Chemical Characterisation and a Comparative Study on Multi-Target Biological Activities of Root Part Extracts of Coleus barbatus (Andrews) Bent. ex G. Don with its Isolated Compound, Forskolin

Jeewanti Kanyal
G. B. Pant University of Agriculture and Technology
Avneesh Rawat
G. B. Pant University of Agriculture and Technology
Om Prakash
oporgchem@gmail.com

G. B. Pant University of Agriculture and Technology
Kirti Nagarkoti
G. B. Pant University of Agriculture and Technology
Ravendra Kumar
G. B. Pant University of Agriculture and Technology
D. S. Rawat
University of Agriculture and Technology
R. M. Srivastava
G. B. Pant University of Agriculture and Technology
R. P. Singh
G. B. Pant University of Agriculture and Technology

Research Article

Keywords: Forskolin, Chemical composition, Antifeedant, Curvularia lunata, Coleus barbatus

Posted Date: November 21st, 2025

DOI: https://doi.org/10.21203/rs.3.rs-7690438/v1

Additional Declarations:

No competing interests reported.

Tables are available in the Supplementary Files section.

Chemical Characterisation and a Comparative Study on Multi-Target Biological Activities of Root Part Extracts of *Coleus barbatus* (Andrews) Bent. ex G. Don with its Isolated Compound, Forskolin

Jeewanti Kanyal[1], Avneesh Rawat[1], Om Prakash[1*], Kirti Nagarkoti[1], Ravendra Kumar[1], D. S. Rawat[2], R. M. Srivastava[3] and R. P. Singh[4]

[1]Department of Chemistry, College of Basic Sciences and Humanities, G. B. Pant University of Agriculture and Technology, Pantnagar, U. S. Nagar 263145, Uttarakhand, India
[2]Department of Biological Sciences, College of Basic Sciences and Humanities, G. B. Pant University of Agriculture and Technology, Pantnagar, U. S. Nagar 263145, Uttarakhand, India
[3]Department of Entomology, College of Agriculture, G. B. Pant University of Agriculture and Technology, Pantnagar, U. S. Nagar 263145, Uttarakhand, India
[4]Department of Plant Pathology, College of Agriculture, G. B. Pant University of Agriculture and Technology, Pantnagar, U. S. Nagar 263145, Uttarakhand, India

Corresponding author: *oporgchem@gmail.com

Abstract

Objective The present study was aimed on the comparative investigation of chemical composition and biological activities of *Coleus barbatus* root part ethyl acetate extract (CBREE) with its non-polar fraction, polar fraction and isolated compound, forskolin.

Methodology The analysis of extracts was carried out by gas chromatography-mass spectrometry (GC-MS). Along with the biochemical assay, the biological activities were investigated in terms of antioxidant, *in vitro* anti-inflammatory, herbicidal, antibacterial, antifungal and anti-feedant properties.

Results GC-MS analysis led to the identification of 73 compounds contributing 83.3% in CBREE, while 74 and 57 components contributing 87.2% and 78.7% were identified in non-polar and polar fraction of ethyl acetate extract respectively. CBREE was dominated by bornyl acetate (6.5%), decanal (6.5%), n-hexatriacontane (6.4%) and forskolin (4.6%), while the non-polar fraction was dominated by 1-(1-heptadecynyl) cyclopentanol (7.7%), bornyl acetate (6.7%) and decanal (5.5%) and the polar fraction was dominated by forskolin (36.0%) and spiro[4.5]decan-7-one, 1,8-dimethyl-8,9-epoxy-4-isopropyl (6.1%). The extracts exhibited good to moderate biochemical assay quantified in terms of total phenolic, total flavonoid and total orthodihydric phenol content. The extracts and isolated compound forskolin showed

significant antioxidant potential. Forskolin showed good anti-inflammatory activity (89.40%) compared to CBREE (86.37%), polar fraction (82.78%) and non-polar fraction (79.92%) of ethyl acetate extract. Forskolin showed good herbicidal potential in term of root and shoot growth inhibition than the extracts against *Raphanus raphanistrum* subsp. *sativus* seeds. Forskolin exhibited more antibacterial potential against *Bacillus cereus* and *Escherichia coli* than the extracts. The extracts and isolated compound forskolin exhibited good to moderate antifungal activity against *Alternaria alternata* and *Curvularia lunata*.Isolated compound forskolin exhibited a strong anti-feedant activity than extracts against third instar larvae of *Spilosoma obliqua*.

Conclusions The isolated compound forskolin has much more potential than the extracts to act as an anti-inflammatory, herbicidal, antibacterial and anti-feedant agent. Based on these observation it can be inferred that for the development of environmentally benign drugs and pesticides the herb *Coleus barbatus* can be used as natural source due to its abundant growth and world over distribution.

Key words Forskolin; Chemical composition; Antifeedant; *Curvularia lunata*; *Coleus barbatus*

1 Introduction

Plants are the backbone of life on Earth and an essential resource for human beings that used for food, fodder, fiber and medicinal purposes from ancient time. The plant natural products are the active components of many traditional medicines [1, 2]. Lamiaceae (Labiatae) also known as the mint family is one of the most diverse and widespread flowering plant family in terms of ethno medicine [3], that comprise of about 236-240 genera and 6,700-7,200 species [4] world over. Most species of this family are highly aromatic due to the presence of external glandular structures that produce volatile oils [5]. *Coleus barbatus* (Andrews) Bent.ex G. Don, an aromatic perennial succulent shrub commonly known as Makandi, Patharchur (Hindi) and Pashan bhedi (Sanskrit) is an important species of family Lamiaceae. On account of overlapping generic characters of *Coleus* and *Plectranthus* genera, the taxonomy of this species is quite ambiguous, as it has been placed in either *Coleus* or *Plectranthus*. Different authors have used different names for this species as *Plectranthus forskalaei*, *C. barbatus* (Andrews) Benth., *Solenocarpus barbatus* (Andr.) Codd., *C. forshohlii* (Willd.) Briq., *Plectranthus barbatus* Andrews etc [6]. **Paton *et al.*** [7] have finally resolved the generic limits between these two genera and recognised *Plectranthus*, *Coleus* and *Equilabium* as distinct genera. Accordingly, the correct name today is *Coleus barbatus* (Andrews) Bent. ex G. Don and all other names mentioned above are synonyms. This herb is distributed in many places of the world including India, China, Africa, Pakistan, and Sri Lanka [8]. In India, it grows wild in the sub-tropical

Himalayas, Deccan peninsula, Bihar, and Gujarat and cultivated on a commercial scale in some parts of Maharashtra, Rajasthan, Gujarat, Karnataka, and Tamil Nadu due to its nutritive and medicinal values [6, 9]. This herb is used as cough expectorant and diuretic in Africa, in treating intestinal and stomach disorders in Brazil and in treatment of cardiac disorders, insomnia, convulsions and respiratory disorders in India [10, 11]. It has been found useful in accelerating the breakdown of existing fat stores, promoting healthy cardiovascular function and lowering the elevated blood pressure [12, 13].

Till now, majority of the literature have focused on biochemical assay [14-17], chemical composition, antioxidant [15, 18, 19], antimicrobial [15, 20-25], insecticidal [16, 26] and pharmacological activities [27-34] on root part extract of this plant in different solvent like alcohol, acetone, chloroform, methanol, aqueous etc.

Very few reports on root part ethyl acetate extract of this plant have focused on chemical composition [35] and antifungal activity [19]. However, no reports exist on biochemical analysis, antioxidant, *in-vitro* anti-inflammatory, herbicidal, antibacterial and anti-feedant activities on this plant extract. Based on these facts the present study was aimed for the comparison in chemical composition and biological activities of *C. barbatus* root part ethyl acetate extract (CBREE), *C. barbatus* root part ethyl acetate extract non polar fraction (CBREN), *C. barbatus* root part ethyl acetate extract polar fraction (CBREP) and isolated major compound forskolin.

2 Materials and methods

2.1 Collection of plant material

C. barbatus (2180 g) was collected from Lohaghat, Champawat, Uttarakhand, India (29.42°N, 80.10°E, ~1514 m. a. s. l.) in the month of October 2018 and taxonomically identified by Dr. D. S. Rawat (Plant Taxonomist). The herbarium (Specimen No. GBPUH-982/16-01-2019) has been submitted in the Department of Biological Sciences, College of Basic Sciences and Humanities, Pantnagar, Uttarakhand for future reference.

2.2 Preparation of plant extract

Shade dried powder of the root part of *C. barbatus* (≈568 g) was subjected for extraction in ethyl acetate by Soxhlet apparatus. The extract so obtained was concentrated by evaporating the solvents under reduced pressure with the help of vacuum rotary evaporator to get

concentrated crude extract. The extract yield was recorded and preserved at low temperature (4 °C in refrigerator) until used for further studies.

2.3 Chemical composition

The chemical compositions of the plant extracts were analysed by GC-MS using GCMS-QP 2010 Ultra equipment with DB-5 silica capillary column (30m×0.25mm and film thickness 0.25 μm) in the following experimental conditions like carrier gas: helium, column flow rate: 1.21 mL/min, injection temperature: 260°C, injection mode: split, pressure: 73.3 kPa, split ratio: 20.0, ion source temperature: 230°C, interphase temperature: 270 °C. The oven temperature was initially programmed at 60°C for 3 min then increased to 250 °C at the rate of 7°C/min, isothermal for 5 min and finally raised to 280°C at a rate of 20°C/min and isothermal for 29 min. The constituents of the extracts were identified by matching their mass spectra and retention indices with those in NIST14, FFNSC 2 and Wiley8 Library and comparing the data with literature reports [36].

2.4 Isolation and characterization of major compound

The dried polar fraction of ethyl acetate extract of *Coleus barbatus* was subjected to column chromatography. The column was packed with activated silica gel (mesh size 60-120). Then the extract was loaded on to the column and eluted first with 100% of n-hexane. The polarity of the mobile phase was gradually increased by adding varying percentage of ethyl acetate starting from 1% to 75% and continued to elute the column until TLC showed no more spots of compounds. Finally, the column was eluted with 100% of ethyl acetate. Different fractions of the column of about 50 mL each were collected and monitored by TLC. The fractions (at 20% for isolated compound) showing similar RF values were mixed together and purified by re-column chromatography. A pale yellow coloured crystalline solid compound was obtained from fractions (obtained after re-column) by removing the solvents in vacuum rotary evaporator. The structural analysis of the isolated compound was carried out by using spectroscopic techniques (FT-IR, ^{1}H NMR, ^{13}C NMR and DEPT-135).

2.5 Biochemical Assay

The biochemical assay of extracts of *Coleus barbatus* was studied quantitatively by spectrophotometer (Thermo Scientific Evolution 201 series) in terms of total phenolics, flavonoids and orthodihydric phenols.

2.5.1 Total phenolic contents

The total phenolic contents were estimated by using the Folin-Ciocalteu method [37] and recently being practiced [38] with slight modification**.** 0.1 mL of the extract sample of 1 mg/mL concentration was mixed with 0.4 mL of 80% methanol, 8 mL of distilled water and 0.5 mL of 1 N Folin-Ciocalteu reagent. After 5 min of incubation, 1 mL of saturated Na_2CO_3 was added to the reaction mixture and allowed to reach for 60 min. Absorbance of the test sample was recorded at 650 nm in a UV-visible spectrophotometer (Thermo Scientific Evolution 201 series). The total phenolic contents in extract were quantified from the standard calibration curve obtained from gallic acid. The results expressed in terms of mg of gallic acid equivalent per g of dry weight of extract (mg/g).

2.5.2 Total flavonoid contents

The total flavonoid contents were determined using aluminium chloride colorimetric method [39]. 0.1 mL of extract sample of 1 mg/mL concentration was mixed with 1.25 mL of distilled water and 750 µL of 5% $NaNO_2$. The reaction mixture was incubated for 5 min followed by the addition of 150 µL of 10% $AlCl_3$. After 6 min 500 µL of 1 N NaOH and 275 µL of distilled water was added and the absorbance of the mixture was recorded at 510 nm. The total flavonoid contents were calculated from standard calibration curve which was established using different concentrations of catechin under the same procedure. The results expressed as mg of catechin equivalent per g of dry weight of extract (mg/g).

2.5.3 Ortho-dihydric phenols

To estimate the orthodihydric phenols,0.1 mL of the extract (1mg/mL concentration) was mixed with 0.4 mL of distilled water, 1 mL of 0.05 N HCl, 1 mL of Arnow's reagent (10 g $NaNO_2$ and 10 g sodium molybdate were mixed in 100 mL of distilled water), 10 mL of distilled water and 2 mL of 1 N NaOH. The solution was mixed thoroughly and the absorbance was measured at 515 nm. Similar procedure was repeated using various concentrations of catechol for establishing the standard working curve. The results expressed as mg of catechol equivalent per g of dry weight of extract [40, 41].

2.6 Antioxidant activity

2.6.1 Radical scavenging activity

2.6.1.1 DPPH (2,2-diphenyl-2-picrylhydrazyl) radical scavenging activity

DPPH radical scavenging activity of the test samples was evaluated according to the method developed by **Blois** [42] and are being practiced. In brief, 1 mL of different concentrations of

tested samples (50-250 µg/mL) were mixed with 5 mL of freshly prepared 0.004% methanol solution of DPPH and kept in the dark for incubation for 30 min. The absorbance was recorded at 517 nm in a UV-visible spectrophotometer using BHT (butylated hydroxyl toluene) and catechin as the standard. The inhibition of free radical by DPPH was calculated in term of IC % by using the equation:

$$IC\% = \frac{(A_0 - A_t)}{A_0} \times 100$$

A_0= Absorbance of control, A_t= Absorbance of test sample or standard, IC= Inhibitory concentration

The equation obtained by plotting percent inhibition against concentrations was used for the determination of the IC_{50} value. A lower IC_{50} value indicates more radical scavenging activity.

2.6.1.2 Hydroxyl radical scavenging activity

The hydroxyl radical scavenging capacity was evaluated by the method described by **Olabinri *et al.*** [43] and **Ramalingam *et al.*** [44]. 60 µL of $FeSO_4.7H_2O$ (1 mM) was added to 90 µL of aqueous 1, 10 phenanthroline (1 mM). After that 2.4 mL of 0.2 M phosphate buffer (pH 7.8) was added to the above mixture, followed by the addition of 150 µL of 0.17 mM hydrogen peroxide and 1.5 mL of different concentrations of tested samples (50-250 µg/mL). The mixture was incubated for 5 min at room temperature and the absorbance of the resultant mixture was recorded at 560 nm using ascorbic acid as the standard. The % hydroxyl radical scavenging capacity was calculated in term of IC % by using the equation:

$$IC\% = \frac{(A_0 - A_t)}{A_0} \times 100$$

A_0= Absorbance of control, A_t= Absorbance of test sample or standard, IC= Inhibitory concentration

2.6.1.3 Nitric oxide radical scavenging activity

Nitric oxide radical scavenging activity of the extracts and isolated compound was evaluated by the method used by **Naskar *et al.*** [45]. In brief the mixture of 1mL of 10 mM sodium nitroprusside in phosphate buffer (pH 7.4) and 1 mL of different concentrations of tested samples (50-250 µg/mL) were incubated at 25 °C for 150 min. From the incubated mixture, 1 mL was taken out and 1 mL of Griess reagent (1% sulphanilamide, 2% o-phosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride) was added into it. Absorbance of resultant solution

was read at 546 nm using ascorbic acid as a standard. The % nitric oxide radical scavenging capacity was calculated in term of IC % by using the equation:

$$IC\% = \frac{(A_0 - A_t)}{A_0} \times 100$$

A_0= Absorbance of control, A_t= Absorbance of test sample or standard, IC= Inhibitory concentration

2.6.1.4 Superoxide radical scavenging activity

Superoxide radical scavenging activity of the extracts and isolated compound was determined by the nitroblue tetrazolium reduction method [38, 46]. 1 mL of 156 µM NBT (nitroblue tetrazolium) and 1 mL of 468 µM NADH (Nicotinamide adenine dinucleotide) in 100 mM phosphate buffer (pH 7.4) were mixed with 0.1 mL of different concentrations of tested samples (50-250 µg/mL). After that 0.1 mL of 60 µM phenazine methosulphate (PMS) solution in 100 mM phosphate buffer (pH 7.4) was added to the mixture and incubated at 25 °C for 5 min. The absorbance was recorded at 560 nm using ascorbic acid as a standard. The % superoxide radical scavenging capacity was calculated in term of IC % by using the equation:

$$IC\% = \frac{(A_0 - A_t)}{A_0} \times 100$$

A_0= Absorbance of control, A_t= Absorbance of test sample or standard, IC= Inhibitory concentration

2.6.2 Reducing power activity

The reducing power of essential oils and various extracts was performed by the method reported by **Yen and Duh,** [47] and are being practiced [6, 48] with slight modifications. Varying concentrations of tested samples (5-25 and 50-250 µg/mL) were mixed with 2.5 mL of 200 mM phosphate buffer (pH= 6.6) and 2.5 mL of 1% potassium ferricyanide and incubated at 50°C for 20 min. After incubation 2.5 mL of trichloroacetic acid was added to the mixture, followed by centrifugation at 650 RPM for 10 min. The supernatant (2.5 mL) of mixture was taken out and mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride. The absorbance of the resultant mixture was recorded at 700 nm, using BHT and ascorbic acid as the standards. The % reducing power of the test samples was calculated in term of RP % by using the equation:

$$RP\% = \frac{(A_0 - A_t)}{A_0} \times 100$$

A_0= Absorbance of control, A_t= Absorbance of test sample or standard, RP= Reducing power

2.6.3 Metal chelating activity

The metal chelating activity of tested samples was examined by the method reported by **Hsu *et al.*** [49] and is being practiced [4, 50]. In brief 1 mL of different concentrations of tested samples (50-250 µg/mL) was mixed with 0.1 mL of 2mM $FeCl_2.4H_2O$, 0.2 mL of 5 mM ferrozine and 3.7 mL of methanol. The mixture was incubated for 10 min. The absorbance of resultant mixture was recorded at 562 nm using EDTA (Ethylene diamine tetraacetic acid) as a standard. The % metal chelating capacity was expressed in term of IC % by using the equation:

$$IC\% = \frac{(A_0 - A_t)}{A_0} \times 100$$

A_0= Absorbance of control, A_t= Absorbance of test sample or standard, IC= Inhibitory concentration

2.7 *In-vitro* anti-inflammatory activity

In-vitro anti-inflammatory activity of tested samples was carried out by using inhibition of albumin denaturation technique as reported by various researchers [38, 51] with minor modifications. The reaction mixture was consisting of 2 mL of varying concentrations of tested samples (100-500 µg/mL), 0.2 mL of egg albumin (from hen's fresh egg) and 2.8 mL of phosphate buffered saline (PBS, pH 6.4). The mixture was incubated at 37±2 °C in a BOD incubator for 15 min and then heated at 70 °C for 5 min in a water bath. After cooling of the mixture, the absorbance was recorded at 660 nm using diclofenac sodium as a standard anti-inflammatory drug. The % inhibition of protein denaturation was calculated in term of IC % by using the equation:

$$IC\% = \frac{(A_0 - A_t)}{A_0} \times 100$$

A_0= Absorbance of control, A_t= Absorbance of test sample or standard, IC= Inhibitory concentration.

2.8 Herbicidal activity

Different concentrations of the test samples (1-5 %) were prepared in sterilised distilled water for testing the seed germination inhibition. Seeds of *Raphanus raphanistrum* subsp. *sativus* (L.) Domin (syn. *Raphanus sativus* L.) (Radish) were used as a test plant, that were surface sterilized in 5% hypochlorite solution for 15 seconds prior to use. The petri plates were layered with ordinary filter papers on which ten sterilized seeds of *R. raphanistrum* subsp. *sativus* were taken. Then 2 mL of different concentrations of test samples was poured on that plates and

allowed to germinate at 25±1ºC in an incubator with 12 hours of photoperiod. The total seed germination, percent seed germination inhibition and length of root and shoot were measured on 5th days of incubation taking pendimethalin as a standard herbicide [6].

2.9 Antibacterial activity

Agar well diffusion method [52] was used to study the antibacterial activity of test samples. The screening was tested against two pathogenic, gram positive and gram negative bacterial strains namely *Bacillus cereus* (MTCC No. 430) and *Escherichia coli* (MTCC No. 443), respectively. A loopful amount from nutrient agar petri plate of bacterial culture was inoculated in 10 mL of nutrient broth and left overnight in shaking condition in an incubator for its growth. For screening 20 mL of molten nutrient agar was poured into the petri plates and left to solidify. The inoculum of different bacterial strains (100 µL) was spread evenly on the entire surface of nutrient agar plates with a sterilized spreader under aseptic conditions. After solidifying the plates, a well/ hole with a diameter of 5 mm was punched aseptically using sterilised cork borer and a volume of 50 µL of different concentrations (200-1000 ppm) of test samples was introduced into each well. Then agar plates were incubated at 32±2°C for 24 hours. After the incubation, the diameter of zone of inhibition of bacterial growth was measured in mm. Rifamycin a reference commercial antimicrobial was used to compare the antimicrobial potential.

2.10 Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) is the lowest concentration of the antimicrobial agent that inhibits the microbial growth after 24 hours of incubation. MIC was determined by the agar dilution susceptibility test which was based on modified methods of NCCLS and CLSI [53, 54]. Serial dilutions of the test samples were prepared by diluting them with sterilized distilled water to achieve a decreasing concentration ranges from 1000-100 ppm. A 100 µL suspension of bacterial strains were spread on nutrient agar plates. The wells, punched using sterilised cork borer were filled with 50 µL of different concentrations of test samples in the inoculated nutrient agar plates. The bacterial plates were incubated at 37±2°C and 62±5% RH for 24 h. The lowest concentration of each test samples showing a clear zone of inhibition was taken as the MIC. distilled water was used as the negative control, while rifamycin was used as positive control.

2.11 Antifungal activity

Antifungal activity of the test samples against two phytopathogenic fungi namely *Alternaria alternata* and *Curvularia lunata* was assisted by using poisoned food technique as recently adopted by **Kanyal *et al.*,** [4]. Potato dextrose agar, distilled water and chloramphenicol were used for the preparation of PDA (Potato dextrose agar) media. The test fungi were revived and grown on PDA medium by transferring the fungal colonies aseptically on the petri plates containing the media and incubated for one week at 25± 2°C. The stock solutions of 1000 ppm of the test samples was prepared in sterilized distilled water from which different concentrations (200-1000 ppm) of test samples were prepared in PDA medium and poured into the petriplates. After the solidification of the media, these prepared PDA plates of test samples were inoculated aseptically with assay discs (diameter= 5 mm) of the7 days old culture of test fungus and incubated for 7 days in an incubator at 26±2°C and 68±2% RH until the growth in control plates reached at the edge of the plates. Clear zones of mycelia growth inhibition around the petri plate indicated the presence of antifungal activity which was recorded in millimetre. Carbendazim was used as standard fungicide and percent inhibition was calculated by using following formula as given by **McKinney,** [55].

$$\text{Percent inhibition} = \frac{X - Y}{X} \times 100$$

Where, X= Radial growth in control, Y= Radial growth in treatment

2.12 Antifeedant activity

Antifeedant activity of the extracts and isolated compound was assayed against third instar larvae of Bihar hairy caterpillar (*Spilosoma obliqua*) using a leaf disc bioassay in no-choice situation [56]. The fresh leaf discs of 25 sq.cm of soybean (*Glycine max*) were treated with different concentrations (1-5%) of test samples and kept in the pre sterilized glass petri dishes containing an inner lining of moist filter paper. After that, prestarved (24 hours) and freshly molted uniform sized larvae were released in each petri dish (n=1) and allowed to feed until more than 75% leaf discs were eaten away in control (sterilized distilled water). The observations were recorded by measuring the consumed leaf area with the help of graph paper and the calculations were carried out on the following parameter: feeding percentage [57], antifeedant activity [58], feeding inhibition [59, 60], preference index [61] and antifeedant category [62].

2.13 Statistical analysis

All the experiments were performed in triplicate. The data were statistically analysed as mean ± SD subjected to one way-ANOVA by using SPSS (Statistical Package for the Social Science) 16.0 software package. Means were separated by the Tukey's multiple range test. The difference was considered significant when $p < 0.05$.

3 Results and discussion

3.1 Chemical composition

The yield of extracts in CBREE was found to be 6.9% (w/w) whereas in nonpolar and polar fraction it was found to be 4.4% (w/w) and 2.6% (w/w) respectively. By GC-MS analysis over 73 compounds contributing 83.3% of the total extracts were identified in CBREE while 74 and 57 components were identified in nonpolar and polar fraction contributing 87.2% and 78.7% of the total extract respectively. CBREE was dominated by bornyl acetate (6.5%), decanal (6.5%), n-hexatriacontane (6.4%), forskolin (4.6%) and β-sesquiphellandrene (4.1%). While 1-(1-heptadecynyl) cyclopentanol (7.7%), bornyl acetate (6.7%), decanal (5.5%) and 2-methylhexacosane (4.2%) were as found as major component in nonpolar fraction and forskolin (36.0%), spiro[4.5]decan-7-one,1,8-dimethyl-8,9-epoxy-4-isopropyl (6.1%) and methyl 3,4α,7,10α-tetramethyl-3-vinyldodecahydro-1H benzo [f] chromene-7-carboxylate (4.1%) were found as major components in polar fraction of extract. Isolated compound forskolin, a major component of CBREE was also present as a major component in polar fraction of extract and completely absent in nonpolar fraction of extract**.** The detailed comparative chemical compositions of nonpolar and polar fraction of CBREE have been illustrated in **Table 1**. In previous studies **Wen *et al.*** [35] isolated and identified 14-deoxycoleon U, forskolin H, chamaecydin, 16-diacetyl forskolin and β-sitosterol from ethyl acetate extract of *C. forskohlii*. But in our study instead of these compounds forskolin and γ-sitosterol were found in CBREE. A study by **Nidiry *et al.*** [19] revealed the presence of maximum amount of forskolin in ethyl acetate extracts (4.6%) followed by hexane extract (3.9%) of *C. forskohlii* roots and in our study forskolin was also found quantity (4.6%) in CBREE.

3.2 Characterization of compound #01: (Physiochemical data)

i) Yield: 4.53g

ii) Physical character: Pale yellowish crystal

iii) Odour: odourless

iv) Solubility: Ethanol, chloroform, ethyl acetate, and DMSO

The compound #01 was obtained in solid state. The EI-MS of the compound displayed a molecular ion peak at m/z 410 [M^+] corresponding to the molecular formula $C_{22}H_{34}O_7$. The FT-IR spectra at 3241.18 cm^{-1} stretching frequency corresponded to the presence of OH group. The other stretching peak at 2921.18, 1694.18, 1270.43 and 1160.06 cm^{-1} corresponded to the presence of C-H, C=O, C-O-C, and C-O bond, respectively (**Fig 1(a)**). Its ^1H NMR showed signals at δ 1.04, 1.26, 1.34, 1.42, 1.71 and 2.16 (s, 18H, 6 ×CH3) for methyl groups, a down field signal at δ 6.27 (s, H) for hydroxyl group and at δ 2.16 and 5.95 (dd, 2H) for methine groups. Signals at δ 1.96, 2.47, 3.20, 3.50, 4.98, 5.29 and 5.47 (d) at δ 1.12, 1.71, 4.47 and 4.56 (ddd) and at δ 1.42 and 2.16 (m) for the different groups have been shown in **Table 2** and **Fig. 1(b).** The ^{13}C NMR of the compound #01 showed a total of twenty-two carbons and their multiplicity assignment marked the presence of six CH_3, four CH_2, five CH and seven quaternary carbon atoms (**Fig 1(c)**). The DEPT-135 of the compound #01 showed eleven +ve peaks marked the presence of eleven CH_3 and CH carbon atoms and four –ve peaks marked the presence of four CH_2 carbon atoms. In DEPT-135 fifteen peaks were present and in ^{13}C NMR twenty-two peaks were present that also revealed the presence of seven quaternary carbons in compound #02 (**Fig 1(d)**). On the bases of these spectral data the compound #01 has been identified as forskolin ($C_{22}H_{34}O_7$). The structure of the compound has been presented in (**Fig 1(e)**).

3.3 Biochemical Assay

The total phenolic content in the various extracts from root part of *Coleus barbatus*was quantified by gallic acid calibration curve. Nonpolar fraction of the extract (47.72±0.26 mg/g GAE) showed good quantity of phenolic content followed by polar fraction (21.14±0.13 mg/g GAE) and CBREE (17.68±0.26 mg/g GAE). The total flavonoid content was determined by catechin calibration curve. CBREE (71.73±0.55 mg/g CNE) exhibited significantly higher quantity of total flavonoids as compared to polar fraction (34.93±0.69 mg/g CNE) and nonpolar fraction of extracts (26.82±0.41 mg/g CNE). The orthodihydric phenols were estimated by catechol calibration curve. Significant quantity of total orthodihydric phenol content was found

in CBREE (7.07±0.25 mg/g CLE) followed by polar fraction (6.80±0.52 mg/g CLE) and nonpolar fraction of extracts (4.55±0.43 mg/g CLE). **Table 3** revealed the detailed quantitative results of biochemical assay in different extracts from root part of *C. barbatus*.

3.4 Antioxidant activity

In present study strong to moderate antioxidant potential of the extracts and isolated compound forskolin from the root part of *C. barbatus* were observed in terms of six antioxidant parameters in a dose dependent manner. In case of DPPH radical scavenging activity polar fraction of extract showed the maximum scavenging potential with average IC_{50} of 57.55±0.12 µg/mL. Whereas CBREE (62.18±0.20 µg/mL), nonpolar fraction of extract (167.38±0.08 µg/mL) and forskolin (169.89±0.15 µg/mL) exhibited good to moderate antioxidant potential. In case of hydroxyl radical scavenging activity, maximum scavenging potential was shown by CBREE (IC_{50}= 106.00±0.35 µg/mL) and moderate potential shown by nonpolar fraction of extract (152.09±1.36µg/mL), polar fraction of extract (118.30±1.30 µg/mL) and forskolin (123.92±1.71µg/mL). In case of nitric oxide radical scavenging activity, forskolin (IC_{50}= 129.60±0.41 µg/mL) exhibited the more antioxidant potential than CBREE (159.39±0.59 µg/mL), nonpolar fraction of extract (151.20±0.13 µg/mL) and polar fraction of extract (134.92±0.36 µg/mL). Superoxide radical scavenging activity of forskolin (IC_{50}=115.82±0.45 µg/mL) showed more antioxidant potential than CBREE (161.68±0.25µg/mL), nonpolar fraction of extract (168.84±0.87 µg/mL) and polar fraction of extract (144.47±0.47µg/mL). Polar fraction of extract (RP_{50}=124.21±0.33 µg/mL) exhibited more reducing power activity among the all tested samples CBREE (198.33±0.84 µg/mL), polar fraction of extract (209.44±0.91 µg/mL) and forskolin (260.26±0.76 µg/mL). In the case of metal chelating activity polar fraction of extract (IC_{50}=180.96±0.18 µg/mL) exhibited good chelating activity than CBREE (216.47±0.25 µg/mL), nonpolar fraction of extract (243.48±1.08 µg/mL) and forskolin (186.92±0.32 µg/mL).The minimum IC_{50}/RP_{50} value reflects higher antioxidant activity of test samples, hence revealed good antioxidant potential of test samples under investigation. **Table 4** represents the antioxidant potential of the all the extracts and forskolin in term of their IC_{50}/RP_{50} along with the respective standards. Polar fraction of extract and forskolin exhibited good antioxidant activity as compared to CBREE and nonpolar fraction of extract. The difference in the antioxidant activity of extracts might be possibly due to the different biochemical makeup of the extracts in terms of total phenolic, flavonoid and orthodihydric compounds and their different concentrations.

3.5 *In-vitro* anti-inflammatory activity

In present study extracts and isolated compound forskolin from the root part of *C. barbatus* exhibited *in vitro* anti-inflammatory activity in a dose dependent manner at all tested concentrations (100-500 µg/mL). At higher dose of 500 µg/mL of concentration forskolin exhibited the maximum (89.40%) inhibition of protein denaturation, whereas CBREE, nonpolar and polar fraction exhibited 86.37%, 79.92% and 82.78% inhibition respectively while comparing with standard diclofenac sodium (94.32%) at the same dose level. The anti-inflammatory activity of all the test samples in term of their IC_{50} values (µg/mL) has been depicted in **Table 5**. The literature search did not reveal any report on *in vitro* anti-inflammatory activity of the various extracts from root part of *C. barbatus*. In the present study, forskolin exhibited the maximum anti-inflammatory activity among all test samples. In previous study forskolin which was isolated from *Solena amplexicaulis* leaf has also been reported to exhibit the potential anti-inflammatory activity [63]. The anti-inflammatory activity of various extracts from the root part of *C. barbatus* might be attributed due to several biologically active components reported of having anti-inflammatory activity like palmitic acid [64], *trans*-nerolidol [65], bornyl acetate [66] present in CBREE and nonpolar fraction, *p*-cymene [67], β-caryophyllene [68] present in CBREE, nonpolar fraction and polar fraction etc. or the synergistic effects among the major and minor constituents.

3.6 Herbicidal activity

All the test samples exhibited the herbicidal activity in a dose dependent manner. Forskolin exhibited the maximum herbicidal activity by inhibiting 83.33% of seed germination at higher concentration level (5 %) while CBREE, nonpolar fraction and polar fraction showed the significant herbicidal potential by inhibiting 50.00 %, 56.67 % and 63.33 % respectively of seed germination at this dose level. The mean percent seed germination inhibition of extracts and isolated compound forskolin from root part of *C. barbatus* at different concentrations (1-5 %) has been represented in **Table 6**. The inhibitory effect of test samples on root and shoot growth (length) of *Raphanus raphanistrum* subsp. *sativus* in term of percent inhibition has been depicted in **Figure 2** and **Figure 3** respectively. The maximum inhibition in root growth was found in forskolin (97.34 %) followed by polar fraction (88.60 %), nonpolar fraction (85.92 %) and CBREE (76.05 %) at higher concentration (5%). Among the all forskolin exhibited excellent

shoot growth inhibition up to 100% from 2 % to above concentration levels. Nonpolar and polar fraction both exhibited 100 % of shoot growth inhibition while CBREE exhibited 97.72 % of inhibition at highest concentration (5 %).From the above results, it was observed that isolated compound forskolin from root part of *C. barbatus* exhibited maximum herbicidal potential. The mode of action by which the seed germination inhibition was exhibited by various extracts from root part of *C. barbatus* is unknown and yet to be ascertained as there is no literature report available on it. Compound like *p*-cymene, β-pinene, α-terpinene, γ-terpinene, α-terpineol, borneol, β-linalool, α-pinene, camphene, β-phellandrene etc. have been reported to possess the herbicidal property, phytotoxic effect and allelopathic effect [69-72]. Some of these compounds were present in extracts of root part of *C. barbatus* as major or minor constituents that might be responsible for the herbicidal property.

3.7 Antibacterial activity

The extracts and isolated compound forskolin from root part of *C. barbatus* have been evaluated for their antibacterial activity against two pathogenic bacterial strains at different concentrations (200-1000 ppm). All the samples exhibited antibacterial potential in a dose dependent manner as expressed in term of zone of inhibition (ZOI) in millimetre. Among the all tested samples the maximum antibacterial potential was exhibited by forskolin against both the bacterial strains *B. cereus* (ZOI= 28.00 mm) and *E. coli* (ZOI= 27.67 mm) at 1000 ppm of concentration. Against *B. cereus* the antibacterial potential of all tested samples in term of zone of inhibition (ZOI) at similar dose was compared with rifamycin and found in the order: rifamycin (ZOI= 30.67 mm) > forskolin (ZOI= 28.00 mm) > polar fraction (ZOI=16.67mm) > CBREE (ZOI=15.67 mm) > nonpolar fraction (ZOI=15.00 mm). Against *E. coli* at 1000 ppm concentration the antibacterial activity of test samples was compared with rifamycin and found in the order: rifamycin (ZOI= 29.00 mm) > forskolin (ZOI= 27.67 mm) >nonpolar faction (ZOI=16.33 mm) > CBREE (ZOI=16.00 mm) > polar fraction (ZOI=14.33 mm).The results of the antibacterial activity have been represented in **Table 7.**

3.8 Minimum inhibitory concentration (MIC)

Against *B. cereus* and *E. coli* forskolin exhibited MIC of 100 ppm. Nonpolar fraction of ethyl acetate extracts from root part of *C. barbatus* exhibited MIC of 200 ppm for both *B. cereus* and *E. coli*. CBREE and polar fraction both exhibited MIC of 150 ppm against *B.* cereus and 200

ppm against *E. coli*. **Table 8** depicts the minimum inhibitory concentration (MIC) of the samples under study.

The significant antimicrobial activity of different extracts (petroleum ether, ethanol, chloroform, diethyl ether, methanol and distilled water) of *Coleus forskohlii* roots against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens, Escherichia coli*, *Bacillus subtilis*, *Bacillus pumilus*, *Aspergillus niger*, *Sericea* and *Kelebsiella pneumonia* have been reported previously [23-25]. But there is no literature report on the antibacterial activity of root part ethyl acetate extract of *C. barbatus*.

3.9 Antifungal activity

In the present study, extracts and isolated compound forskolin from root part of *C. barbatus* were examined for the antifungal potential against two phytopathogenic fungi at different concentrations (200-1000 ppm). All the tested samples exhibited antifungal activity in a dose dependent manner by inhibiting the growth of fungal mycelia. At higher concentration (1000 ppm) the maximum antifungal activity against *A. alternate* was exhibited by isolated compound forskolin (80.92%), whereas against *C. lunata* maximum antifungal activity was exhibited by nonpolar fraction (80.50%). The order of antifungal activity of all tested samples at 1000 ppm concentration against *A. alternata* was observed as: Carbendazim (100.00%) > forskolin (80.92 %) > CBREE (73.45 %) > nonpolar fraction (68.47 %) > polar fraction (31.95 %). At the similar concentration the order of antifungal activity of all tested samples against *C. lunata* was observed as follows: Carbendazim (100.00%) > nonpolar fraction (80.50 %) > CBREE (76.76 %) > forskolin (74.69 %) > polar fraction (55.60 %). **Table 9** represents the antifungal activity of the extracts and isolated compound in term of percent mycelial growth inhibition.

In the present study, isolated compound forskolin exhibited maximum antifungal potential against *A. alternata* and a strong antifungal action against *C. lunata*. This was also supported by a previous study reported by **Nidiry *et al.*** [19] which revealed that forskolin, a diterpene present in *Coleus forskohlii* roots exhibited dose dependent spore germination inhibition of *Alternaria solani.* It has also reported the mycelial growth inhibition of *Colletotrichum gloeosporioides* and spore germination inhibition of *Alternaria solani* by hexane, ethyl acetate and methanol roots extracts of *Coleus forskohlii.* The reported study and present observation reveals that the plant possess the antifungal activity which might be due to the

presence of major compound forskolin or due to the rich biochemical make-up of secondary metabolites like terpenoids, alkaloids etc.

3.10 Anti-feedant activity

In the present study, extracts and isolated compound forskolin from root part of *C. barbatus* were tested for their antifeedant potential against third instar larvae of *S. obliqua* at different concentrations (1-5 %). All the test samples exhibited antifeedant activity in a dose dependent manner as evaluated on the bases of mean leaf area consumed (MLAC) by the third instar larvae of *S. obliqua*. **Table 10** represents the mean leaf area consumed by the larva at different concentrations of test samples along with other parameters (feeding percentage, antifeedant activity, feeding inhibition, preference index and antifeedant category). Among all the tested samples at higher concentration (5 %), the isolated compound forskolin exhibited excellent antifeedant activity (87.60 %) as the MLAC was found to be minimum in forskolin (2.35 cm^2). The MLAC in test samples at higher concentration (5 %) was found in the order: control (19.15 cm^2) > polar fraction (5.11 cm^2) > CBREE (4.85 cm^2) > nonpolar fraction (3.97 cm^2) > forskolin (2.35 cm^2). Thus the order of antifeedant potential of test samples was found in the order: forskolin (87.60 %) > nonpolar fraction (78.18 %) > CBREE (74.65 %) > polar fraction (73.29 %), as the least value of MLAC showed the strong antifeedant character.

Previously **Nayini *et al.*,** [27] reported the repellent efficiency of *Coleus forskohlii* acetone root extract and isolated compound forskolin against Uzi fly (*Exorista bombycis*). In the present study, the isolated compound forskolin exhibited maximum antifeedant activity among all tested samples and this finding was also supported by **Vattikonda *et al.*,** [73] which reported significant antifeedant activity of forskolin against fourth instar larvae of *Papilio demoleus*. There is no literature report on root part ethyl acetate extract of *C. barbatus*. The antifeedant efficiency of the extracts might be attributed due to the presence of biologically active constituents in the extracts such as α-pinene, α-terpinolene, *p*-eugenol, α-limonene [74] and β-caryophyllene [75] besides forskolin.

4 Conclusions

On the bases of present study, it can be concluded that the plant extract obtained from *C. barbatus* roots part possessed multi target activities. It exhibited good quantity of phenolic and flavonoids contents that enhanced the antioxidant property of the herb. The antioxidant findings show more antioxidant potential in polar fraction and isolated compound forskolin that contributed by different antioxidant components. Thus the use of this herb as a natural antioxidant in replacement of synthetic (because of implications for human health) may be advantageous. Among all test samples isolated compound forskolin exhibited maximum *in vitro* anti-inflammatory activity against the denaturation of protein. Further definitive studies are necessary to ascertain the mechanisms behind its anti-inflammatory actions to make it promising agent for drug research. Herbicidal activity of forskolin among all test samples was found maximum that might be helpful for the development of natural herbicide as natural pesticides and herbicides are effective, selective, biodegradable and less toxic to the environment. The remarkable antimicrobial effect of root part extract of *C. barbatus* against tested strains of bacteria and fungi showed the possibility of this plant as a source of natural and organic antimicrobial agent. Isolated compound forskolin exhibited dose dependent activity against the bacterial and fungal strains and has been identified as one of the antimicrobial compounds present in the plant. It can play a role in the defence mechanism against bacterial and fungal pathogens. Synthesis of forskolin and its structure-activity relationship studies can lead to the development of new antibiotics and fungicides. All the extracts of *C. barbatus* exhibited strong antifeedant activity and that might be helpful for the management of pest. Thus, the plant extract may have their great impact in future integrated pest management (IPM) programmes due to their safety to non-target organisms and the environment. The excellent antifeedant property of isolated compound forskolin may lead to the development of suitable botanical formulations for controlling the insect pest.

Thus, along with the forskolin the future study obviously needs the isolation of active constituents followed by their spectral analysis for quality control of the compounds responsible for antioxidant, herbicidal, antibacterial, antifungal and antifeedant activity which could possibly facilitate the new formulations for their bioactivity at lower concentrations.

Conflicts of interest

The authors declare no conflicts of interest.

Authorship contribution statement

Jeewanti Kanyal: Planning original draft, collated the literature and prepared manuscript. The study was part of his Ph.D. thesis work. **Avneesh Rawat:** Collated the literature and prepared manuscript. **Om Prakash**: Advisor of the student, Planned the study of present work, provided research guidance. **Kirti Nagarkoti**: Helped in preparing the manuscript, Formal analysis. **Ravendra Kumar**: Co-advisor of the student, helped in executing the experiments. **D.S. Rawat**: Member of research advisory committee, guided to identify the plant. **R.M. Srivastava**: Member of research advisory committee, guided to conduct the entomological studies. **R.P. Singh**: Member of research advisory committee, guided to conduct the anti-microbial studies.

Acknowledgments

Authors are thankful to G. B. Pant University of Agriculture and Technology, Pantnagar for providing all necessary research facilities and AIRF (Advance Instrumentation Research Facility), Jawaharlal Nehru University, New Delhi, India for GC-MS analysis.

Funding

The authors did not receive support from any organization for the submitted work.

Data availability

Data will be made available upon request from the corresponding author

Declarations

Permissions to collect the plants/plant parts

The authors confirm that the necessary permission to collect and cultivate the samples have been obtained. The herbarium (Specimen No. GBPUH-982/16-01-2019) has been identified and submitted to Dr. D.S. Rawat, Plant Taxonomist in the Department of Biological Sciences, College of Basic Sciences and Humanities, Pantnagar, Uttarakhand for future reference.

Ethics approval and consent to participate

Not Applicable

Consent for publication

Not Applicable

Competing interests

The authors declare no competing interests

References

1. Beghyn T, Deprez-Poulain R, Willand N, Folleas B, Deprez B. Natural compounds: leads or ideas? Bioinspired molecules for drug discovery. Chem Biol Drug Des. 2008;72(1): 3-15.
2. Li J WH, Vederas JC. Drug discovery and natural products: end of an era or an endless frontier? Science. 2009;325(5937): 161-165.
3. Sarac N, Ugur A, Antimicrobial activities and usage in folkloric medicine of some Lamiaceae species growing in Mugla, Turkey. Eur Asian J BioSci. 2007;4: 28-37.
4. Kanyal J, Prakash O, Kumar R, Rawat DS, Singh RP, Srivastava RM, Pant AK. Essential oil composition and biological activities determination of *Mosla dianthera* (Buch.-Ham. ex Roxb.) Maxim. and its major isolated component, carvone. Braz J Pharm Sci. 2022; 58.
5. Giuliani C, Bini LM. Insight into the structure and chemistry of glandular trichomes of Labiatae, with emphasis on subfamily Lamioideae. Plant Syst Evol. 2008;276(3-4): 199-208.
6. Kanyal J, Prakash O, Kumar R, Rawat DS, Srivastava RM, Singh RP, Pant AK. Study on comparative chemical composition and biological activities in the essential oils from different parts of Coleus barbatus (Andrews) Bent. ex G. Don. J Essent Oil Bear Plants. 2021; 24(4), 808-825.
7. Paton AJ, Mwanyambo M, Govaerts RHA, Smitha K, Suddee S, Phillipson PB, Wilson TC, Forster PI, Culham A. Nomenclatural changes in *Coleus* and *Plectranthus* (Lamiaceae): a tale of more than two genera. PhytoKeys. 2019;129: 1-158.
8. Shukla PK, Misra A, Kumar M, Jaichand SK, Akhtar J, Srivastava S, Agrawal PK, Rawat AKS. Simultaneous quantification of forskolin and iso-forskolin in *Coleus forskohlii* (Wild.) Briq and identification of elite chemotype, collected from Eastern Ghats (India). Pharmacogn Mag. 2018;13: 881-885.
9. Singh R, Gangwar SP, Singh D, Singh R, Pandey R, Kalra A. Medicinal plant Coleus forskohlii Briq.: disease and management. Med Plants - Int J Phytomed Relat Ind. 2011;3(1): 1-7.
10. Ammon HP, Muller AB. Forskolin: from an ayurvedic remedy to a modern agent. Planta Med. 1985;51(06): 473-477.

11. Valdes L, Mislankar SG, Paul AG. *Coleus barbatus* (*C. forskohlii*) (Lamiaceae) and the potential new drug forskolin (Coleonol). Econ Bot. 1987;41(4): 474.

12. Kramer W, Thormann J, Kindler M, Schlepper M. Effects of forskolin on left ventricular function indilated cardiomyopathy. Arzneimittel Forschung. 1987;37(3): 364-367.

13. Schlepper M, Thormann J, Mitrovic V. Cardiovascular effects of forskolin and phosphodiesterase-III inhibitors. Basic Res Cardiol. 1989;84(1): 197-212.

14. Bajpai VK, Park YH, Agrawal P. Studies on phytochemical analysis, antioxidant and lipid peroxidation inhibitory effects of a medicinal plant, *Coleus forskohlii*. Front Life Sci. 2015;8(2): 139-147.

15. Rajkumar K, Malathi R. Phytochemical investigation, GC-MS analysis and in vitro antimicrobial activity of *Coleus forskohlii*. Bangladesh J Pharmacol. 2015;10(4): 924-930.

16. Lawi Y, Saria J, Kidukuli AW. Brine shrimp cytotoxicity, phytochemical screening and larvicidal activities of *Plectranthus barbatus* extracts. *Res Rev Insights.* 2018;2(1): 1-4.

17. Shanmugam S, Usha Rani V, Pradeep BV. Antioxidant activity of rhizome extract of *Coleus forskohlii* Briq. Asian J Pharm Clin Res**.** 2018;11(11): 275-279.

18. 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: 1469-1473.

19. Nidiry ESJ, Ganeshan G, Lokesha AN. Antifungal activity of the extractives of *Coleus forskohlii* roots and forskolin. Pharm Chem J. 2015;49(9): 624-626.

20. Baskaran C, Ratha bai V. Green Synthesis of silver nanoparticles using *Coleus forskohlii* roots extract and its antimicrobial activity against bacteria and fungus. *Int J* Drug Develop Res. 2013;5: 1-10.

21. Manikandan D, Prakash DG, Gandhi NN**.** A rapid and green route to synthesis of silver nanoparticles from *Plectranthus barbatus* (*Coleus forskohlii*) root extract for antimicrobial activity. Int J Chem Tech Res. 2014;6: 4391-4396.

22. Naraginti S, Sivakumar A. Eco-friendly synthesis of silver and gold nanoparticles with enhanced bactericidal activity and study of silver catalyzed reduction of 4-nitrophenol. Spectrochim Acta A. 2014;128: 357-362.

23. Atulkar P, Thakur R, Singh P. Antimicrobial activities of root extracts of *Coleus forskohlii* Briq. Int J Recent Sci Res. 2015;6 (12): 7732-7734.

24. Saklani S, Gahlot M, Kumar A, Singh R, Patial R, Kashyap P. Antimicrobial activity of extracts of the medicinal plant *Coleus forskohlii*. Int J Drug Res Technol. 2017;1(1): 52-59.

25. Nguta JM, Kiraithe MN. *In vitro* antimicrobial activity of aqueous extracts of *Ocimum suave* Willd., *Plectranthus barbatus* Andrews and *Zanthoxylum chalybeum* Engl. against selected pathogenic bacteria. Biomed Biotechnol Res J. 2019;3(1): 30-34

26. Nayini SY, Nalagoni CSR, Banala RR, Raju S. A comparitative study to evaluate the efficiency of *Coleus forskohlii* root extract and forskolin as a repellent against Uzi fly (*Exorista bombycis*). Int J Bio-Pharma Res. 2019;8(6): 2623-2629.

27. Varma N, Srivastava V, Tandon JS, Krishna Prasad BN, Chitravanshi VC. Effect of *Coleus forskohlii* against caecal amoebiasis of rats. Int J Crude Drug Res. 1990;28(1): 1-3.

28. Jagtap M, Chandola HM, Ravishankar B. Clinical efficacy of *Coleus forskohlii* (Willd.) Briq. (Makandi) in hypertension of geriatric population. Ayu. 2011;32(1): 59-65.

29. Ahsan F, Siddiqui HH, Mahmood T, Srivastav RK, Nayeem A. Evaluation of cardioprotective effect of *Coleus forskohlii* against isoprenaline induced myocardial infarction in rats. Indian J Pharm Biol Res. 2014;2(1): 17-25.

30. Shivaprasad HN, Gopalakrishna S, Mariyanna B, Thekkoot M, Reddy R, Tippeswamy BS. Effect of *Coleus forskohlii* extract on cafeteria diet-induced obesity in rats. Pharmacogn Res. 2014;6(1): 42-45.

31. Malarvizhi A, Srinivasan S. Effect of *Coleus forskohlii* root extracts on liver marker enzymes. Int J Life Sci Biotechnol Pharma Res. 2015;4(3): 158.

32. Kamohara S. An evidence-based review: Anti-obesity effects of *Coleus forskohlii*. Personalized Medicine Universe. 2016;5: 16-20.

33. Kiraithe MN, Nguta JM, Mbaria JM, Kiama SG. Evaluation of the use of *Ocimum suave* Willd. (Lamiaceae), *Plectranthus barbatus* Andrews (Lamiaceae) and *Zanthoxylum chalybeum* Engl. (Rutaceae) as antimalarial remedies in Kenyan folk medicine. J Ethnopharmacol. 2016;178: 266-271.

34. Rajkumar K, Malathi R. Anticancer activity of an ethanol extract of *Coleus forskohlii* root against gastric cancer cells *in-vitro*. Int J Pharm Sci Res. 2016;7(4): 1603-1611.

35. Wen OY, Mei YANG, Geng-gui LIU, Gui-xia HE, Gang PEI, Fang-lu DU. Study on chemical constituents of wild *Coleus forskohlii* Briq. from Yunnan. J Tradit Chin Med Hunan Univ. 2010;9.

36. Adams RP. Identification of essential oil components by gas chromatography/ mass spectrometry, 4th edn. Allured Publishing Corp., Carol Stream, Illinois. 2007.

37. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic. 1965;16(3): 144-158.

38. Kanyal J, Prakash O, Kumar R, Rawat DS, Pant AK. *Mosla dianthera* (Buch.-Ham. ex Roxb.) Maxim: Chemical compositions, biochemical assay, *in-vitro* antioxidant and anti-inflammatory activity of chloroform extract. Int J Chem Stud. 2019;7(4): 2807-2813.

39. Woisky RG, Salatino A. Analysis of propolis: some parameters and procedures for chemical quality control. J Apic Res. 1998;37(2): 99-105.

40. Sethi S, Prakash O, Pant AK. Antioxidant potential and total phenolic content of essential oil and various extracts from *Alpinia malaccensis* (Burm. f.) Roscoe. Asian J Chem. 2015;27(11): 4085-4088.

41. Joshi A, Pant AK, Prakash O, Stocki M, Isidorov VA. Phytochemical analysis, phenolic content and antioxidant activity of methanolic extract of *Caryopteris odorata* D. Don. Robin. J Herbal Drugs (An International Journal on Medicinal Herbs). 2018;9(4): 189-196.

42. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature. 1958;181(4617): 1199-1200.

43. Olabinri BM, Odedire OO, Olaleye MT, Adekunle AS, Ehigie LO, Olabinri PF. *In vitro* evaluation of hydroxyl and nitric oxide radical scavenging activities of artemether. Res J Biol Sci. 2010;5(1): 102-105.

44. Ramalingam R, Sushruthi S, Bindu BM, Ravinder AN. Antioxidant, free radical scavenging and invitro cytotoxic studies of ethanolic extract of *Leucas indica* var lavandulifolia and *Leucas indica* var nagalapuramiana. Asian Pac J Trop Biomed. 2012;2(3): 1637-1642.

45. Naskar S, Islam A, Mazumder UK, Saha P, Haldar PK, Gupta M. *In vitro* and *in vivo* antioxidant potential of hydromethanolic extract of *Phoenix dactylifera* fruits. J Sci Res. 2010;2(1): 144-157.

46. Fu W, Chen J, Cai Y, Lei Y, Chen L, Pei L, Zhou D, Liang X, Ruan J. Antioxidant, free radical scavenging, anti-inflammatory and hepatoprotective potential of the extract from *Parathelypteris nipponica* (Franch. et Sav.) Ching. J Ethnopharmacol. 2010;130(3): 521-528.

47. Yen GC, Duh PD. Antioxidative properties of methanolic extracts from peanut hulls. J Am Oil Chem' Soc. 1993;70(4): 383-386.

48. Goswami S, Kanyal J, Prakash O, Kumar R, Rawat DS, Srivastava RM, Pant AK. Chemical composition, antioxidant, antifungal and antifeedant activity of the *Salvia reflexa* Hornem. essential oil. Asian J Appl Sci. 2019;12(4): 185-191.

49. Hsu CL, Chen W, Weng YM, Tseng CY. Chemical composition, physical properties, and antioxidant activities of yam flours as affected by different drying methods. Food Chem. 2003;83(1): 85-92.

50. Parki A, Chaubey P, Prakash O, Kumar R, Pant AK. Seasonal variation in essential oil compositions and antioxidant properties of *Acorus calamus* L. accessions. Medicines. 2017;4(4), 81.

51. Ullah HA, Zaman S, Juhara F, Akter L, Tareq SM, Masum EH, Bhattacharjee R. Evaluation of antinociceptive, *in-vivo* & *in-vitro* anti-inflammatory activity of ethanolic extract of *Curcuma zedoaria* rhizome. BMC Complemen Altern Med. 2014;14(1): 346.

52. Javed MS, Kumar P, Kumar R, Tiwari AK, Bisht KS. *In vitro* antifungal, antibacterial and antioxidant activity of essential oil from the aerial parts of *Agrimonia aitchisonii* Schonbeck Temesy from Himalayan Region. World J Pharm Res. 2016;5(3): 1747-1763.

53. Wayne PA. National Committee for Clinical Laboratory Standards (NCCLS), Methods for dilution antimicrobial susceptibility test for bacteria that grow aerobically. Approved Standard-NCCLS document 6th edn. M7-A6. 2003.

54. Wayne PA. Clinical and Laboratory Standards Institute (CLSI), Methods for dilution antimicrobial susceptibility test for bacteria that grow aerobically. Approved Standard-NCCLS document 8th edn. M07-A8. 2009.

55. McKinney HH. Influence of soil temperature and moisture on infection of wheat seedlings by *Helminthosporium sativum*. J Agric Res. 1923;26(5): 195-218.

56. Belles X, Camps F, Coll J, Piulachs MD. Insect antifeedant activity of clerodane diterpenoids against larvae of *Spodoptera littoralis* (Boisd.) (Lepidoptera). J Chem Ecol 1985;11(10): 1439-1445.

57. Purwar JP, Srivastava RP. Toxicity and antifeedant activity of diflubenzuron (Dimilin 25 WP) against *S. litura*. Indian J Appl Entomol. 2003;17(1): 28-32.

58. Singh RP, Pant NC. Lycorine- A resistance factor in the plants of subfamily Amaryllidoideae (Amaryllidaceae) against desert locust, *Schistocerca gregaria* F. Experientia. 1980;36(5): 552-553.

59. Isman MB, Koul O, Luczynski A, Kaminski J. Insecticidal and antifeedant bioactivities of neem oils and their relationship to azadirachtin content. J Agr Food Chem. 1990;38(6): 1406-1411.

60. Pande D, Srivastava RP. Toxicity and antifeedant activity of indoxacarb (Avaunt 14.5 SC) against tobacco caterpillar, *Spodoptera litura* (Fab.). Insect Environ. 2003;9(2): 69-70.

61. Kogan M, Goeden RD. The host-plant range of *Lema trilineata daturaphila* (Coleoptera: Chrysomelidae). Ann Entomol Soc Am. 1970;63(4): 1175-1180.

62. Sharma RK, Bisht RS. Antifeedant activity of indigenous plant extracts against *Spodoptera litura* Fabricius. J. Insect Sci. (Ludhiana). 2008;21(1): 56-60.

63. Karthika K, Jamuna S, Abinaya G, Venkatachalapathi A, Thenmozhi K, Paulsamy S. Evaluation of anti-inflammatory and antioxidant properties of crude extract and forskolin from *Solena amplexicaulis* leaf. Indian J Pharm Sci. 2016;78(3): 377-387.

64. Aparna V, Dileep KV, Mandal PK, Karthe P, Sadasivan C, Haridas, M. Anti-inflammatory property of n-hexadecanoic acid: structural evidence and kinetic assessment. Chem Biol Drug Des. 2012;80(3): 434-439.

65. Fonseca DV, Salgado PR, de Carvalho FL, Salvadori MGS, Penha ARS, Leite FC, Borges CJS, Piuvezam MR, Pordeus LCDM, Sousa DP, Almeida RN. Nerolidol exhibits antinociceptive and anti-inflammatory activity: involvement of the GABA ergic system and proinflammatory cytokines. Fund Clin Pharmacol. 2016;30(1): 14-22.

66. Wu X, Li X, Xiao F, Zhang Z, Xu Z, Wang H. Studies on the analgesic and anti-inflammatory effect of bornyl acetate in volatile oil from *Amomum villosum*. Zhong Yao Cai. 2004;27(6): 438-439.

67. Bonjardim LR, Cunha ES, Guimaraes AG, Santana MF, Oliveira MG, Serafini MR, Araujo AA, Antoniolli AR, Cavalcanti SC, Santos MR, Quintans-Junior LJ. Evaluation of the anti-inflammatory and antinociceptive properties of p-cymene in mice. Z Naturforsch C. 2012;67(1-2): 15-21.

68. Vijayalaxmi A, Bakshi V, Begum N, Kowmudi V, Yadav NK, Reddy Y. Anti-arthritic and anti-inflammatory activity of β caryophyllene against freund's complete adjuvant induced arthritis in wistar rats. J Bone Rep Recommend. 2015;1(2): 1-10.

69. Abrahim D, Braguini WL, Kelmer-Bracht AM, Ishii-Iwamoto EL. Effects of four monoterpenes on germination, primary root growth, and mitochondrial respiration of maize. J Chem Ecol. 2000;26(3): 611-624.

70. Singh HP, Batish DR, Kaur S, Ramezani H, Kohli RK. Comparative phytotoxicity of four monoterpenes against *Cassia occidentalis*. Ann Appl Biol. 2002;141(2): 111-116.

71. Singh HP, Batish DR, Kaur S, Arora K, Kohli RK. α-pinene inhibits growth and induces oxidative stress in roots. Ann Bot. 2006;98(6):1261-1269.

72. Martino LD, Mancini E, Almeida LFRD, Feo VD. The antigerminative activity of twenty-seven monoterpenes. Molecules. 2010;15(9): 6630-6637.

73. Vattikonda SR, Amanchi NR, Raja SS. Antifeedant activity of forskolin, an extract of *Coleus forskohlii*, against *Papilio demoleus* L. (Lepidoptera: Papilionidae) larvae. Eur J Exp Biol. 2014;4(1): 237-241.

74. Nerio LS, Olivero-Verbel J, Stashenko E. Repellent activity of essential oils: a review. Bioresour Technol. 2010;101(1): 372-378.

75. Gillij YG, Gleiser RM, Zygadlo JA. Mosquito repellent activity of essential oils of aromatic plants growing in Argentina. Bioresour Technol. 2008;99(7): 2507-2515.

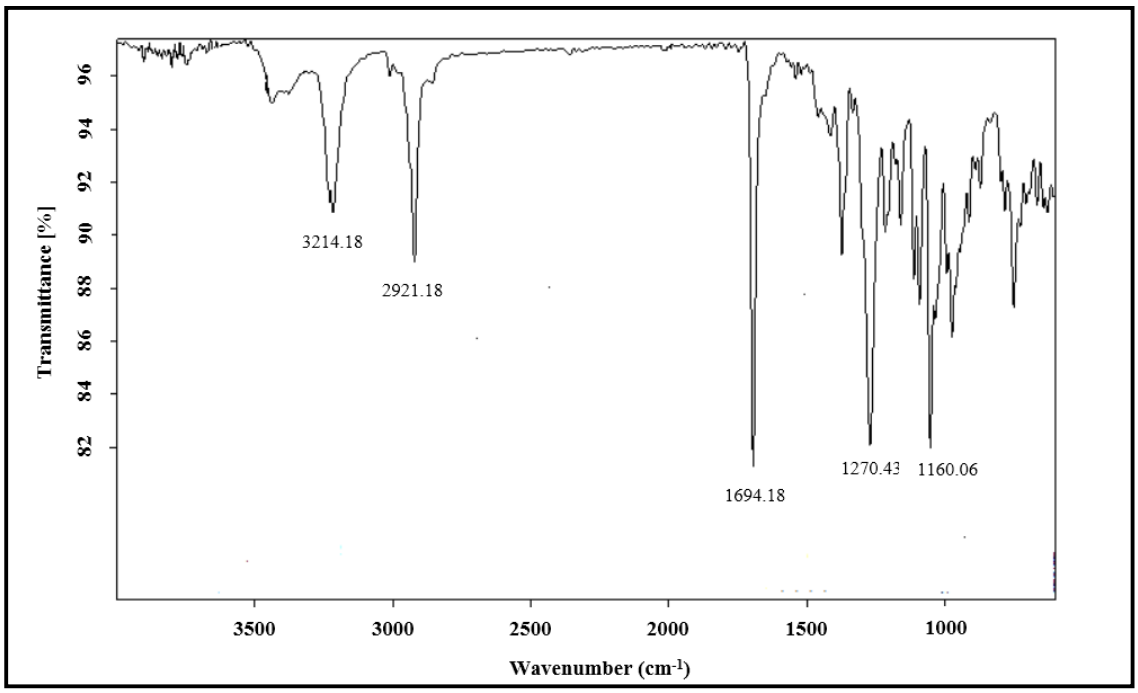


Fig 1(a): FT-IR spectrum of compound #01

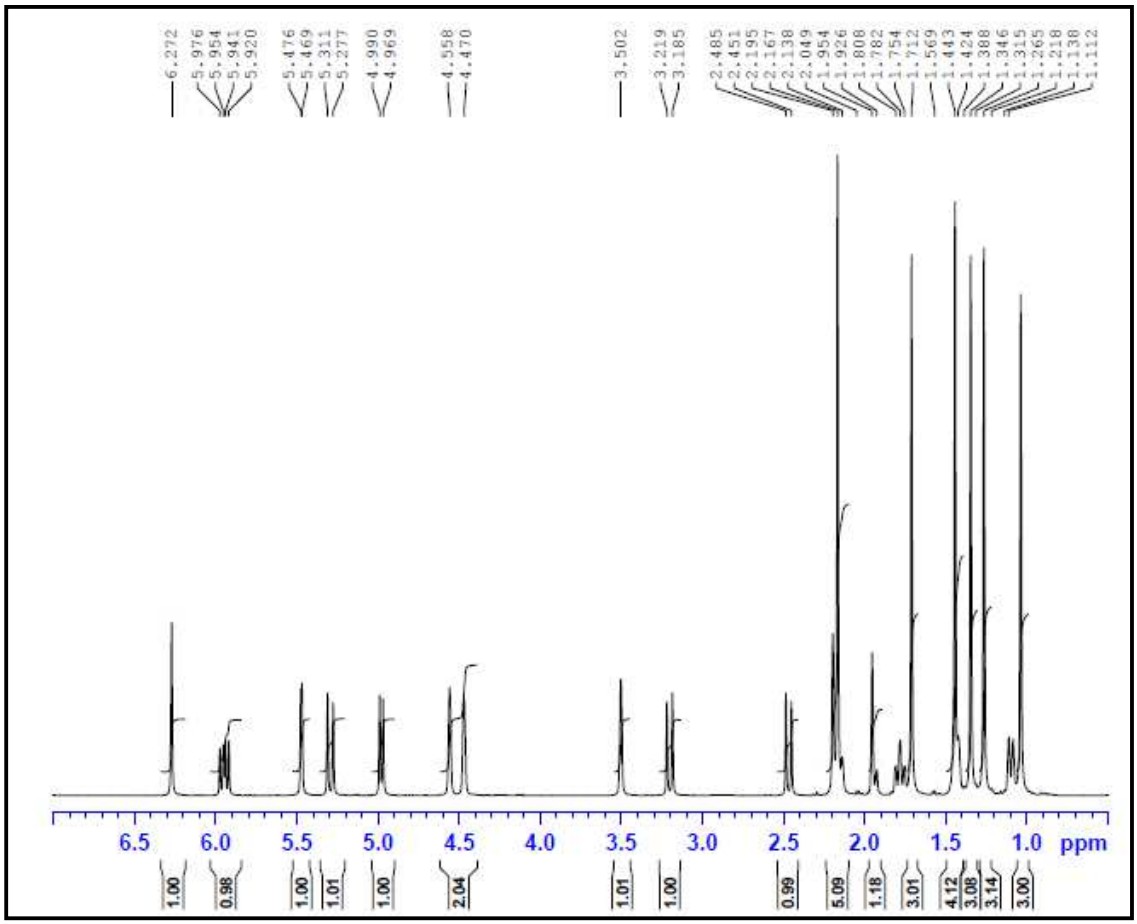


Fig 1(b): ^{1}H NMR spectrum of compound #01

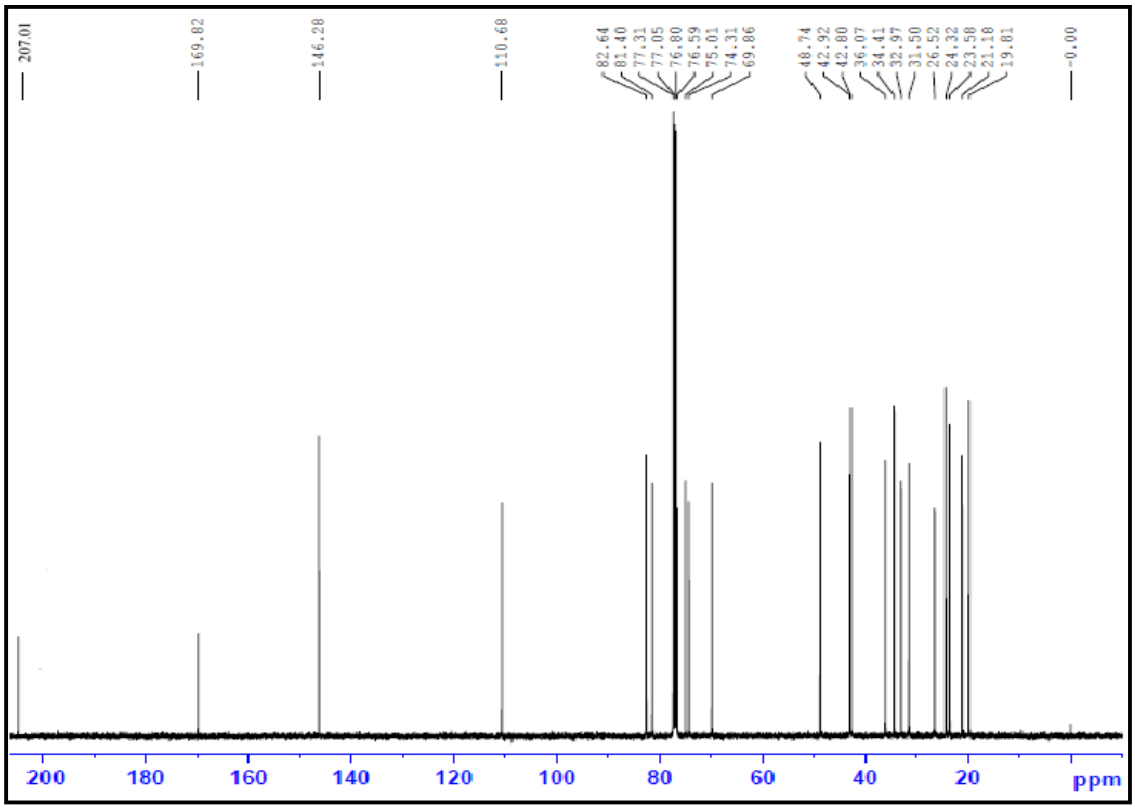


Fig 1(c): ^{13}C NMR spectrum of compound #01

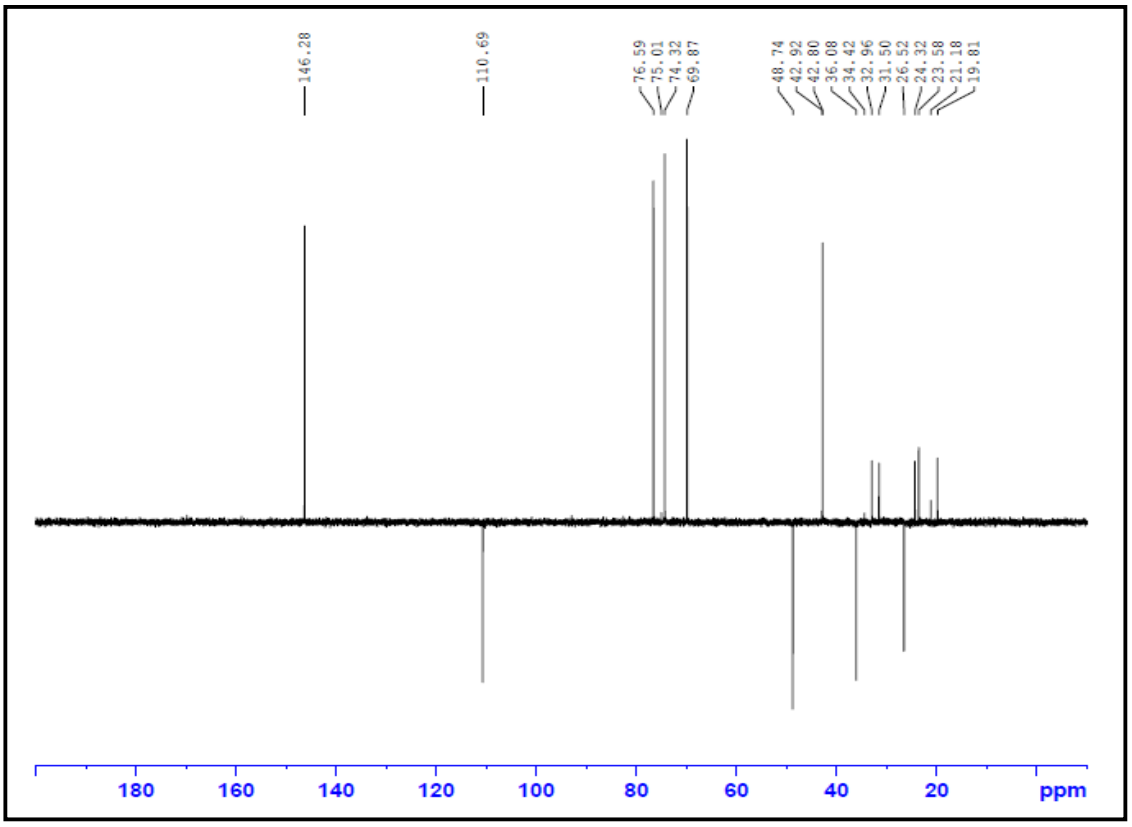


Fig 1(d): DEPT-135 spectrum of compound #01

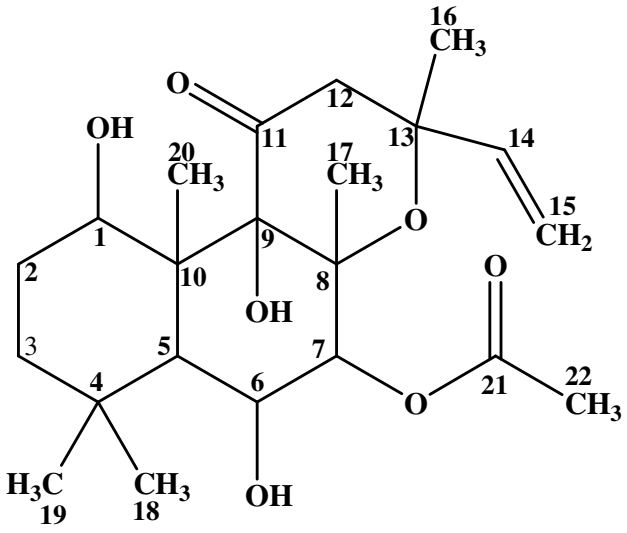
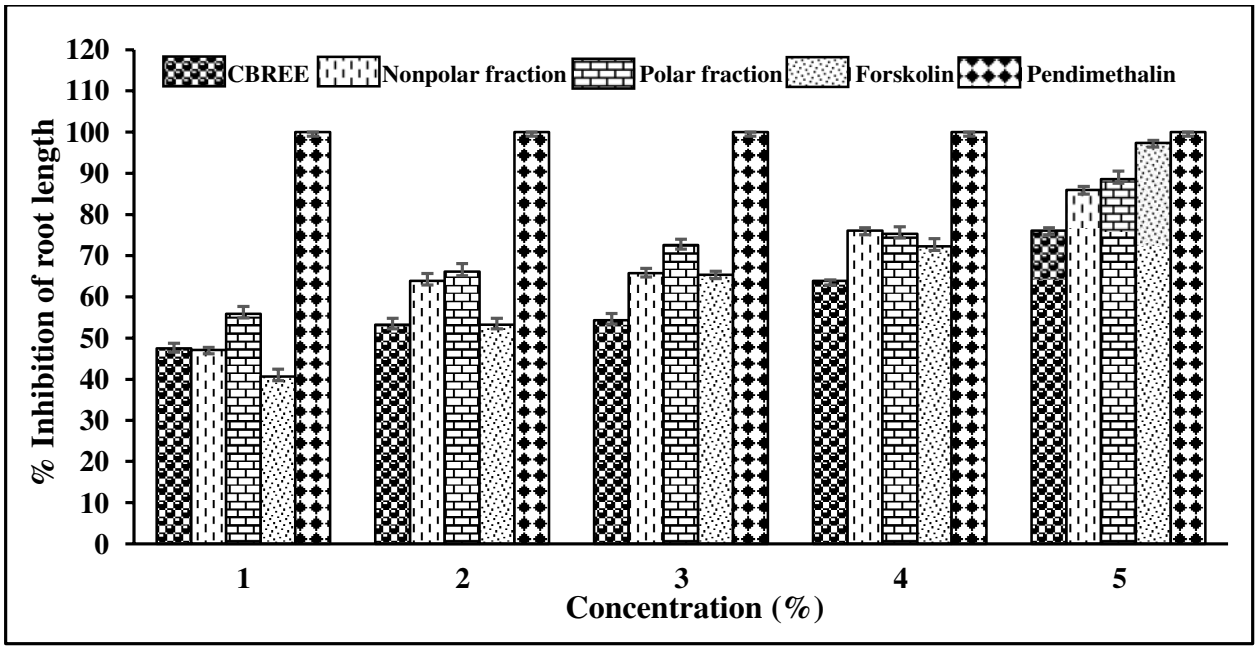
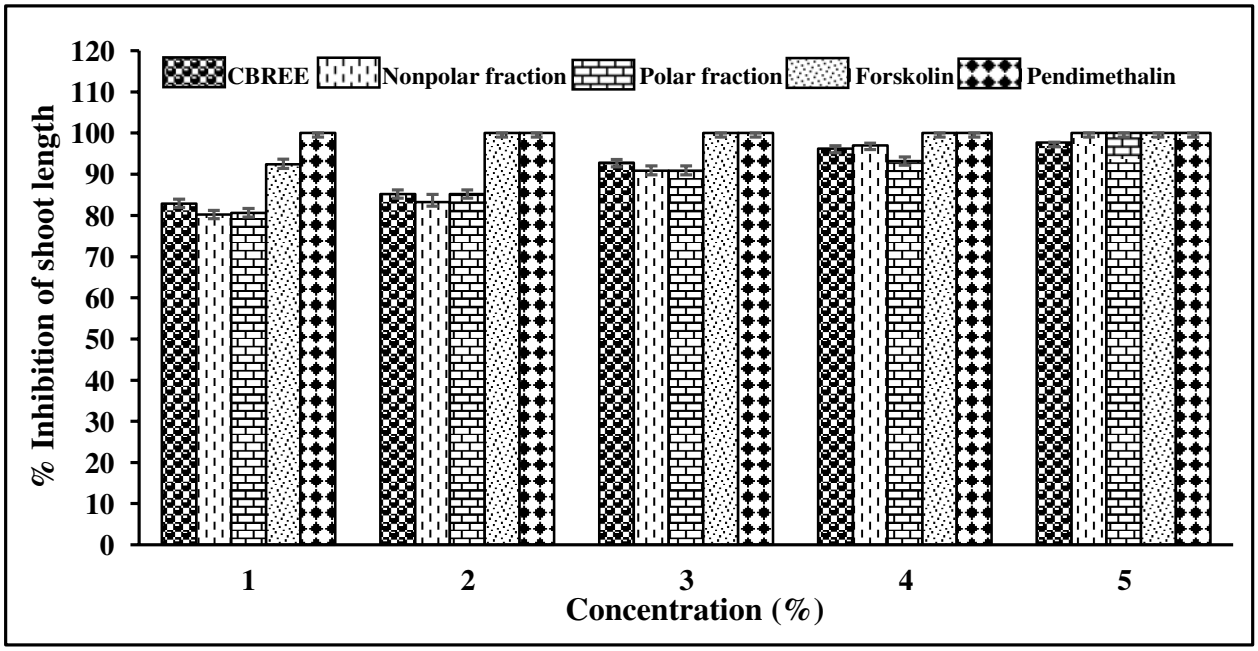


Fig 1(e): Structure of compound #01



CBREE= *Coleus barbatus* root part ethyl acetate extract

Figure 2. Mean percent inhibition of root length by extracts and isolated compound from root part of *C. barbatus*



CBREE= *Coleus barbatus* root part ethyl acetate extract

Figure 3. Mean percent inhibition of shoot length by extracts and isolated compound from root part of *C. barbatus*

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Tables.docx