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Chemical composition, antimicrobial, and antioxidant activities of the essential oils from stem, leaves, and seeds of *Caryopteris foetida* (D. don) Thell.

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Caryopteris foetida (D. Don) Thell. also known as stinking blue beard is an aromatic shrub of family verbenaceae. The essential oils from the leaf, stem, and seeds of *C. foetida* were extracted and analyzed in search of a novel compound. Over 73 constituents were identified from the essential oils of leaf, seeds, and stem. The major compound identified in the leaf oil was δ-cadinene (15.4%) followed by β-caryophyllene (7.8%), (E)-β-farnesene (8.3%), γ-cadinene (7.5%), spathulenol (7.2%), and τ-muurolol (5.1%). The stem oil predominantly consisted of methyl 7-methylcyclopenta[c]pyran-4-carboxylate (15.8%) along with a significant amount of δ-cadinene (11.6%) and γ-cadinene (5.6%), whereas seed oil was characterized by the presence of β-caryophyllene (14.3%) along with α-humulene (8.6%) and β-bisabolene (9.4%). The essential oils were screened for their *in vitro* antioxidant potential in terms of radical scavenging, metal chelating and reducing assay. The leaf oil exhibited strong DPPH radical scavenging (IC_{50} =5.1±0.2 µg/mL) and reducing activity (IC_{50} =3.6±0.5 µg/mL). The antibacterial potential was tested against *Salmonela typhimurium, Escherichia coli, Staphylococcus Aureus*, and *Bacillus megaterium*. Maximum activity was exhibited by the essential oils from the leaf and seed. The essential oils were also analyzed for their *in vitro* anti-inflammatory activity by the protein denaturation method. Both the leaf and stem essential oils exhibited good anti-inflammatory activity with IC_{50} value of 12.8±0.0 µg/mL and 17.3±0.0 µg/mL respectively.

Keywords: Anti-inflammatory, Antioxidant, BHT, *Caryopteris foetida*, δ-cadinene.

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Introduction

Caryopteris foetida (D. Don) Thell (Stinking bluebeard) syn. *Caryopteris grata* is an aromatic shrub of the family verbenaceae[1]. *C. foetida*, is a straggling shrub often purplish or brownish and commonly found in the central Himalaya, from Kumaun to Nepal, and in other parts of India and Pakistan, at altitudes of 1200-2200 m. Fragrant foliage and violet-blue flowers formed in the late summer and autumn are the characteristics of *Caryopteris* species[2].

The genus *Caryopteris* consists of 16 species native to China and East Asia[3]. Three species of genus *Caryopteris* have been reported from India[4]. Several species of genus *Caryopteris* such as *C. forrestii* Diels, *C. incana* (Thunberg ex Houttuyn) Miquel**,** *C. mongholica* Bunge, *C. tangutica* Maximowicz**,** and *C. trichosphaera* W. Smith have been explored for phytochemical composition[5-9]. New glycosides have been identified from *C. incana*[10] and new alkaloids have been isolated from *C. mongholica*[11]. Estragole (24.8%), linalool (14.0%), 1, 8-cineol (5.2%) and δ-guaiene (4.1%) were reported from the essential oil of *C. incana*[5]. A report on the essential oil composition of *C. odorata* revealed β-caryophyllene (37.67%), α-longipinene (13.39%), β-longipinene (10.34%), α-humulene (9.79%) and caryophyllene oxide (8.46%) as the major constituents[12]. The essential oil of *Caryopteris clandonensis* contained α-copaene (8.3%), limonene (7.2%), δ-cadinene (6.3%), *trans*-p-mentha-2,8-dien-1-ol (4.6%), *trans*-p-mentha-1(7),8-dien-2-ol (4.5%), *cis*-p-mentha-2,8-dien-1-ol (4.0%), and hotrienol (3.8%)[13]. The antimicrobial potential of methanolic extract of *Caryopteris grata* was reported against *Pseudomonas aureginosa*, *Staphylococcus aureus* and *Micrococcus luteus*[14].

A study on the composition of *C. foetida* essential oil from the whole aerial part has been reported[15], but to the best of the authors' knowledge, no previous

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study on the chemistry, antioxidant and antimicrobial activities of the essential oils from the stem, seed, and leaves of *C. foetida* has been published in the literature till date. In the present communication, the composition of the essential oils obtained separately from stem, leaves, and seeds of *C. foetida* growing wildly in North India have been studied along with its antioxidant and antimicrobial activities.

Materials and Methods

Plant material

The fresh aerial parts of *C. foetida* were collected in the month of February 2018 from Nainital (Altitude 2117 m, Latitude 29.39743 ^{o}N, Longitude 79.44686 ^{o}E) in their seeding stage. The plant identification was done at the Dept. of Biological Sciences, G. B. Pant University and the voucher specimens (GBPUH specimen Acc. No. 1011) deposited in the herbarium. Fresh leaves, seeds, and stem were collected for oil extraction.

Extraction of essential oil

Fresh aerial parts (leaves, seeds, and stem) were crushed and transferred to a Clevenger apparatus separately for essential oil extraction using the hydro-distillation method for 5-6 hours. The essential oils were extracted with hexane and dried over anhydrous Na_2SO_4. The oils were stored in a refrigerator at 4 ^{o}C for further analysis.

GC-MS analysis

The analyses were performed on an Agilent 7890A chromatograph with Agilent 5975C mass detector, on a capillary non-polar column HP-5MS (30 m x 0.25 mm x 0.25 μm) with helium as carrier gas and flow rate of 1 mL/min. An injection of a 1.0 μL sample was performed using an Agilent 7693A auto sampler. The injector worked in a split (1:10) mode at an injector temperature of 300 °C. The initial column temperature was 50 °C, rising to 325 °C, at 3 °C/min; the final temperature was held in an isothermal mode for 10 minutes. The ion source temperature was 230 °C. Electron ionization mass spectrum was obtained at ionization energy 70 eV. The detection was performed in a full scan mode from 41 to 800 a.m.u. After integration, the content (%) of each component in the total ion current was calculated. The identification of compounds was based on a comparison of MS spectra with computer mass library NIST 2012 along with the relative retention indices (RI, non-polar column). The experimental retention times of the compounds were compared to the RTs of the *n*-alkane standard mixture (C_{10}-C_{40} Sigma Aldrich).

Antioxidant activity

DPPH radical scavenging activity

DPPH radical scavenging activity was determined by following the reported protocols with some modifications[16-17]. The assay mixture contained 5 mL of 0.004% methanol solution of DPPH and different amount (5-25 μg/mL) of essential oils. The solutions were rapidly mixed and scavenging capacity was measured spectrophotometrically using Thermo Scientific Evolution 201 series by monitoring the decrease in absorbance at 517 nm. BHT was used as positive control while reaction mixture (DPPH radical solution) minus essential oil solution was taken as control. Inhibition of free radical by DPPH in per cent (IC %) was calculated by using the formula

$$IC\% = \frac{Ao\text{-}As}{Ao} \times 100$$

where A_0= absorbance value of control sample, A_s= absorbance value of test sample, and IC= Inhibitory concentration.

Per cent inhibition was plotted against concentration and the standard curve was drawn using standard antioxidant (BHT) to calculate the IC_{50} values for standard and essential oils. A lower IC_{50} value indicated more radical scavenging activity.

Metal chelating activity

The metal chelating activity of Fe^{2+} was examined by the standard methods, generally being practiced and based on the principle of the Fe^{2+} chelating ability[18]. The absorbance of the ferrous iron-ferrozine complex formed was measured at 562 nm. The reaction was performed by adding 0.1 mL of 2 mM $FeCl_2.4H_2O$, 0.2 mL of 5 mM ferrozine and 4.7 mL of methanol with different concentrations of test samples (5-25 μg/mL). After incubation, the absorbance of test samples was measured at 562 nm. The metal chelating activity of test samples in percentage was calculated by the formula as follows

$$IC\% = \frac{Ao\text{-}As}{Ao} \times 100$$

where A_0= absorbance value of control sample, A_s= absorbance value of test sample, and IC= Inhibitory concentration.

Per cent inhibition was plotted against concentration and the standard curve was drawn using standard antioxidant (citric acid) to calculate the IC_{50} values for standard and essential oils.

Reducing power

The reducing power of essential oils was determined by the method developed earlier with some modifications[19]. The different concentrations of essential oils (5-25 µg/mL) were mixed with 2.5 mL of phosphate buffer (200 mM, pH= 6.6) and 2.5 mL of 1% potassium ferricyanide, $K_3[Fe(CN)_6]$. After 20 minutes of incubation at 50±1ºC, 2.5mL of trichloroacetic acid was added to the mixtures, followed by centrifugation at 650 RPM for 10 minutes. The supernatant (1.0 mL) was mixed with 5 mL distilled water and 1 mL of 0.1% ferric chloride. The absorbance of the resultant solution was measured at 700 nm using a UV spectrophotometer (Thermo Scientific Evolution 201 series). All the readings were taken in triplicate and BHT was used as the standard.

The reducing power of samples was calculated using the formula

$$\text{Reducing activity \%} = \frac{\text{Ao-As}}{\text{Ao}} \times 100$$

where A_0= absorbance value of control sample and A_s= absorbance value of the test sample.

Per cent inhibition was plotted against concentration and the standard curve was drawn using standard antioxidant (BHT) to calculate the RP_{50} values for standard and essential oils. The lower RP_{50} value indicates greater reducing power ability.

Assay of nitric oxide scavenging activity

Nitric oxide (NO) was generated from sodium nitroprusside (SNP) and was measured by the Griess reagent (1% sulphanilamide, 0.1% naphthylethylenediamine dichloride and 2 mL orthophosphoric acid). It is based on the principle that SNP in the aqueous solution at physiological pH spontaneously generates NO, which interacts with oxygen to produce nitrite ions that can be estimated by the use of Griess Reagent. Scavengers of NO compete with oxygen leading to reduced production of NO. Exactly 2 mL of SNP (10 mM) in phosphate buffer saline (PBS) pH 7.4 was mixed with different concentrations of essential oil (5–25 µg/mL) and incubated at 25 °C for two and a half hours. The samples were reacted with 1 mL of Griess reagent. Absorbance was measured at 546 nm. Ascorbic acid was taken as standard[20].

Nitric oxide scavenging activity was calculated by the following equation

$$\text{IC\%} = \frac{\text{Ao-As}}{\text{Ao}} \times 100$$

where, A_0= absorbance value of control sample, A_s= absorbance value of test sample, and IC= inhibitory concentration.

Per cent inhibition was plotted against concentration and the standard curve was drawn using standard antioxidant (ascorbic acid) to calculate the IC_{50} values for standard and essential oils.

Super oxide radical scavenging activity

Exactly 1 mL of nitroblue terazolium (156 Mm), 1 mL nicotinamide adenine dinucleotide (reduced) (468 Mm), and 0.1 mL of phenanzine methosulphate solution (PMS) in 0.1 M of phosphate buffer solution (pH 7.4) were added to different concentration of essential oils and incubated at 25 °C for 5 minutes. The absorbance was read at 560 nm against a blank containing all reagents except PMS. Ascorbic acid was taken as standard[21].

Super oxide radical scavenging activity was calculated by the following equation

$$\text{Superoxide radical scavenged (\%)} = \text{IC\%} = \frac{\text{Ao-As}}{\text{Ao}} \times 100$$

where, A_0= absorbance value of control sample, A_s= absorbance value of test sample, and IC= inhibitory concentration.

Per cent inhibition was plotted against concentration and the standard curve was drawn using standard antioxidant (ascorbic acid) to calculate the IC_{50} values for standard and essential oil.

***In-vitro* anti-inflammatory activity**

Inhibition of albumin denaturation

In vitro anti-inflammatory activity was carried out by the method developed earlier[22]. The reaction mixture (5 mL) consisted of 0.2 mL of egg albumin (from fresh hen's egg), 2.8 mL of phosphate buffered saline (PBS, pH 6.4) and 2 mL of varying amount of essential oils (5-25 µg/mL respectively). A similar volume of double-distilled water served as a control. The mixtures were incubated at (37±2 °C) in an incubator for 15 minutes and then heated at 70 °C for 5 minutes. After cooling, the absorbance was measured at 660 nm. Diclofenac Sodium was used as a reference drug and treated similarly for

the determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the following formula

$$\% \text{ Inhibition} = \left(\frac{Vt}{Vc} - 1\right) \times 100$$

where, V_t = absorbance of the test sample and V_c = absorbance of control.

Per cent inhibition was plotted against concentration and the standard curve was drawn using standard (Diclofenac Sodium) to calculate the IC_{50} values for standard and essential oil.

Antibacterial activity

Antibacterial activity of essential oils was evaluated by the agar well diffusion with some modifications on nutrient agar[23,24]. The antimicrobial screening was done against *Salmonella typhimurium*, *Escherichia coli*, *Bacillus megaterium* and *Staphylococcus aureus.* 20 mL of molten nutrient agar was poured into the Petri plates and left to solidify. Nutrient agar plates were inoculated with bacterial strain under aseptic conditions and wells (diameter = 6 mm) were filled with 50 µL of the test samples and incubated at 37 °C for 24 hours. After the incubation period, the diameter of the growth inhibition zones was measured. Single colonies developed after 18 to 24 hours on agar plates were used to prepare the bacterial suspension containing 1.0×10^6 colony-forming units (CFU)/mL. After incubation, confluent bacterial growth was observed. Inhibition of the bacterial growth was measured in mm. Reference commercial antimicrobial (Refamycin) was used to compare the antimicrobial potential. All the experiments were performed in triplicate.

Determination of the minimum inhibitory concentration (MIC)

The applied agar dilution susceptibility test was based on modified methods of NCCLS and CLSI[25]. In the agar-well diffusion technique, serial dilutions of the essential oils were prepared by diluting the oil with dimethyl sulfoxide (DMSO) to achieve a decreasing concentration range from 25 to 5 µg/mL. A 100 µL suspension containing 1×10^6 CFU/mL of bacteria spread on nutrient agar plates. The wells were filled with 50 µL of essential oils solutions in the inoculated nutrient agar plates. The bacterial plates were incubated at 37±2°C for 24 hours. The MIC was defined as the minimum concentration of the oil inhibiting the visible growth of each bacterium on the agar plate, so the lowest concentration of each essential oil showing a clear zone of inhibition was taken as the MIC. DMSO was used as the negative control, while rifamycin was used as positive control.

Statistical analysis

All experiments were performed thrice and the results averaged data were expressed as mean±SD. Linear regression analysis was used to calculate IC_{50} for each essential oils. For statistical analysis, SPSS 16.0 software was used.

Results and Discussion

Phytochemical analysis

The results of GC-MS analysis of essential oil from leaf, stem, and seeds of *C. foetida* have been presented in Table 1. The yield of oils was found to be 0.10, 0.09, and 0.06% w/v for leaf, seed, and stem essential oil, respectively. Over 73 components were identified collectively from the leaves, seed, and stem essential oils. In the leaf essential oil of *C. foetida*,

Table 1 — Comparative chemical composition of leaf, seed and stem oil of *Caryopteris foetida* (D.Don) Thell.

S. No.	Compound	$RI_{cal.}$	$RI_{lib.}$	% Contribution		
				Leaf	Stem	Seed
1	1-octen-3-ol	-	979	4.2	0.6	0.4
2	3-octanone	-	987	0.2	-	-
3	β-myrcene	-	991	-	-	0.1
4	(z)-3-hexen-1-yl acetate	1007	1007	0.7	-	t
5	limonene	1027	1027	-	-	t
6	(*Z*)-β-ocimene	1037	1037	0.1	-	0.3
7	(*E*)-ocimene	1047	1048	2.2	1.5	6.9
8	rosefuran	1097	1098	-	-	0.1
9	linalool	1100	1100	0.6	1.4	1.2
10	nonanal	1104	1105	-	-	0.1
11	(*E*)-myroxide	1141	1143	-	-	0.1

(*Contd.*)

Table 1 — Comparative chemical composition of leaf, seed and stem oil of *Caryopteris foetida* (D.Don) Thell. (*Contd*.)

S. No.	Compound	$RI_{cal.}$	$RI_{lib.}$	% Contribution	S. No.	Compound
12	α-terpineol	1188	1191	-	-	0.1
13	methyl salicylate	1192	1193	0.3	0.4	0.9
14	decanal	1206	1206	-	0.4	-
15	neral	1240	1241	-	-	0.1
16	α-citral	1269	1272	-	-	0.2
17	dihydroedulan	1291	1288	0.3	-	0.1
18	bicycloelemene	1336	1339	0.9	0.5	0.4
19	α-cubebene	1349	1348	0.5	0.4	0.2
20	α-ylangene	1370	1372	-	-	0.1
21	α-copaene	1374	1376	3.3	1.5	1.1
22	β-bourbonene	1383	1387	1.3	1.1	0.5
23	β-cubebene	1389	1392	0.2	-	0.1
24	β-elemene	1391	1392	-	-	0.2
25	α-gurjunene	1408	1412	0.3	-	0.1
26	β-caryophyllene	1417	1416	7.8	6.4	14.3
27	β-copaene	1427	1432	0.8	0.5	0.7
28	aromadendrene	1436	1440	0.6	0.4	0.3
29	isogermacrene D	1442	1445	0.2	-	0.3
30	α-humulene	1451	1457	3.7	3.2	8.6
31	(*E*)-*β*-famesene	1458	1458	8.3	3.1	6.3
32	*allo*-aromadendrene	1460	1464	-	2.5	-
33	cis-muurola-4(14),5-diene	1460	1465	0.3	-	0.2
34	trans-cadina-1(6),4-diene	1472	1475	0.2	0.4	0.1
35	γ-muurolene	1475	1480	1.8	1.6	1.1
36	D-germacrene	1479	1480	5.1	3.1	6.4
37	trans- muurola-4(14),5-diene	1489	1493	0.4	0.3	0.2
38	4-epi-cubebol	1492	1495	1.2	0.5	0.8
39	bicyclogermacrene	1494	1500	1.5	0.7	0.9
40	α-muurolene	1498	1502	2.1	1.8	1.0
41	α-farnesene	1508	1510	2.1	3.3	-
42	β-bisabolene	1510	1511	-	-	9.4
43	cubebol	1513	1516	-	-	3.2
44	γ-cadinene	1512	1517	7.5	5.6	-
45	δ-cadinene	1522	1527	15.4	11.6	7.4
46	trans-cadina-1,4-diene	1530	1533	0.3	0.4	0.2
47	α-cadinene	1536	1541	0.4	0.4	0.3
48	α-calocorene	1540	1544	0.2	0.6	0.1
49	isocaryophyllene epoxide	1549	1556	-	-	0.1
50	salviadienol	1552	1555	-	-	0.1
51	β-calocorene	1561	1565	-	-	0.1
52	(*E*)-nerolidol	1563	1565	0.3	0.6	0.4
53	spathulenol	1574	1580	7.2	4.8	4.0
54	caryophyllene oxide	1579	1586	1.8	3.9	2.7
55	methyl 7-methylcyclopenta[c]pyran-4-carboxylate	1588	-	-	15.8	-
56	salvial-4(14)-en-1-one	1591	1598	0.2	0.3	0.1
57	humulene-2,3-epoxide	1595	1603	-	-	0.2
58	copaborneol	1600	1605	1.0	1.1	0.7

(*Contd*.)

Table 1 — Comparative chemical composition of leaf, seed and stem oil of *Caryopteris foetida* (D.Don) Thell. (*Contd*.)

S. No.	Compound	$RI_{cal.}$	$RI_{lib.}$	% Contribution	S. No.	Compound
59	humulene-6,7-epoxide	1605	1612	0.7	1.1	1.3
60	1,10-di-*epi*-cubenol	1612	1618	0.2	-	0.3
61	1-*epi*-cubenol	1625	1628	0.6	0.7	0.4
62	isospathulenol	1635	1640	0.3	-	-
63	τ-muurolol	1639	1644	5.1	4.8	3.4
64	δ-cadinol	1643	1649	0.8	0.9	0.6
65	α-cadinol	1652	1658	5.3	4.4	4.0
66	(*Z*)-3-caryophylla-3,8(13)-dien-5β-ol	1668	1676	-	0.2	0.3
67	cadina-3,10(15)-dien-5β-ol	1682	1684	-	0.4	0.4
68	3-hydroxy-β-muurolene	1699	1701	0.2	-	0.1
69	oplopanone	1733	1739	-	-	0.2
70	benzyl benzoate	1759	1764	-	-	0.3
71	hexahydrofarnesyl acetone	1845	1846	-	0.4	0.5
72	benzyl salicylate	1862	1867	-	-	0.1
73	palmitic acid	1962	1962	-	0.3	-
74	n-tricosane	2300	2300		0.3	
75	n-pentacosane	2500	2500	-	0.4	-
Total				98.7	94.6	95.4
Monoterpene hydrocarbons				2.3	1.5	7.3
Oxygenated monoterpenes				0.6	1.4	1.8
Sesquiterpene hydrocarbons				65.2	49.4	60.6
Oxygenated sesquiterpenes				24.9	23.7	23.3
Others				5.7	18.6	2.4

Mode of identification = RI, MS, t = trace (< 0.05%)

over 46 components were identified corresponding to 98.7% of the total essential oil composition. The essential oil of the leaf contributed 93% of terpene derivatives (65.2% sesquiterpenes and 24.9% sesquiterpinoides, respectively). Sesquiterpene hydrocarbons (65.2%) were prevalent compared to oxygenated sesquiterpene (24.9%). The major compound in the leaf essential oil was δ-cadinene (15.4%) followed by (*E*)-β-farnesene (8.3%), γ-cadinene (7.5%), spathulenol (7.2%), α-cadinol (5.3%), τ-murrolol (5.1%) and germacrene-D(5.1%).The other constituents present in minor quantity in leaf oil were (*E*)-ocimene (2.2%), α-copaene (3.3%), β-bourbonene (1.3%), α-humulene (3.7%), α-farnesene (2.1%), γ-murrolene (1.8%), α-murrolene (2.1%), bicyclogermacrene (1.5%) and caryophyllene oxide (1.8%). Forty-five compounds accounting for 94.6% of total composition were identified in the stem essential oil. Sesquiterpene hydrocarbons (49.4%) constituted the major fraction of essential oil composition. The stem essential oil of *C.foetida* contains pyran ring containing compound, methyl-7-methylcyclopenta[c]pyran-4-carboxylate (15.8%) along with the major presence of δ-cadinene (11.6%), γ-cadinene (5.6%), spathulenol (4.8%), τ-murrolol (4.8%) and α-cadinol (4.4%), caryophyllene oxide (3.9%), α-farnesene (3.3%), (*E*)-β-farnesene (3.1%), germacrene-D (3.1%), α-humulene (3.2%), *allo*-aromadendrene (2.5%).

Over sixty-four constituents contributing 95.4% of the total oils were identified in seeds essential oil. Sesquiterpenes (60.6%) were the dominating compounds in the seed essential oil with β-Caryophyllene (14.3%) β-bisabolene (9.4%), α-humulene (8.6%), δ-cadinene (7.4%), germacrene-D (6.4%) and (*E*)-β-farnesene (6.3%) were the major compounds. Comparing the three essential oils, the highest amount of sesquiterpenes were found in the leaf essential oil (65.2%) followed by seed essential oil (60.6%) whereas lowest amount was found in stem oil (49.4%). For oxygenated sesquiterpenes, the highest percentage were detected in similar amount among the three oils (24.9%, 23.7%, 23.3% for leaf, stem and seed oil, respectively). The major components common in all three oils were β-caryophyllene, E-ocimene, α-humulene, (*E*)-β-

farnesene, germacrene-D, δ-cadinene, spathulenol, τ-murrolol and α-cadinol, whereas common constituents in minor quantity were 1-octen-3-ol, methyl salicylate, bicycloelemene, α-cubebene, linalool, α-copaene, β-bourbonene, β-copaene, aromadedrene, trans-cadina-1(6),4-diene, γ-murrolene, trans-murrola-4(14),5-diene, 4-epi-cubebol, bicyclogermacrene, α-murrolene, trans–cadina-1,4-diene, α-cadinene, α-calacorene, (*E*)-nerolidol, caryophylleneoxide, salvia-4(14)-en-1-one, copaborneol, humulene-6,7-epoxide,1-epi-cubenol and δ-cadinol.

Interestingly β-bisabolene (9.4%), cubebol (3.2%), β-myrcene (0.1%), rosefuran (0.1%), nonanal (0.1%), E-myroxide (0.1%), α-terpineol (0.1%), neral (0.1%), α-citral (0.2%), α-ylangene (0.1%), β-elemene (0.2%), isocaryophyllene epoxide-A (0.1%), salviadienol (0.1%), β-calocorene (0.1%), oplopanone (0.2%), benzyl benzoate (0.3%) and benzyl salicylate (0.1%) were exclusively present in the seed essential oil. Methyl-7-methylcyclopenta[c]pyran-4-carboxylate, the major constituent of stem oil was not found in leaf and seed oil. In addition, *allo*-aromadendrene found in stem oil (2.5%) was completely absent in leaf and seed oil. Palmitic acid (0.3%), n-tricosane (0.3%), n-pentacosane (0.4%) although present in minor quantity in seed oil were not found in leaf and stem oil. The only report on essential oil composition of whole aerial parts of *C. foetida* revealed the dominance of spathulenol (30.1%) followed by humulene epoxide II (8.4%), epi-α-cadinol (6.8%), α-muurolol (6.1%), α-humulene (5.0%), α-selinene (3.6%), (*Z*)-α-bisabolene (3.1%), germacrene D-4-ol (3.0%) and β-caryophyllene (2.5%)[15]. Our results revealed that spathulenol although present in a significant amount 7.2, 4, and 4.8% in leaf, seed, and stem oil, respectively was not the major compound in essential oil from any of the parts of *C. foetida*.

These qualitative and quantitative differences in the chemical composition of essential oils could be attributed to several factors such as geographical location, climatic effects on the plants, harvest season, nature of the soil, age of the plant parts (young or adult), the state of plant material used (dried or fresh), the part of the plant used, time of collection, *etc*.

Antioxidant activity

DPPH Radical scavenging activity

The DPPH radical scavenging activity was performed and IC_{50} was calculated (Table 2). The leaf oil (IC_{50}=5.1±0.2 µg/mL) and seeds oil (IC_{50}=6.2±0.0 µg/mL) was found most effective in scavenging radicals whereas stem essential oil (IC_{50}=18.6±0.2 µg/mL) was least effective when compared to synthetic standard BHT (IC_{50}=0.3±0.1 µg/mL). All the tested essential oils showed radical scavenging activity in a dose-dependent manner.

Reducing power activity

The tested essential oils exhibited reducing potential in a dose-dependent manner. The leaf oil was found most effective with IC_{50}= 3.6±0.5 µg/mL followed by stem essential oil (IC_{50}=4.8±0.8 µg/mL) and seeds essential oil (IC_{50}= 5.3±1.4 µg/mL) (Table 2). The ferric reducing antioxidant power of all essential oils showed a different order from the DPPH assay results. These differences could be attributed to the different stoichiometry of the DPPH and reducing power assay reactions. In addition, the compositional differences in essential oils and their different solubility may also affect their antioxidant activity.

Metal chelating activity

Ferrous ions, the most effective pro-oxidants, are commonly found in food systems[26]. In the present study, the chelating ability of the essential oil of *C. foetida* towards ferrous ions was investigated. Table 2 shows the chelating effects of the samples compared with citric acid as standard. The results revealed moderate chelating activity shown in leaf (IC_{50}=

Table 2 — Antioxidant activity IC_{50} (µg/mL) of different parts of *Caryopteris foetida*

Samples	Antioxidant assay				
	DPPH assay	Metal chelating assay	Reducing power assay	NO Radical scavenging	SO Radical scavenging
Leaf oil	5.1±0.2	11.4±0.0	3.6±0.5	17.9±0.0	27.1±0.2
Stem oil	6.2±0.0	13.7±1.1	4.8±0.8	17.7±0.4	42.2±0.6
Seed oil	18.6±0.2	28.3±0.9	5.3±1.4	24.6±1.1	28.6±0.1
BHT	0.3±0.1	-	2.5±0.3	-	-
Citric acid	-	4.0±0.6	-	-	-
Ascorbic acid	-	-	-	10.4±0.5	9.9±0.2

Values are Mean ±S.D.

11.4±0.5 µg/mL) and seed essential oil (IC_{50}= 13.7±1.1 µg/mL) however stem oil did not show any significant chelating activity compared to positive control citric acid (IC_{50}= 4.0±0.6 µg/mL).

Nitric oxide radical scavenging activity

The stem oil (IC_{50} =17.7±0.4 µg/mL) and leaf oil (IC_{50} =17.9±0.0 µg/mL) was found most active in nitric oxide scavenging activity compared to seed oil (IC_{50} =24.6±1.1 µg/mL). Ascorbic acid was used as a positive control (IC_{50} =10.4±0.5 µg/mL) (Table 2). In addition to reactive oxygen species, nitric oxide is also implicated in inflammation, cancer and other pathological conditions[27]. The components of essential oils have the property to counteract the formation of NO and in turn, may be of considerable interest in preventing the ill effect of NO formation in the human body.

Superoxide radical scavenging activity

The superoxide radical scavenging activity was evaluated and leaf oil was found most effective with IC_{50} =27.1±0.2 µg/mL followed by seed oil IC_{50} =28.6±0.1 µg/mL whereas stem oil demonstrated weak activity with IC_{50} value of 42.2±0.6 µg/mL.

The antioxidant activity of essential oils could be due to the major compounds or the synergic effect of minor components present in the essential oils. The antioxidant effect of β-caryophyllene, *β*-caryophyllene oxide, linalool have been reported earlier and these compounds have a significant presence in our essential oils[28-30]. The antioxidant activity of essential oil could be attributed to these molecules. Further, the good antioxidant activity of leaf and stem essential oil could be due to components present in leaf and stem oil such as α-farnesene, γ-cadinene,3-octanone etc. which are absent in the seed oil.

***In-vitro* anti-inflammatory assay**

As a part of the investigation on the mechanism of the anti-inflammation activity, the ability of essential oils from different parts to inhibit protein denaturation was studied. All the tested oils were found effective in inhibiting heat-induced albumin denaturation. The inhibition per cent for different oils has been shown in Fig. 1. From the results of the present study and calculated IC_{50}, it can be stated that the leaf oil (IC_{50}= 12.8±0.0 µg/mL) was most effective as an anti-inflammatory reagent followed by stem oil (17.3±0.0 µg/mL) and seed oil (22.5±0.0 µg/mL) compared to standard diclofenac Sodium (11.9±0.0 µg/mL). β-caryophyllene, β-caryophyllene oxide, linalool, *E*-ocimene, α-humulene, β-farnesene have been reported to possess anti-inflammatory activity[31-33]. In the present study, all three essential oils possess these constituents as a part of a complex mixture of essential oils. Based on these facts it can be inferred that the *in-vitro* anti-inflammatory activity of leaf, stem and seed essential oils might be due to the presence of these active constituents or synergetic effects of other major/minor constituents.

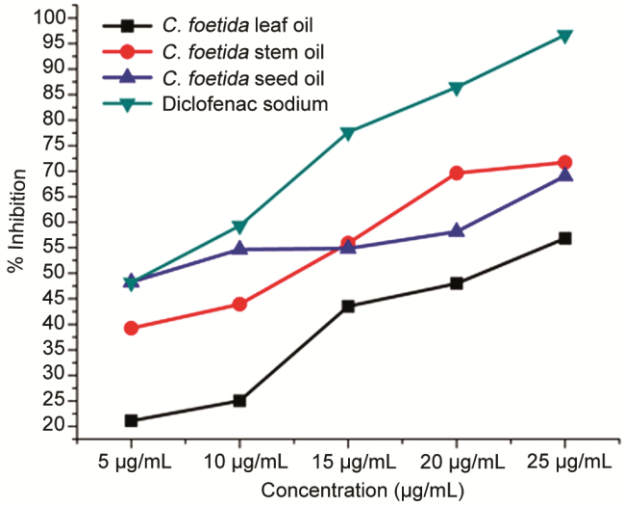

Fig. 1 — Percent inhibition of protein denaturation by leaf, stem, seed oils with standard diclofenac Sodium.

Antibacterial activity

The essential oils under investigation manifested significant antibacterial properties against tested microbes, although the growth inhibition was found to be dependent on essential oil concentration and the bacterial strain (Table 3). The MIC results show that the essential oil had a broad-spectrum activity and was able to inhibit the growth of the tested bacterial strains.

The leaf oil exhibited good antibacterial action against *S. typhimurium* with an inhibition zone (IZ) of 11-21.3 mm followed by stem oil (IZ= 10.3-15 mm) and seeds oil (IZ= 10.3-14 mm). Leaf (IZ= 10.3-17.3 mm) and seed oil (IZ= 12.3-17.7 mm) demonstrated comparable activity against *S. aureus* whereas no activity was shown by stem oil against this bacteria. The leaf oil showed moderate zones of inhibition against *B. megaterium* (IZ= 10-12.7 mm) while it ranged from 10 to 13.7 mm for stem oil and 12.3 to 17.7 mm for seed oil. The stem oil exhibited zone of inhibition in the range of 9-17.7 mm against *E.coli* whereas leaf oil (8-14.7 mm) and seed oil (10.3-16.7 mm) exhibited moderate inhibition. MIC values are presented in Table 4. Leaf oil exhibited

Table 3 — Antibacterial activity (zone of inhibition) of different parts of essential oils of *Caryopteris foetida*

Sample	Conc. (µg/mL)	Zone of Inhibition (mm)			
		B. megaterium	*S. typhimurium*	*E. coli*	*S. aureus*
	20				
	25				
Leaf oil	10	10±0.02	11±1.00	8±1.00	10.3±1.5
	15	11±0.02	16.3±0.6	11.3±0.6	13.3±0.6
	20	11.7±0.6	20±0.01	13.3±0.6	14.7±0.6
	25	12.7±0.6	21.3±0.6	14.7±0.6	17.3±0.6
Stem oil	10	10±1.00	10.3±0.6	9±1.00	
	15	12±0.02	12±0.01	13.7±1.2	NA
	20	13±0.03	13.7±0.6	14.7±0.6	
	25	13.7±0.6	15±0.00	17.7±0.6	
Seed oil	10	12.3±0.6	10.3±1.5	10.3±1.5	12.3±1.5
	15	14±0.01	12.3±1.5	13.7±0.6	15.3±0.6
	20	15.7±0.6	12.7±1.5	15.7±0.6	16.3±0.6
	25	17.7±0.6	14±0.01	16.7±0.6	17.7±0.6
Rifamycin	10	24.3±0.6	22.7±1.5	25±1	20.7±0.6
	15	27±1.6	25.3±1.5	28±1.2	22.3±1.2
	20	29.7±1.6	29.3±1.5	32±1.00	25±1.00
	25	31.3±1.2	35±1.00	34±1.2	27±1.00

Zone of inhibition is given in mm, negative control = DMSO, positive control = Rifamycin NA = not active in tested range, Results are mean±S.D.

Table 4 — Minimum inhibitory concentration of leaf, stem and seed essential oil of *Caryopteris foetida*

Test samples	MIC (µg/mL)			
	B. megaterium	*S. typhimurium*	*E. coli*	*S. aureus*
Leaf oil	10	10	5	10
Stem oil	10	10	10	NA
Seed oil	5	10	10	10
Rifamycin	5	5	5	5

higher MIC against *B. megaterium*, *S. aureus* and *S. typhimurium* but a lower MIC (5 µg/mL) for *E.coli*, whereas stem oil revealed higher MIC (10 µg/mL) for all tested bacteria. Seed oil showed a lower MIC (5 µg/mL) for *B. megaterium.*

The antimicrobial potential of essential oils could be attributed to sesquiterpenes and their oxygenated derivatives[34-35]. These low molecular weight compounds easily diffuse across cell membranes to induce biological reactions, hence responsible for the biological properties of essential oil. Further, the differences in the antibacterial activity of the essential oils and their corresponding MIC's may result from different chemical composition and percentage content of active compounds in essential oils. Several factors such as choice of bacterial strains, their sensitivity, concentration of test samples and different plant parts used for oil extraction could also be related to variation in the results among tested essential oils. The inactivity of stem oil against *S. aureus* might be due to the absence of certain minor components such as β-cubebene, α-gurjunene, isogermacrene-D, cis-murrola-4(14),5-diene, 1, 10-di-epi-cubenol, 3-hydroxy- β-murrolene which are present in leaf and seed oil. Thus, results indicated significant antibacterial activity against all microorganisms. However, none of the oil showed more potency than that of the standard drugs.

Conclusion

The results of the present study revealed different chemical compositions in the essential oils from different part of the plant *C. foetida.* The difference in chemical composition might be because of the need-based biosynthesis of secondary metabolites, towards defense mechanism against various environmental factors or stress conditions. As the biological properties of extracts/ oils depend upon the major/ minor components, hence the biological activity of essential oils from different parts of *C. foetida* in the present study might be directly associated with their major/minor constituents or synergic/ antagonistic behaviour among the major and minor constituents within the oil. It can be inferred from the findings of the current study that the plant *C. foetida* can be explored as a herbal alternative to synthetic nutraceutical because of its antioxidant property,

potent anti-inflammatory and anti-bacterial activities after proper clinical trials.

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Conflict of interest

The authors declared no conflict of interest.

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