-Selinene (11.77%), Geroniol (11.71%), Valencene/Eremophyllene (5.98%), m-cymene (4.59%), Ledol (4.35%), α-Bergamotene (3.61%), 10S,11S Himachala-3(12) 4-diene (3.0%), α-Cypenene (2.74%), Fencyl acetate (2.62%), α-Pinene (2.57%), Azulene (2.25%), β-Bisabolene (2.25%), 2-Camphonyl acetate (2.08%) were found to be the major phytocomponents. The oil revealed a weak to strong antibacterial activity against Pseudomonas fluorescence, Pseudomonas aeruginosa, Micrococus luteus, Escherichia coli, Staphylococcus aureus, Salmonella typhi and Bacillus cereus. The oil was also tested for its antifungal property against two fungal strains Aspergillus niger and Candida albicans. The highest antifungal activity was found by Candida albicans.

Key words: *Coleus zeylanicus, essential oil (EO), GC-MS, antimicrobial activity, phyto component*

INTRODUCTION

Medicinal plants play a vital role from ancient to modern medicine. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi and herbivorous mammals. Medicinal plants are the reservoirs of potentially useful chemical compounds which

could serve as newer leads and clues for modern drug design. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, phenols and volatile compounds. The phytochemical constituents and its specific activity are exploited to treat various health ailments and chronic diseases as well [1]. Chemical compounds in plants mediate their effect on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs; thus herbal medicines do not differ greatly from conventional drugs in terms of how they work. This enables herbal medicines to have beneficial pharmacology.

Coleus zeylanicus is an important medicinal plant of Lamiaceae, which contains about 300 species found in tropical and subtropical regions of Asia, Africa, Australia, Madagascar, India and the Pacific islands. C. zeylanicus is cultivated on the river banks and sandy loams of Tamil Nadu and Kerala. In Kerala (India), this species is widely cultivated as a pot herb in homestead gardens. The genus Coleus comprises a number of herbaceous medicinal plants which are particularly employed in home remedies for various ailments. The active constituents contributing to these protecting effects are naturally occurring phytochemicals, vitamins and minerals that give plants their color and flavor. This plant contains number of medicinally important compounds such as Alkaloids, tannins, flavonoids, phenols, volatile compounds and bioactivities like anti-inflammatory, anti-thrombotic, anti-oxidative and anti-carcinogenic activities play a major role in preventing number of chronic diseases in human beings [2]. The essential oils of Lamiaceae are regarded as Mother Nature's chemical factory, which are non-toxic have shown good fighting potential against drug resistance pathogens [3,4]. In traditional medicinal practice, this plant leaves are used as a single drug remedy for children's cough and cold. This species is reported as an endemic taxon of Sri Lanka, where it is known as Iruveriya in Sinhalese. The juice of stem and leaves of the plant is mixed with honey and taken as a remedy for diarrhea [5]. Many members of the family are widely cultivated not just for their aromatic qualities but also due to their ease of cultivation. This plant especially is among the easiest plants to propagate by stem cuttings [6]. To our knowledge there is no report available on Coleus zeylanicus plant species. In the present work, we have, for the first time, for phytochemical analysis and antimicrobial activity of leaf essential for further research on the biochemical composition and medicinal importance of this valuable medicinal herb.

MATERIALS AND METHODS

Plant material

The source plant material of *Coleus zeylanicus* was collected from Agro Biotech Industries Pvt. Ltd., in Kerala. They were then cultivated for the study by vegetative propagation. The plant was allowed to grow for one to two months and then its leaves were harvested to facilitate the extraction of essential oils.

Extraction of essential oil

The extraction of essential oil was carried out by the technique of hydro distillation on a Clevenger type apparatus [7]. 100 g of *Coleus* plant fresh leaves was distillated in 200ml of water. The plant extraction time was about 2-3 hrs. Three repetitions were performed to standardize the protocol. The oil obtained was yellowish in color and liquid at room temperature. The oil recovered was dried over anhydrous sodium sulphate and kept in the refrigerator at 4°C before use. The oil extracted had a pleasant odor of the plant [8].

Gas chromatography mass spectrometry (GC-MS) analysis

The oils were analyzed by capillary GC-MS by using a Perkin Elmer 8500 instrument with a FID and a capillary column Chrompack CP-WAX-58CB, 25 mx0.32 mm, film thickness 0.2 mm. Running conditions were: initial column temperature, 50°C; temperature rise rate, 3°C/min to 190°C and 60 min hold; injector temperature, 250°C; detector temperature, 270°C; helium flow rate, 20 cm/s. Identification of the major components of the oils was obtained by GC/MS using a Hewlett-Packard 5890 Gas chromatograph equipped with a fused silica capillary column Supelcowax 10 (30 m x 0.25 mm internal diameter ; 0.25 mm film thickness) connected to a Mass Selective Detector 5971A (Hewlett-Packard). Operating conditions were: temperature program, 40°C for 5 min, 40°C–240°C at 2.5°C/min and 15 min hold; injector temperature, 250°C; transfer line temperature, 240°C; carrier gas, helium; head pressure, 8 psi; linear flow rate, 34 cm/s at 100°C; injection volume, 2 ml; split ratio 1:30; electron voltage, 70 eV; temperature of ion source, 150°C; scanning range, 40–450 *m/z*; scan speed, 1.8 scan/s. Components were first identified comparing their retention index with that of available authentic standards. Further identification was achieved by GC/MS Wiley and NIST Library, Hewlett-Packard PBM search [9,10].

Microbial strains and culture medium

The test microorganism gram positive (*Staphylococcus aureus, Micrococcus luteus and Bacillus cereus*), gram negative (*Pseudomonas fluorescence, Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi) and two fungal strains (Aspergillus niger and Candida albicans)* were collected from culture repository of culture collection centre, Chennai, India. The organisms were inoculated onto nutrient agar medium for bacterial cultures and Sabourauds Dextrose Agar (SDA) medium for fungal cultures and incubated at 37°C for 24 hrs [11].

Antimicrobial activity by disc diffusion method

The study of antimicrobial activity was performed on the gram positive bacteria: *Micrococcus luteus, Staphylococcus aureus and Bacillus cereus* and on the gram negative bacteria: *Pseudomonas fluorescence, Pseudomonas aeruginosa, Escherichia coli and Salmonella typhi* by agar diffusion method (Kirby-Bauer disk susceptibility test). Hi-media sterile discs of size 5mm was used for

loading the essential oil. Lawn culture of the respective bacteria were made in different petri plates with Mueller-Hinton agar medium and the discs impregnated with 10 µl of the essential oil was placed on it. The petri plates were incubated at 37°C for 24 hrs. After incubation the diameters of zone of inhibition were measured. Similarly the study of anti-fungal activity was performed on two fungal strains: *Aspergillus niger* and *Candida albicans* [12].

The tested bacteria are pathogenic and are known for their invasiveness and toxicity to humans. They are frequently encountered in many infections like food borne illness (*Salmonella typhi*), intestinal infections and opportunistic pathogen *(Escherichia coli*), as a contaminant in sick patients (*Micrococcus luteus*), skin and endovascular infections (*Staphylococcus aureus*), nosocomial infections (*Staphylococcus and Pseudomonas*). Some bacteria are also responsible of food alteration (*Bacillus sp.*).The fungi that is been tested is also known to cause food contamination (*Aspergillus niger*) and an opportunistic oral and genital tract infections (*Candida albicans*). The present study on isolation of components of *Coleus zeylanicus* would be useful as a source of drug in chemotherapy to cure such routine infections.

Statistical analysis

The percentage of response, antimicrobial activity indicated by zone of growth inhibition was monitored as growth parameters. Data of three independent experiments represented by 3 replicates from each experiment were subjected to statistical analysis (mean ± SE), according to New Duncan's Multiple Range Test [13].

RESULT AND DISCUSSION

Yield of essential oil

A yield of 0.5% of essential oil has been obtained by hydro distillation of leaves of *Coleus zeylanicus*. The formula used for the calculation of essential oil yield is:

Essential oils yield (%) = W_1 / W_2 X 100

W1 = Net weight of oils (grams)

W2 = Total weight of fresh leaves (grams)

GC-MS analysis

The dried leaves of *Coleus zeylanicus* yielded 0.5% of a pale yellowish oil with a characteristic odor. The qualitative and quantitative comparison of both essential oils is reported (Table 1) Around 62 components were identified in the dried leaves of *Coleus* plant. The main compounds identified include Geroniol/lemanol (11.71), Geraniol (7.16), Eremophylene/Valencene (5.98), m-cymene (4.59), Ledol (4.35), α-Bergamotene (3.61), Fenchyl acetate (2.62), α-Pinene (2.57).

Table 1: GC-MS analysis of *Coleus zeylanicus.*

Peak	Compound Name	Retention Time (Min)	Area (%)
1	α-Thujene	15.57	0.17
2	α-Pinene	15.81	2.57
3	Camphene	16.17	0.67
4	β-myrcene	16.41	0.15
5	β-Pinene	16.61	0.29
6	α-Phellandrene	16.88	1.54
7	1,3-cyclohexadiene	17.02	0.11
8	m-cymene	17.23	4.59
9	β-Phellandrene	17.31	1.36
10	Eucalyptol	17.40	0.02
11	Linalool	17.96	1.55
12	o-Isoropenyltoluene	18.12	0.14
13	Fenchol	18.58	0.65
14	Cis-p-2-menthen-1-ol	18.82	0.17
15	Fenchyl acetate	19.38	2.62
16	Borneol	19.43	1.39
17	Terpinolene	19.54	0.38
18	Fenchyl acetate	19.79	1.14
19	α-Copaen-11-ol	19.84	0.24
20	1,4-cyclohexadiene,1-methyl-	19.90	0.29
21	Geroniol/lemanol	20.09	11.71
22	2-camphonyl acetate/ isobornyl formate	20.22	0.55
23	β-Myrcene	20.33	0.16
24	Citral	20.65	0.25

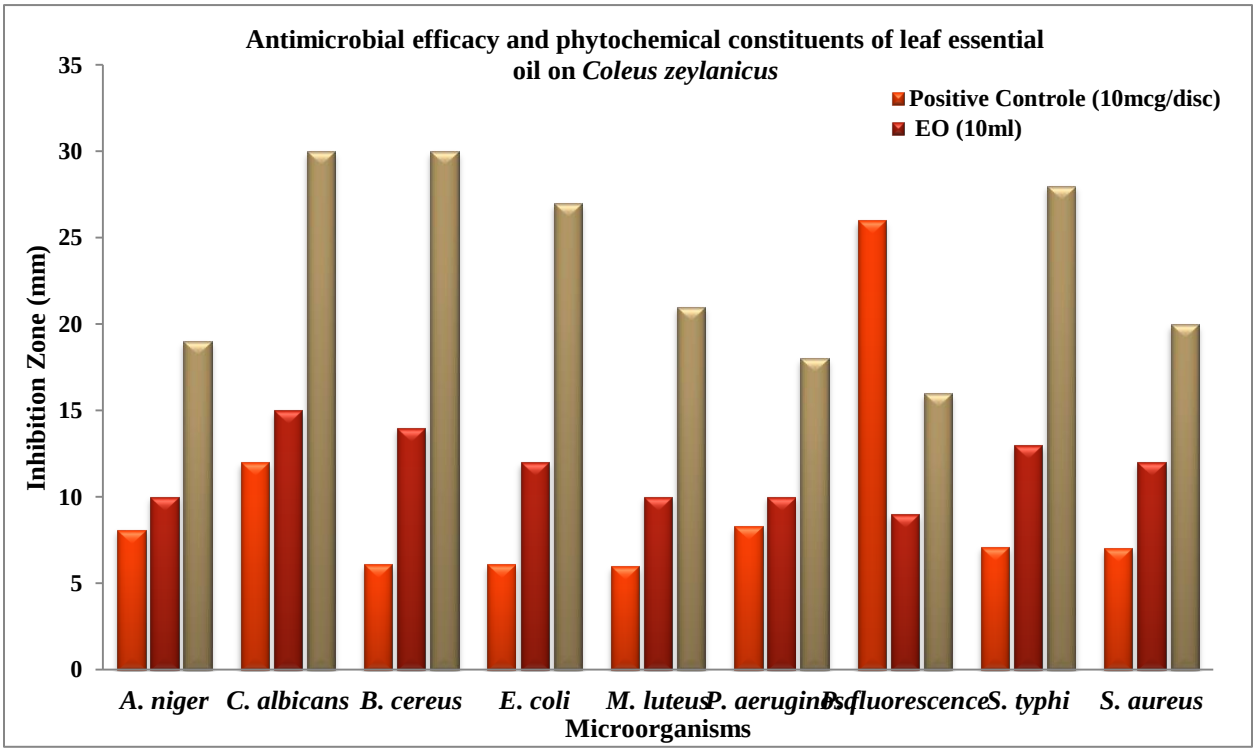
25	2-camphonyl acetate/ isobornyl formate	20.87	2.08
26	Carvacrol	21.08	0.27
27	Cuminol	21.19	0.20
28	Copaene	21.59	0.02
29	Geraniol	21.85	7.16
30	α-Cedrene	22.02	0.52
31	3,4-dimethoxystyrene	22.31	0.20
32	β-Elemene	22.45	0.25
33	α-Bergamotene	22.77	0.29
34	Tricyclo [2.2.1.0(2.6)]heptanes,1,7	22.92	0.86
35	(Z)-β-Farnesene	23.02	0.77
36	α-Bergamotene	23.14	3.61
37	Caryophyllene	23.28	0.29
38	Azulene	23.50	2.25
39	Cedrene	23.71	0.10
40	Armadendrene	23.92	0.28
41	α-Cypenene	24.02	2.74
42	α-Himachala	24.18	0.16
43	α-curcumene	24.31	1.91
44	α-caryophyllene	24.40	1.81
45	β-Patchoulene	24.58	1.33
46	β-Bisabolene	24.74	2.25
47	Neoisolongifolene	24.91	1.47
48	γ-Selinene	25.17	11.77
49	Eremophylene/Valencene	25.32	5.98

50	7-epi-α-silenene	25.43	1.60
51	10s,11s-himachala-3(12),4-diene	25.66	3.00
52	Thujopsene	25.89	0.65
53	bicyclo[4.4.0]dec-1-ene,2-isoprop	26.20	1.69
54	1H-cyclopropa[a]naphthalene, decah	26.57	0.23
55	β-panasinsene	26.68	0.29
56	Naphthalene,1,2-dihydro-1,1,6-tri	26.98	0.53
57	γ-Maaliene	27.43	0.35
58	Trimethylsilyl acetylene/TMSA	27.84	0.99
59	3,4-Pentadienal,2,2-dimethyl	28.20	1.71
60	Spathulenol	28.45	0.87
61	Ledol	29.06	4.35
62	Globulol	29.40	0.68

Antimicrobial activity

In the present study, the antimicrobial activity of essential oil extracted from *Coleus zeylanicus* against gram positive, gram negative *bacteria and fungi* was performed by disc diffusion method. The diameter of growth inhibition zones was measured. The results showed that the leaf oil extracts possessed strong antibacterial and antifungal activity. We found that the highest antibacterial activity was found in *Bacillus cereus* with a zone of inhibition of (14mm) followed by *Salmonella typhi* with (13mm) zone of inhibition. It could also be noted from the result that the antifungal activity of essential oil of *Coleus zeylanicus* is the highest as in *Candida albicans* with a zone of inhibition of (15mm). The overall activity results from our experiments indicated that the essential oil has strong inhibitory activity against *Bacillus cereus, Salmonella typhi, Staphylococcus aureus and Escherichia coli*. The essential oil also revealed an extraordinary inhibitory effect against the fungi *Candida albicans* and the activity is thus depicted in (Figure 1).

Figure 1: Antimicrobial efficacy and phytochemical constituents of leaf essential oil on *Coleus zeylanicus*.



Coleus zeylanicus is an important ornamental and medicinal plant. It is a valuable addition in medicinal plants biodiversity. This plant contains several medicinal components and high medicinal activity like, colic, congestive heart failure, convulsions, eczema, hypertension, insomnia, painful urination and respiratory disorders [14]. In the present study essential oil extracted from *Coleus zeylanicus* has a comparatively low yield of 0.5% ml/g of 100g of dried leaves, while *Coleus amboinicus* was reported to yield an average of 0.89% [12]. It is thus clear that different plants containing essential oils have different yield. The GC-MS analysis revealed the presence of constituents of essential oil of the plant. As it is reported genus *Coleus*, the main components which characterized were γ-Selinene (11.77%), Geroniol (11.71%), valencene/Eremophyllene (5.98%), m-cymene (4.59%), Ledol (4.35%), α-Bergamotene (3.61%),10S,11S Himachala-3(12) 4-diene (3.0%), α-Cypenene (2.74%), Fencyl acetate (2.62%), α-Pinene (2.57%), Azulene (2.25%), β-Bisabolene (2.25%), 2-Camphonyl acetate (2.08%) out of the sixty two components fractioned.

Many phytochemicals viz. terpenoids, tannins, flavonoids, phlobatannins, saponins and cardiac glycosides were reported in to the species of *Coleus* [15]**.** The gas chromatogram of *Coleus forskohlii* showed a total of 30 compounds out of which Bornyl acetate (Acetic acid isobornyl ester; endo-1, 7, 7-Trimethylbicyclo [2.2.1] hept-2-yl acetate) was found in high quantity. The essential oil composition of *Coleus aromaticus* was examined by capillary GC and GC-MS analyses revealed the presence of 28

constituents, of which 16 were identified. Thymol (83.39%) was found to be the major compound. However, according to the results of the present study, γ-selinene (11.77%) and Geroniol (11.71%) were found to be the major component of the essential oil of *Coleus zeylanicus*. Selinenes are a group of closely related isomeric chemical compounds which are classified as sesquiterpenes. Geraniol is a monoterpenoid and an alcohol which is of great importance in environmental and food industry. The high content of polyphenols can account for the use of this plant in traditional medicine [16]. The presence of β-ionone, α-humulene in *C. laniniatus* and β-thujone, α-farnesene has been reported in *Coleus parviflorus* [2].

This study also throws light on the antimicrobial activity of essential oil of *Coleus zeylanicus*. In general maximum activity was observed against gram positive bacteria when compared to that of gram negative bacteria. The antimicrobial activity of EO has been studied by many authors. The location or mechanisms thought to be sites of action for EO components are: degradation of the cell wall, damage to cytoplasm membrane, damage to membrane proteins, leakage of cell contents, and coagulation of cytoplasm and depletion of the proton motive force [17]. Essential oil of *Coleus* plant is proven to have great anti-microbial activities against various microorganisms. Similar type of activity is proven in *Coleus aromaticus* which is found to be effective against *Candida albicans*, but showed comparatively low anti-microbial activities against *Pseudomonas aeruginosa, Bacillus subtilis, Escherichia coli, Staphylococcus aureus and Aspergillus niger* [18]. *Coleus aromaticus* has been reported of highest anti-microbial activity against *Salmonella typhi* with a zone of inhibition of 11mm and 14mm in its ethanolic and methanolic extracts respectively [19]. Antifungal activity was reported to be in many species of *Coleus*, as in *C. forskohlii, C. blumei* and *C. barbatus* by testing against pathogenic fungi like *A. niger, A. fumigates, A. ruantii, Proteus vulgaris* and *Candida albicans*. The study showed that extract of *C. forskohlii* and *C. barbatus* exhibited significant antifungal activity against all selected organisms while extract of *C. blumei* did not show any significant effect [20]. Thus the present study, with a comparative analysis of the antimicrobial activity of leaf essential oil in *Coleus zeylanicus* plant species, could prove itself to be a potent medicinal aid for treatment of various enteric pathogens.

This research work on phytochemical analysis of *Coleus zeylanicus* and its antimicrobial activity is first of its kind. The essential oil showed a strong inhibitory effect on enteric pathogens like *Salmonella typhi* and also a greater zone of inhibition of 14mm against genito urinary tract infection causing fungi *Candida albicans*. The encouraging result proves itself as a potent antimicrobial agent especially against enteric pathogens and also against *Candida* infections. The future research of the work could be to purify and isolate the compound that is responsible for the inhibition of the micro organism and develop it into a potential herbal product. This work will have importance in the field of pharmaceutical and cosmetic industry which will be cost effective and there will be no side effects by the usage of this valuable medicinal plant.

CONCLUSION

The present study demonstrates that *Coleus zeylanicus* EO has moderate activity against bacterial and fungal strains tested, with different degrees of effectiveness. Our findings suggest feasibility of application of this essential oil in treatment of the infections caused by microorganisms.

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