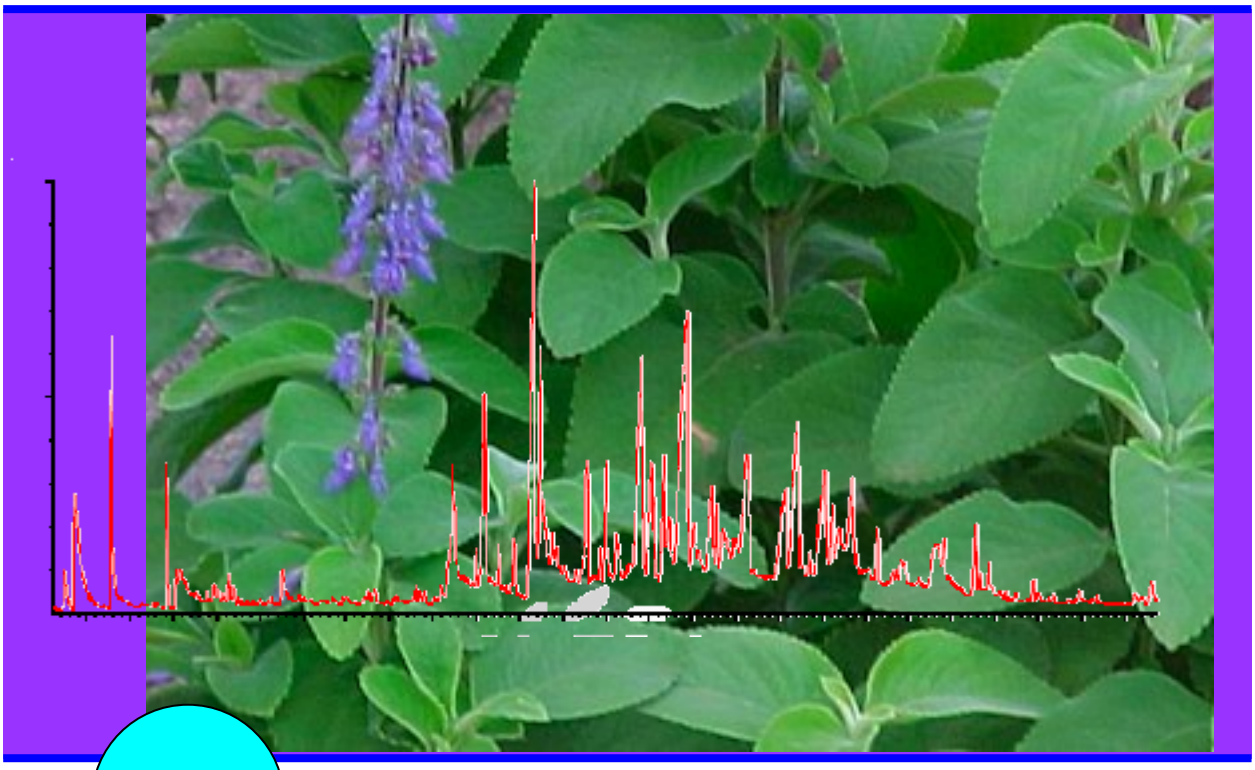

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Phytochemical investigation, GC-MS analysis and *in vitro* antimicrobial activity of *Coleus forskohlii*

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Abstract

The aim of this study was to investigate the phytochemical constituents, gas chromatography-mass spectrometry (GC-MS) analysis and antimicrobial activity of *Coleus forskohlii*. The different solvents such as ethanol, chloroform, acetone and aqueous extracts were identified pharmacologically as important bioactive compounds and their antimicrobial properties were studied. In the phytochemical investigation almost all the ethanol extract of leaf, stem and root having secondary metabolites like alkaloids, flavonoids, tannins, saponins, terpenoids, and steroids. The active constituents of the ethanol extract of *C. forskohlii* root was studied by GC-MS analysis. According to the antimicrobial results ethanol extract of *C. froshkolii* root showed highest antibacterial activity compared with stem and leaf. The highest antimicrobial activity was observed against *Klebsiella pneumonia* (19 mm) and *Candida albicans* (16 mm) in ethanol extract of root. Among the above extracts of leaf, stem and root, ethanol extract of root having antimicrobial activities due to the presence of phytoconstituents.

Introduction

Plants are the major source of medicines and foods which are play a vital role in the conservation of human health. The importance of plants in medicine remains even the greater significance with the current global trends to obtain drugs from the plant sources. The medicinal value of these plant sources lies in some chemical substances that produce a definite physiological action on the human body (Edeoga et al., 2005). These plants are main source of certain bioactive molecules which act as antioxidants and antimicrobial agents (Sengul et al., 2009). Multiple factors are responsible for the development of antibiotic resistance including the specific nature of the relationship of bacteria to antibiotics, the usage of antibacterial agent, host characteristics and environmental factors (Abiramasundari et al., 2011). This situation has forced scientists to search for new antimicrobial substances from various sources as novel antimicrobial chemotherapeutic agents (Ranjan et al., 2012). Various researches demonstrated that plants contain some bioactive phytochemical constituents which are mainly responsible for combating against disease (Deshmukh et al., 2012).

Coleus forskohlii Briq. (Family: *Lamiaceae*) has a very long history of use in many traditional herbal medicines, with special reference to Ayurveda. It possesses antianaphylactic, antiobesity, amebicidal, gastroprotective, bronchodilating, antiaging, antioxidant, anti-inflamma-tory, and anticancer activities. It is being used exclusively for weight management and hypotension (Alasbahi, 2013; Murugesan et al., 2012; Shivaprasad et al., 2014). In this study, the antimicrobial efficacy of various organic extracts of *C. forskohlii* was studied and phytochemical constituent present in extracts were identified by GC-MS method.

Materials and Methods

Collection of plant materials

Plant material free from infection was collected from Kaikalathur village of Perambalur District in Tamilnadu. The whole plants (leaf, stem and root) were used to prepare extracts. The plants collected were washed with water to remove the soil and dust particles. Then they were dried thoroughly in shaded place and blended to form a fine powder and stored in airtight containers. Then it was transported to the Laboratory of PG and Research Department of Biotechnology, Sri Vinayaga College of Arts and Science, Ulundurpet, where the study was carried out.

Extraction of plant material

Whole plant parts including leaf, stem and root were separated and made free from soil matter. They were dried and powdered by using hand pulveriser to a course powder. Then the powder was extracted with different solvents like aqueous, ethanol, chloroform and acetone by using sohxlet apparatus at a temperature of 50-55°C for 8 hours. The extracts were concentrated using vacuum evaporator and stored for further analysis.

Phytochemical screening

Phytochemical evaluation for major phytochemicals was done using standard qualitative methods (Sowofora, 1993; Tiwari et al., 2011). Tests for presence of reducing sugars, alkaloids, anthraquinones, tanins, terpenoids, saponins, oils and fats, flavonoids and cardiac glycosides were carried out on both extracts. The methods used are briefly described below.

Alkaloids (Wagner's test)

The extract (5 mL) was added Wagner's reagent. A reddish-brown precipitate indicates the presence of alkaloids.

Carbohydrates

In a test tube, 5 mL of the filtrate was treated with 5 mL Fehling's solutions (A and B) and was heated. The appearance of a red precipitate indicates the presence of reducing sugars.

Cardiac glycosides (Keller-Killiani test)

The extract (0.5 mL) was diluted in 5 mL of water. Then 2 mL of glacial acetic acid was added with a drop of ferric chloride solution. 1 mL of concentrated sulfuric acid was used to underplay this. A brown ring at interface shows presence of cardenolides.

Saponins (Foam test)

Distilled water (5 mL) was added to 0.5 mL of the extract in a test tube and shaken vigorously. The solution was observed for the formation of a persistent froth. Emulsion formation on mixture of the froth with olive oil indicates the presence of saponin.

Flavonoids

Sodium hydroxide (5 mL) was added to 5 mL of the extract. Formation of a deep yellow color that lessens when a few drops of dilute sulfuric acid are added indicates presence of flavonoids.

Amino acid

The filtrate (2 mL) was treated with 2-5 drops of ninhydrin solution placed in a boiling water bath for 1-2 min and observed for the formation of purple color.

Reducing sugars (Fehling's test)

Extract (1 mL) in 10 mL of water was mixed with 5 mL of boiling Fehling's solution (A and B). A brick-red, orange or yellow precipitate showed the presence of reducing sugars.

Steroids

The powder was dissolved in 2 mL of chloroform in a dry test tube. Ten drops of acetic anhydride and 2 drops of concentrated sulfuric acid were added. The solution became red, then blue and finally became bluish which indicates the presence of steroids.

Terpenoids (Salkowski's test)

Extract (0.5 mL) was added to 2 mL of chloroform. 3 mL of concentrated sulfuric acid was carefully added to the sides of the test tube to form a layer. Reddish-brown color at the interface shows the presence of terpenoids.

Phenolic compounds

Extract (1 mL) was added to 2 mL of distilled water and a few drops of 10% ferric chloride. Appearance of blue or green color indicates the presence of phenols.

Tannins (Ferric chloride test)

0.5 mL of the extract was diluted with 10 mL water and boiled in a test tube. After boiling it was filtered and a few drops of 0.1% ferric chloride added. A yellow to red precipitate indicated presence of tannins.

GC-MS analysis

The root of *C. forskohlii* was extracted with ethanol and analyzed by GC-MS for the identification of different compounds. GC-MS was performed by using column: Elite-5MS (5% diphenyl /95% dimethyl polysiloxane), 30 x 0.25 mm x 0.25 µm df, equipment: GC Clarus 500 Perkin Elmer carrier gas: 1 mL per min, split: 10:1 detector: mass detector Turbo mass gold-Perkin Elmer software: turbomass 5.2. 2 µL of extract was injected in injection port of GC column. Oven temperature program, no hold for up to 200°C at the rate of 10°C/min, 9 min hold for up to 280°C at the rate of 5°C/min, Injector temperature 250°C and total GC running time

36 min. Helium gas was used as the carrier gas at a constant flow rate of 1.0 mL/min. MS program: Library used NIST Version-Year 2005, inlet line temperature 200° C, source temperature 200°C, electron energy: 70 eV, mass scan (m/z): 45-450, solvent delay: 0-2 min and total MS running time: 36 min. (Woo et al., 2012)

Identification of the compounds

Compound identification was done by comparing the NIST library data of the peaks with those reported in literature, mass spectra of the peaks with literature data. Percentage composition was computed from GC peak areas on BP-I column without applying correction factors.

Culture media and strains

The media used for antibacterial test was nutrient agar of Hi media Pvt., India. The media used for antifungal test was potato dextrose agar media of Hi media Pvt. India. Pathological strains, i.e. Bacteria *Escherichia coli, Salmonella typhi, Streptococcus bovi, Enterococcus feacali, Klebsiella pneumonia* and fungi *Aspergillus flavus, A. parasiticus, Trichoderma rubrum, Candida albicans* were tested for antimicrobial activity of the extracts. These pure cultures of strains were collected from Pondicherry Center for Biological science, Pondicherry, India.

Antimicrobial activity

The antimicrobial activity of the leaf, stem and root extracts was determined by disc diffusion method (NCCLS, 1997) in petriplates containing NA and PDA medium (20 mL media/plate), respectively. The paper discs (6 mm in diameter) were separately impregnated with 15 µL of extracts placed on the agar which had previously been inoculated with the selected test microorganism. Plates were kept at 4°C for 1 hour. The plates were incubated at 37°C for 24 hours for bacteria and at 27°C for 48 hours for fungal strains. Antimicrobial activity was assessed by measuring the diameter of the growth-inhibition zone in millimeters for the test organisms comparing to the controls.

Results

The results of phytochemical screening of extracts revealed the presence of alkaloids, flavonoids, phenolic compounds, terpenoids, protein and cardiac glycosides in the leaf, stem and root extracts of *C. forskohlii* (Table I). Particularly, aqueous, ethanol, acetone and chloroform extracts of *C. forskohlii* were good sources of different classes of compounds. This indicates that these solvents are effective to isolate active biological compounds due to their high polarity. Flavonoids were detected in ethanol, acetone and aqueous extract of root absence of chloroform extract, acetone and ethanol extracts of leaf, chloroform and ethanol extract of stem also having the flavonoid. Cardiac glycosides are present in all the extracts of *C. forskohlii* except aqueous and chloroform extract of stem and root respectively. The alkaloid was observed in all the extract except chloroform extracts of the stem and root, but absence of aqueous in leaf extract. Ethanol and chloroform extract of leaf, stem and root of *C. forskohlii* having the terpenoids, amino acids were present in all root extract but absent in chloroform extract. Among all the tested extracts of leaf, stem and roots, chloroform extract has the lowest number of phytochemicals present. The ethanol extract of the leaf, stem and root was found rich

Table I

Phytochemical analysis of *Coleus forskohlii* with different solvent

SL. No.	Test Name	Leaf extract with different solvent				Stem extract with different solvent				Root extract with different solvent			
		Aq	Chl	Eth	Ace	Aq	Chl	Eth	Ace	Aq	Chl	Eth	Ace
1	Alkaloids	-	+	+	+	-	-	+	+	+	-	+	+
2	Carbohydrates	+	+	+	+	+	+	+	+	+	-	+	+
3	Cardiac glycosides	+	+	+	+	-	+	+	+	+	-	+	+
4	Saponins	+	-	+	-	+	-	+	+	-	+	+	-
5	Flavonoids	+	-	+	-	-	+	+	-	+	-	+	+
6	Aminoacids	-	-	+	-	-	-	-	-	+	-	+	+
7	Reducing sugar	-	+	+	+	+	+	+	+	+	-	+	+
8	Steroids	+	+	+	+	-	+	+	+	-	+	+	-
9	Terpenoids	-	+	+	-	-	+	+	-	+	-	+	+
10	Phenolic	+	-	+	-	-	+	-	-	+	-	+	+
11	Tannins	+	+	+	+	+	-	+	+	+	-	+	+

Aq- Aqueous, Chl- Chloroform, Eth- Ethanol, Ace- Acetone

source of phytochemicals as compared to the other extracts, whereas, in case of root ethanol extract was the best source of phytochemicals.

The active compounds identified in the ethanol root extract of *C. forskohlii* by GC-MS analysis was shown in (Figure 1). Totally 19 compounds have been detected through GC-MS analysis based on retention time, molecular formula, molecular weight and peak area. The active compounds with their retention time (RT), molecular formula, molecular weight (MW) and concentration (peak area%) are presented in Table II. The major compounds present in the roots were decanal (9.2%), n-hexadecanoic acid (7.51), cedrol (1.74), and betulin (6.67%) etc.; other major and minor compounds were also present.

The antimicrobial activity was determined by measuring the diameter of zone of inhibition. The leaf, stem and root extracts (aqueous, chloroform, acetone and ethanol) of *C. forskohlii* were found to have antimicrobial activity. The results obtained in the evaluation of the antibacterial activity of the different extracts against some bacteria *E. coli, Salmonella typhi, S. bovi, E. feacali* and *K. pneumonia*.

The ethanol extract of root shown maximum zone of inhibition against bacteria *E. coli, Salmonella typhi, S. bovi, E. feacali* and *K. pneumonia*. The inhibition zones are 13 mm, 8 mm, 7 mm, 11 mm and 9 mm respectively (Table III). The results obtained in the evaluation of the antifungal activity of the different extracts against some fungi *A. flavus, A. parasiticus, Trichoderma rubrum, C. albicans*. The ethanol extract of root shown zone of inhibition against fungi *A. flavus, A. parasiticus, Trichoderma rubrum* and *C. albicans* were 16 mm, 12 mm, 14 mm and 13 mm respectively (Table IV).

Table II

GC-MS report for ethanol extract of *Coleus froshkolii* root sample

SL. No.	Chemical constituents	Molecular formulae	Molecular weight	Peak area	RT
1	Bicyclo[2.2.1]heptan-2ol,1,77-trimethyl-,(1S-endo)	$C_{10}H_{18}O$	154	0.12	3.31
2	Decanal	$C_{10}H_{20}O$	156	9.62	3.55
3	Acetic acid, 1,7,7-trimethyl-bicyclo[2.2.1]hept-2-yl	$C_{12}H_{20}O_2$	196	7.49	4.39
4	Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl1-7-(1-methylethenyl)-,[IS-(1a,7a,8aa)]-	$C_{15}H_{24}$	204	2.52	5.68
5	Cedrol	$C_{15}H_{26}O$	222	1.74	8.35
6	1-Heptariacotanol	$C_{37}H_{76}O$	536	0.38	10.50
7	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	7.51	12.29
8	7-isoproapy-1,1,4a-trimehy1-1,4a –trimethl-1,2,3,4,9,10,10a octhy-drophenanthrene	$C_{20}H_{30}$	270	5.17	13.01
9	2- Decen-1-y1(-)succinic anhydride	$C_{16}H_{26}O_3$	266	2.26	14.18
10	2-phenanthrenol,4b,5,6,7,8,8a,9.10-octahydro-4b,.8,8-trimethyl1-1-(1-methylethyl)-,(4bs-trans)-	$C_{20}H_{30}O$	286	13.95	16.65
11	Friedelan-3-one	$C_{30}H_{50}O$	426	4.85	16.88
12	2-pentenoic acid,5-(decahydro-5,5,8a-trimethyl-2- methylene-1-napthalenyl)-3-methyl-,methyi ester[1R-[1a(E),4aa,8aa]]-	$C_{21}H_{34}O_2$	318	20.94	17.72
13	Pregnan-20-one,5,6-epoxy-3-hydroxy-,(3a,5a,6a)-	$C_{21}H_{32}O_3$	332	2.73	18.27
14	1b,4a-Epoxy-2H-cyclopenta[3,4]cyclopenta[8,9]cyclopenta[1,2-b] oxiren-5(6H)-one,7-(acetyloxy)decahydro-2,9,10-trihydroxy-3,6,8,8,10a-pentamethyl-	$C_{22}H_{32}O_8$	424	7.65	19.08
15	Betulin	$C_{30}H_{50}O_2$	442	6.67	20.88
16	Hexadecanoic acid octadecyl ester	$C_{34}H_{68}O_2$	508	1.25	22.10
17	Stigmastane-3,6-dione,(5a)-	$C_{29}H_{48}O_2$	428	3.03	23.66
18	Androstan-9-thiocyanato-3,11,17-trione	$C_{20}H_{25}NO_3S$	359	0.55	25.73
19	Cholesta-22,24-dien-5-ol,4,4-demithyl-	$C_{29}H_{48}O$	412	1.55	28.43

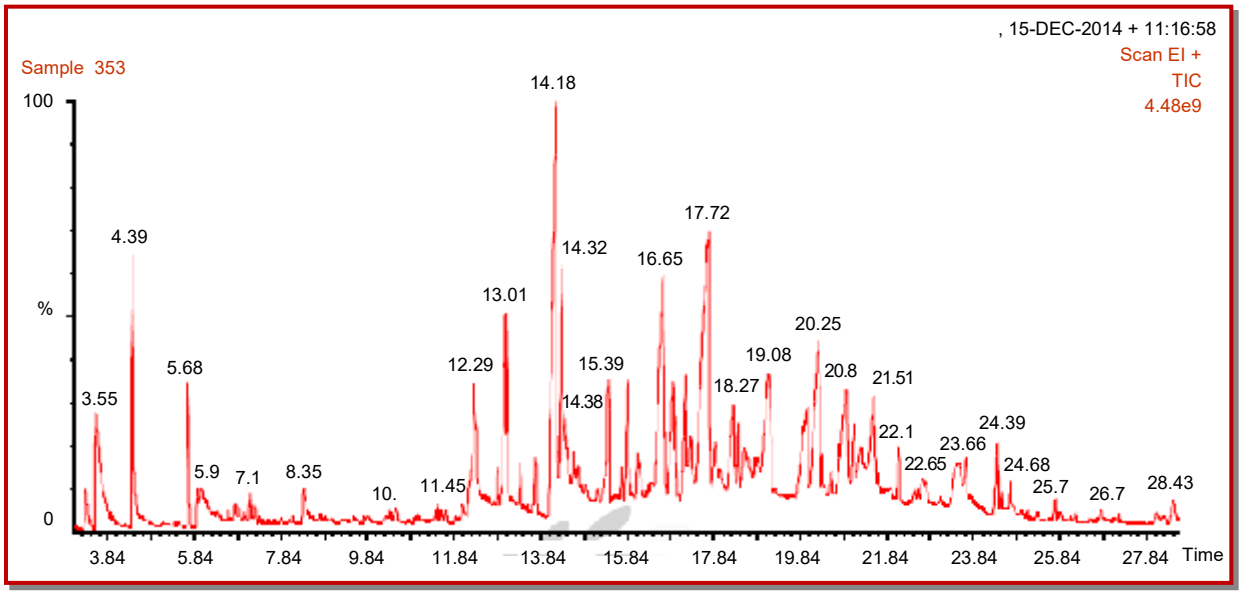

Figure 1: GC-MS analysis report for ethanol extract of *Coleus forskohlii* root

Discussion

Phytochemical analysis of *C. forskohlii* showed the existence of terpenoids, flavonoids, tannins, reducing sugars and alkaloids. Flavonoids belong to the group of polyphenolic compounds and are typically known for health promoting properties such as antioxidant, anti-allergic, anti-inflammatory, antimicrobial and anti-cancer properties (Aiyelaagbe and Osamudiamen, 2009). The plants of *C. forskohlii* have the flavonoids in ethanol, acetone and aqueous extract of root. Antimicrobial activity is often attributed to phytochemicals such as terpenoids, flavonoids, tannins, phenolic compounds or presence of free hydroxyl groups (Rojas et al., 1992).

Flavonoid based antimicrobial activity is thought to be a result of their capacity to disrupt enzymatic action in cell division, platelet aggregation and immunological responses and complex formation in the bacterial cell wall as well as extracellular and soluble proteins (Yadav and Agarwala, 2011). Flavonoids are also used by plants in their own defense against microbial agents. Terpenoid activity is said to be from their ability to disrupt membranes while tannins act by interfering with protein synthesis through binding to proline rich areas (Cowan, 1999). Correspondingly, *C. forskohlii* extracts also tested positive for phenolic compounds. The phenolic compounds are aromatic secondary metabolites that impart color, flavor and associated with health benefits such as reduced risk of heart and cardiovascular diseases (Alothman et al., 2009; Bhat et al., 2011). According to (Aliyu et al., 2009) phenolic compounds account for most of the antioxidant activities in plants. Alkaloids have been reported to possess analgesic, antispasmodic and bactericidal, antimalarial and analgesic activities (Okwu and Okwu, 2004; Oomah, 2003). Moreover, cardiac glycosides commonly used to treat congestive heart failure and cardiac arrhythmia, were discovered in all the extracts of the stem except petroleum ether extract, whereas, none of the root extracts indicated their presence (Hollman, 1985). Terpenoids such as triterpenes,

Table III

Antibacterial activity of *coleus forskohlii* with different solvent

SL. No	Name of the organisms	Zone of inhibition (mm) for leaf				Zone of inhibition (mm) for stem				Zone of inhibition (mm) for root			
		Ace	Aq	Chl	Eth	Ace	Aq	Chl	Eth	Ace	Aq	Chl	Eth
1	*Escherichia coli*	11	-	9	13	9	-	8	13	13	11	10	15.
2	*Salmonella typhi*	08	05	-	08	-	3	6	11	9	08	7	12
3	*Streptococcus bovis*	-	02	05	07	6	4	7	9	10	13	11	14
4	*Enterococcus feacalis*	07	04	06	11	7	5	9	15	12	14	11	17
5	*Klebsiella pneumonia*	12	-	04	09	3	-	9	12	15	13	16	19

Aq- Aqueous, Chl- Chloroform, Eth- Ethanol, Ace- Acetone

Table IV

Antifungal activity of *coleus forskohlii* with different solvent

S. No	Name of the organisms	Zone of inhibition (mm) for leaf				Zone of inhibition (mm) for stem				Zone of inhibition (mm) for root			
		Ace	Aq	Chl	Eth	Ace	Aq	Chl	Eth	Ace	Aq	Chl	Eth
1	*Aspergillus flavus*	06	04	-	9	-	5	5	10	9	7	9	16
2	*Candida albicans*	5	7	4	8	8	6	-	12	10	9	8	13
3	*Aspergillus parasiticus*	06	4	07	10	5	6	4	10	8	10	7	12
4	*Trichoderma rubrum*	08	10	05	11	9	7	5	12	11	13	10	14

Aq- Aqueous, Chl- Chloroform, Eth- Ethanol, Ace- Acetone

sesquiterpenes and diterpenes have been referred to as antibiotics, insecticidal, anthelmintic and antiseptic in pharmaceutical industry (Duke, 1992; Parveen et al., 2010). Proteins are the huge group of macromolecules and act as antibiotic and antimicrobial agents. Plants defend themselves against microbial pathogens by various defense responses including production of antimicrobial proteins which are small molecular mass antimicrobial peptides (Walter, 2012; Garc´ıa-Olmedo et al., 2001). GC-MS result clearly indicated the plant of *C. forskohlii* root ethanol extract have following compounds, n -hexadecanoic acid have antibacterial and antifungal properties (Agoramoorthy et al., 2007), betulin anticancer activities, apoptosis (Patocka, 2003; Ramadoss et al., 2003) and anti-HIV activities (Hashimoto et al., 1997). Cedrol have aromatic (Breitmeier, 2006) and carcinogenic properties (Sabine, 1975). Decanal have fragrances and flavoring properties.

Conclusion

The ethanol root extract of *C. forskohlii* have good fragrances, flavoring antibacterial, antifungal, and anticancer properties and also it induce the apoptosis mechanism due to the presence of phytoconstituents.

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Conflict of Interest

Authors declare no conflict of interest

References

Abiramasundari P, Priya V, Jeyanthi GP, Gayathri D. Evaluation of the antibacterial activity of *Cocculus hirsutus*. Hygeia J Drugs Med. 2011; 3: 26-31.

Agoramoorthy M, Chandrasekaran V, Venkatesalu MJH. Antibacterial and antifungal activities of fatty acid methyl esters of the blind-your-eye mangrove from India. Braz J Microbiol. 2007; 38: 739–42.

Aiyelaagbe OO, Osamudiamen PM. Phytochemical screening for active compounds in *Mangifera indica*. Plant Sci Res. 2009; 2: 11–13.

Alasbahi RH, Melzig MF. *Plectranthus barbatus*: A review of phytochemistry, ethnobotanical uses and pharmacology. Part 2. Planta Med. 2010; 76: 753-65.

Aliyu AB, Musa AM, Sallau MS, Oyewale AO. Proximate composition, mineral elements and anti-nutritional factors of *Anisopus mannii* N.E. Br. (Asclepiadaceae). Trends Appl Sci Res. 2009; 4: 68–71.

Alothman M, Bhat R, Karim AA. Effects of radiation processing on phytochemicals and antioxidants in plant produce. Trends Food Sci Technol. 2009; 5: 201–12.

Bhat R, Ameran SB, Karim AA, Liong MT. Quality attributes of starfruit (*Averrhoa carambola* L.) juice treated with ultraviolet radiation. Food Chem. 2011; 127: 641–44.

Breitmeier, E. Terpenes: Flavors, fragrances, pharmaca, pheromones. Wiley-VCH. 2006, pp 46–47.

Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev. 1999; 12: 564-82.

Deshmukh SR, Ashrit DS, Patil BA. Extraction and evaluation of indole alkaloids from *Rauwolfia serpentina* for their antimicrobial and antiproliferative activities. Int J Pharm Pharm Sci. 2012; 4(Suppl 5): 329-34.

Duke JA. Handbook of phytochemical constituents of grass, herbs and other economic plants. CRC Press, Boca Raton. 1992.

Edeoga HO, Okwo DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. Afr J Biotechnol. 2005; 4**;** 85-88.

Garc´ıa-Olmedo F, Rodriguez-Palenzuela P, Molina A, Alamillo JM, Lopez-Solanilla E, Berrocal-Lobo M, Poza-Carrion C. Antibiotic activities of peptides, hydrogen peroxide and peroxynitrite in plant defense. FEBS Lett. 2001; 498: 219–22.

Hollman A. Plants and cardiac glycosides. Br Heart J. 1985; 54: 258–61.

Hashimoto F, Kashiwada Y, Cosentino LM. Chen CH, Garrett PE, Lee KH. Anti-AIDS agents--XXVII. Synthesis and anti-HIV activity of betulinic acid and dihydrobetulinic acid

derivatives. Bioorg Med Chem. 1997; 5: 2133-43.

Murugesan S, Rajeshkannan C, Sumathi R, Manivachakam P, Suresh Babu D. Bioactivity of root hexane extract of *Coleus forskohlii* Briq. Labiatae: GC/MS/MS characterization and identification. Eur J Exp Biol. 2012; 2: 1469-73.

NCCLS (National Committee for Clinical Laboratory Standards). "Performance standards for antimicrobial disk susceptibility test", 1997, 6th ed. Approved Standard. M2-A6,

Okwu DE, Okwu ME. Chemical composition of *Spondias mombia* Linn plant parts. J Sustain Agric Ecosys Environ. 2004; 6: 140–47.

Oomah, DB. Isolation, characterization and assessment of secondary metabolites from plants for use in human health. PBI Bull. 2003; 1: 13–20.

Parveen M, Ghalib RM, Khanam Z, Mehdi SH, Ali M. A novel antimicrobial agent from the leaves of *Peltophorum vogelianum* (Benth.). Nat Prod Res. 2010; 24: 1268–73.

Patocka J. Biologically active pentacyclic triterpenes and their current medicine signification. J Appl Biomed. 2003; 1: 7-12.

Ramadoss S. Jaggi M, Siddiqui, MJA. U.S. Patents 2003; 6,048,847, 2000, 6,214,814, 2001, 6,670,345.

Ranjan S, Bhavya K, Muntaj SK, Basumata G, Matcha R. Synergistic effect of some medicinal plants and antibiotics against few pathogenic bacteria. Int J Biol Pharm Res. 2012; 3: 1000-04.

Rojas A, Hernandez L, Pereda-Miranda R, Mata R. Screening for antimicrobial activity of crude drug extracts and pure natural products from Mexican medicinal plant. J Ethnopharmacol. 1992; 35: 275-83.

Sabine JR. Exposure to an environment containing the aromatic red cedar, *Juniperus virginiana*: Procarcinogenic, enzyme-inducing and insecticidal effects. Toxicology 1975; 5: 221–35.

Sengul M, Yildiz H, Gungor N, Cetin B, Eser Z, Ercisli S. Total phenolics content, antioxidant and antimicrobial activities of some medicinal plants. Pak J Pharm Sci. 2009 ; 22: 102-06.

Shivaprasad HN, Gopalakrishna S, Mariyanna B, Thekkoot M, Reddy R, Tippeswamy BS. Effect of *Coleus forskohlii* extract on cafeteria diet-induced obesity in rats. Pharmacognosy Res. 2014; 6: 42-45.

Sofowora EA. Phytochemical assays in medicinal plants and traditional medicine in Africa. 3rd ed. Abuja, Spectrum Books Limited, 1993, pp 150-53.

Woo SG, Yoo K, Lee J, Bang S, Lee M, On K, Park J. Comparison of fatty acid analysis methods for assessing biorefinery applicability of wastewater cultivated micro-algae. Talanta 2012; 97: 103–10.

Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and extraction: A review. Internationale Pharmaceutica Sciencia. 2011; 1: 98-106.

Venkatesh P, Arun Kumar N, Hari Prasad R, Krishnamoorthy KB, Hari Prasath K, Soumya V. LC-MS/MS analysis of tetracycline antibiotics in prawns (*Penaeus monodon*) from south India coastal region. J Pharm Res. 2013; 6: 48-52.

Walter A. Plant defense mechanisms are activated during biotrophic and necrotrophic development of *Colletotricum graminicola* in Maize. Plant Physiol. 2012; 158: 1342–58.

Yadav RNS, Agarwala M. Phytochemical analysis of some medicinal plants. J Phytol. 2011; 3: 10-14.

Yang CS, Landau JM, Huang M, Newmark HL. Inhibition of carcinogenesis by dietary polyphenolic compounds. Ann Rev Nutr. 2001; 21: 381–406.

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