Research Article

# Fourier Transform Infrared and Chromatographic Fingerprint of Essential Oil from *Pogostemon benghalensis* (Burm. F.) Kuntze

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## ARTICLE INFO

## ABSTRACT

The purpose of the present research work is to investigate the functional group and category of secondary metabolites present in the essential oil (Eo) from *Pogostemon benghalensis*, using Fourier transform infrared (FTIR) spectrometry, high-performance thin-layer chromatography (HPTLC), and gas chromatography and mass spectrometry (GC-MS) technique. FTIR measures the vibrations of bonds within the functional groups and yields a spectrum that can be considered as a biochemical or metabolic fingerprint of the plant product. Using FTIR spectra, it is possible to find out the minor changes of primary and secondary metabolic characteristic functional groups, which are responsible for their biological feature of the species. FTIR is a non-destructive, cost-effective, user-, and eco-friendly tool. *P. benghalensis*, a wild relative of *Pogostemon cablin*, the highly utilized and adulterated medicinal herb by the native people for the extraction of essential oil patchouli. The fresh leaves were subjected to hydro-distillation for the extraction of the essential oils and were analyzed using the above techniques. The FTIR spectral lines have shown diverse, unique peaks of functional groups. FTIR confirmed the volatile compounds and indicated by their functional groups of the essential oils, such as, C-H (alkene), C-H (aromatic), and C=C. Similarly, the analysis proved the presence of alcohol, p-substituted alcohols or phenols, alkanes, alkynes, alkenes, aldehyde, ester, ether, aliphatic amines, carboxylic acids, aromatics, ketones, disulfide, alkyl halides, halogen, and nitro derivatives. The intensity and estimation of predominant volatiles were analyzed by HPTLC, which showed eight peaks with max Rf values ranged from 0.07 to 0.96. Further, the Eo was fractionated by with GC-MS technique and identified 41 volatile fractions in the oil. Thus, the obtained data provides the biochemical profiles with overlapping signals of a wide array of molecules that are present in the cells. So, the usage of essential oils in pharmacy, cosmetology, and aromatherapy industries may be substantiated. Further advanced spectroscopic analysis is required to identify the structure and nature of active principles present in the Eo.

## INTRODUCTION

Biomedical, environmental, and food industry demands chemical analysis of components directly or indirectly used or consumed by human beings. Modern analytical tasks consist of rapid screening of a wide series of pharmaceutical and food samples for a plausible forgery or contamination. In the chemical evaluation of biological samples, one difficulty is the unpredictability of their chemical composition of a given herbal extract, plus the issue of the costs of authentic phytochemical standards.

Traded herbal products are mostly available in a dried and crumbled or powdered form. FTIR, HPTLC, and GC-MS instrumental systems can provide valuable information for chemotaxonomic analyses of similarities and dissimilarities among individual species. The system must feature efficient fingerprinting of herbal products with the detection system ensuring partial yet immediate identification. A remarkable feature of chromatography is that it can even be used for the separation of the volatile fractions (like terpenes) when employed at the

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lowest temperatures (-20 or -10°C) and using silica gel as a strong enough adsorbent.[1] In many studies, researchers successfully tested this possibility upon the essential oils (Eos) derived from many aromatic species belonging to Lamiaceae. Eos is secondary metabolites that are known for their fragrance and flavor features. They consist of a mixture of mono and sesquiterpenes, phenylpropanoids, and oxygenated molecules. Eos are localized in different plant parts and regions, and their storage is related to specialized secretory structures.[2] Therapeutically, the Eos exert many activities, like antiseptic, stimulant, carminative, diuretic, anthelmintic, analgesic, anti-rheumatic, aromatic, counter-irritant, and many other activities. Apart from food and pharmaceutical applications, they are also used as insect repellents, insecticides, pesticides, and deodorants. FTIR spectroscopic tool, a simple, cost-effective, and eco-friendly tool employed to analyze functional groups and structural hydrocarbon of phyto-molecules in medicinal species. FTIR spectroscopic tool gets popularized and expanding in research fields due to its non-destructive analysis of biological samples.[3] The major focus of the tool is diagnosis of a molecule via spectral images. The present study aims to characterize the essential oil from *P. benghalensis,* using FTIR, HPTLC, and GC-MS techniques leading to the identification of functional groups and individual components present.

## Material and Methods

### Plant Material

Fresh leaves of *P. benghalensis* were used in the present study and were collected from the natural habitats of Munnar Hills of Idukki district, Kerala, India, in July 2019. The collected species were identified using flora and were authenticated at the herbarium of Jawaharlal Nehru Tropical Botanic Garden and Research Institute (JNTBGRI), Palode, Trivandrum, Kerala, India. The voucher specimens were numbered (MGH 1456) and kept in the herbarium of the Department of Botany, M. G. College, Trivandrum, Kerala, India. The collected plants were washed thoroughly with running tap water, air-dried and ground to a coarse powder, and stored in air-tight bottles at 4°C.

### Extraction of Eo

Volatile oil (Eo) was extracted from the fresh leaves of *P. benghalensis* by the process of steam distillation in a Clevenger-type apparatus. The fresh leaves were cut into small pieces for the maximum extraction of oil and placed in a round bottom flask connected to the apparatus. The system was operated continuously for 5 hours for the maximum yield. Water was used as an extraction solvent. Since the oil is immiscible and lighter than water, it is formed as a separate upper layer in the measuring tube. The Eo collected in the measuring tube is carefully separated out and stored in amber-colored glass bottles.

Water is removed using anhydrous $Na_2SO_4$ and stored at 4°C for further experimental studies.[4]

### FTIR Analysis

Absorption spectra were measured in the 500 to 4,000 cm$^{-1}$ domain, using an FTIR technique, model FTIR 640, and JASCO product. This measuring technique has the advantage of a signal/noise ratio, particularly advantageous. For this reason, a full spectrum measuring time is reduced. The high measuring speed (under a second) enables repeated measurements (the device was set for 64 consecutive measurements), following to calculate the average for each value of the wavenumber (operation acquisition of spectra). Due to the measurements of 64 spectra, the signal/noise ratio improved eight times. Samples of essential oil and essential oil used as a reference were compressed in the form of thin-film (about 20 μm) between two slides of calcium fluoride crystal (transparent between 500–4,000 cm$^{-1}$) and interposed in the optical path of the radiation source with infrared emission.[5] Fourier transform spectrophotometer operation is based on light interference and a mathematical transformation (Fourier transform) of the detector signal, which obtains the absorption spectrum of the sample and reference material.[6]

### HPTLC Analysis

The major compounds present in the Eo of *Pogostemon* were estimated using an HPTLC system (CAMAG, Switzerland) connected with the Linomat V sample applicator and CAMAG twin-trough plate development chamber. CAMAG TLC scanner 3 and WinCATS software 4.0 were used for the identification of peaks in the HPTLC system. Silica gel plates (60F-254, Merck, Germany, 20 × 10 cm, 0.2 mm thickness) as stationary phase, and mixture chloroform: ethyl acetate (8:2) (%, v/v) as mobile phase was used to produce a sharp, symmetrical, and well-resolved peak. The present mobile phase composition was developed after testing different solvent systems of varying polarities. The Eo was applied to the silica gel HPTLC plates, using an automatic Linomat V sample applicator, fitted with a Camag microsyringe in $N_2$ flow (application rate-150 nL/s, space between two bands-11 mm, slit dimension-6 × 0.45 mm, scanning speed-20 mm/sec) under saturated conditions (30 minutes). These plates were scanned densitometrically at 366 nm (Hg lamp, K 400 optical cut-off filter), using TLC Scanner 3, and the data were analyzed with WinCATS Software 4.03.[7]

### GC-MS Analysis

The GC-MS analysis was carried out on a Hewlett-Packard 6890 gas chromatograph (Agilent Technologies, USA). 1 μL of Eo was injected into the equipment for the identification of volatile fraction present in the oil. The equipment was fitted with 30 metre (m) × 0.32 mm i.d., and 0.25 μm film thicknesses Hp-5 capillary column

coupled with a model 5973 mass detector. GC-MS operation conditions: carrier gas: helium (1.4 mL/min); injection mode: split (1:1 ratio, v/v); injector temperature: 220°C; detector temperature: 250°C; transfer line temperature: 60 to 246°C (3°C/min); mass spectra electron impact (EI$^+$) mode, 70 eV; ion source temperature: 240°C.[8]

## RESULTS AND DISCUSSION

### Essential Oil

Essential oils are a mixture of volatile, aromatic, and hydrophobic constituents, mainly comprise of terpenes and their derivatives. Eos is extracted from the leaf, flower, seed, twig, wood pulp, or bark tissues of higher plants. These oils are very interesting natural products are used in aromatherapy, pharmacology, pharmaceutical, chemical, and food industries and possess various biological activities. The most common method for extraction of Eos is hydro-distillation, and the volatiles present in the oil are identified using different chromatographic techniques. A 300 grams of *P. benghalensis* fresh leaves yielded 1.15 mL, 0.383% (v/w) brownish-yellow colored oil with clear, transparent, and characteristic medicinal aroma.

### FTIR Spectroscopy of Eos

Fig. 1 represents the absorption spectrum of essential oil obtained from P. benghalensis recorded in the wavelength range 4,000 to 500 cm$^{-1}$, with a resolution of 4 cm$^{-1}$. Each spectrum has been derived as a result of the accumulation of 64 individual spectra. Vegetal samples were gathered from the area Trei Ape-Garana and Arboretum Park Bazos. The 1,400 to 400 cm$^{-1}$ peaks region denotes the infrared (IR) spectral fingerprint, which contains the absorption bands that characterize the molecules' entire molecular structure through vibrations of the spectrum, deformation, and combining, harmonic bands that cannot be usually be attributed by normal vibrations. Fingerprint zones can be employed to discriminate the structure by comparing it with the IR spectrum of a standard molecule. If the two compared spectra (the

sample and the standard molecule) has similar absorption in the fingerprint region, it is strongly confirmed as compared to other methods (e.g., comparing the melting points or thin-layer chromatograms) that the structure of the compound to be analyzed is the same as that of the standard, but the IR spectra must be measured in the same conditions. In the charged peaks of the absorption, bands can be spotted by intense absorptions due to the deformation vibrations of the C-H bond and the valence vibrations of C-O single bonds of alcohols, ethers, esters, and the C-halogen bonds.[9]

The IR spectrum is an ideal tool for providing molecular structural information. *P. benghalensis* Eos spectrum revealed the peaks at 1,683.86 to 597.93 and 2,958.8 to 2,872.01 cm$^{-1}$ range. In the high wavelength range, alkane functional class stretching vibrations were showed by the peaks at 2,958.8 and 2,925.91 cm$^{-1}$, whereas the band at 2,872.01 cm$^{-1}$ was assigned to the C=O stretch stretching vibrations of the terpenoids group. The peak at 1,328.95, 1,136.07, 1,095.57, 1,062.78, 1,028.86, and 736.81 cm$^{-1}$ indicated the presence of alcohol and phenols functional groups. The peak at 786.96 cm$^{-1}$ confirms the presence of arene group assigned with C-H (bending and ring puckering). The remaining peak at 1,683.86 and 1,637.56 cm$^{-1}$ indicated the presence of alkene. 1,450.47 and 1,377.17 cm$^{-1}$ indicated the alkane bending vibrations of $CH_2$ and $CH_3$ deformation. The IR bands were dominated by alkynes, C-H deformation stretching vibration at 597.93 cm$^{-1}$. The C-H and $CH_2$ bending vibrations were seen at 987.55 and 833.4 cm$^{-1}$, whereas the O-H bending vibrations were displayed at 736.81 cm$^{-1}$ with alcohol and phenols functional class (Table 1).

The presence of salient absorption bands for functional groups can be validated by structural assignments but should not be based solely on absorptions seated in this area because they are most often applied only for substantiating the proposed structure by taking into account the characteristic group absorptions in other regions of the spectrum. The $H_2$ bonds change the vibration frequencies of the Eos that possess O-H and N-H bonds. The absorption band's position due to O-H bonds valence vibration is employed to justify the association strength through $H_2$ bonding. When the association through $H_2$ bonding is stronger, the O-H bond length enhances, and bond force constant reduces, so the valence vibration is discriminated at lower frequency values as compared to the values identified in the absence of association with $H_2$ bonds. The vibration absorption band given by the free O-H bond valence occurs in the region of 3,590 to 3,650 cm$^{-1}$, and the association through polymer $H_2$ bonds leads to wide bands in the region 3,200 to 3,600 cm$^{-1}$ (COOH broadband absorption from low frequencies, in 2,500–3,000 cm$^{-1}$ domain, due to associations of strong dimers).

The destruction of the intermolecular $H_2$ bonds by diluting with a solvent not participating in $H_2$ bonding (non-
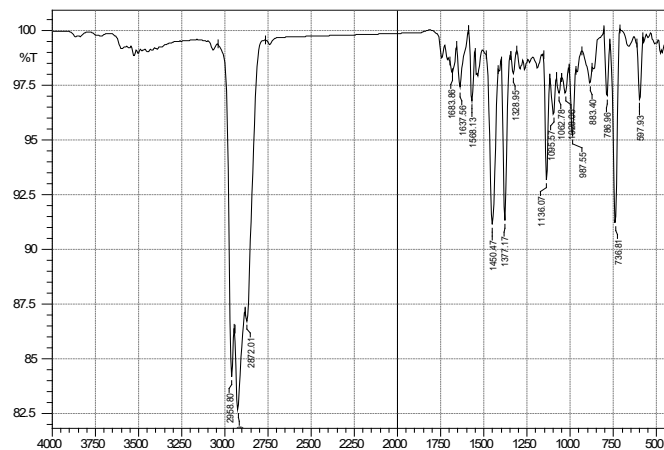


**Fig. 1:** FTIR spectrum of essential oils from *P. benghalensis*

**Table 1:** FTIR interpretation of compounds of Eo of *P. benghalensis*

| Range (cm⁻¹) | Functional class | Intensity | Assignment |
|---|---|---|---|
| 2,958.8 | Alkane | Strong | $CH_3$, $CH_2$, and CH (2 or 3 bands) |
| 2,925.91 | Alkane | Strong | $CH_3$, $CH_2$, and CH (2 or 3 bands) |
| 2,872.01 | Terpenoids | Medium-strong | C=O stretch |
| 1,683.86 | Alkene | Weak, broad | C=O (symmetric reduces intensity) |
| 1,637.56 | | O-H stretching | |
| 1,568.13 | Arene | Medium-weak | C=C (in ring, 2 band: 3, if conjugated) |
| 1,450.47 | Alkane | Medium | $CH_2$ and $CH_3$ deformation |
| 1,377.17 | | | |
| 1,328.95 | | | |
| 1,136.07 | | | |
| 1,095.57 | Alcohol and phenols | Strong | Usually broad C-O |
| 1,062.78 | | | |
| 1,028.86 | | | |
| 987.55 | Alkenes | Strong | =C-H |
| 833.4 | Alkenes | Medium | =$CH_2$ (out of plane bending) |
| 786.96 | Arene | Strong-medium | C-H (bending and ring puckering) |
| 736.81 | Alcohol and phenols | Weak | O-H bending |
| 597.93 | Alkynes | Strong | C-H deformation |

polar organic solvent) results in the reduction of the polymer band intensity and the occurrence of a narrow band range seated at higher frequencies, characteristic to the valence vibration of the O-H free bond (unassociated), so it reflects that the Eos absorption spectra of different species varied substantially.[10] To represent the similarity between the spectra of the two products, the spectra in question are digitized, represented as a set of pairs of values (wavelength number *vs.* transmission%). Each spectrum consists of 3,113 such pairs of values. A comparison of the two spectra from biosystems, which are to be denoted quantitatively for similarity, is a comparison of the absorbance values for each pair of values of the 3,113 wavenumbers *vs.* transmission%. In the case of the theoretical identity of the two spectra, for each value of the wavenumber, the transmission linked with the two spectra should be equal.[11]

Samfira *et al.*[6] analyzed IR spectral features of lavender, peppermint, green Douglas, and chicory Eos. The correlation coefficient values for the oils derived from mint, green Douglas fir, and chicory were lesser than 0.97, the value calculated for lavender. Elzey *et al.*[12] documented the FTIR spectrum of the pure Eos of lemon, revealed the characteristic C-H stretch (~2,900 cm⁻¹), C=O stretch (~1,700 cm⁻¹), broad O-H stretch (~3,400 cm⁻¹), and C-O stretch (~1,100 cm⁻¹) of terpenoid fractions. The compositions and constituents of Eos may vary and depend on the soil environment. In general, Eos is made up of terpenes, like terpineol, cineole, citronellal, and others. Boughendjioua and Djeddi[13] also noticed similar results from the lemon Eo of Algeria.

Luca *et al.*[14] used analytical and preparative tools to identify the various coumarins. Tangpao *et al.*[15] compared aromatic profiles of essential oils from five commonly used at Thai Basils. Foudah *et al.*[16] developed a modified HPTLC validated method for the simultaneous quantification of eucalyptol and α-pinene in Lamiaceae species. Sahayaraja *et al.*[17] screened phytochemicals by FTIR spectroscopic analysis of leaf and stem extracts of *Wedelia biflora*. Tahir and Abbasi[18] validated *Mentha spicata* extracts by FTIR spectroscopic analysis. Elzey *et al.*[12] proved the purity of adulterated essential oils by FTIR spectroscopy and partial-least-squares regression techniques. Morar *et al.*[19] characterized the essential oils extracted from different aromatic plants by FTIR spectroscopy. Adinew[5] studied GC-MS and FTIR analysis of essential oil from *Cinnamon* bark growing in the south-west of Ethiopia. Samfira *et al.*[6] characterized, identified, and confirmed the Eos by mid-infrared absorption spectrophotometry.

## HPTLC Analysis

To analyze the major compounds present in the Eos, HPTLC analysis was carried out. The HPTLC analysis showed eight peaks from *P. benghalensis*. The max Rf values of Eo from *P. benghalensis* ranged between 0.07 to 0.96, and area% were between 2.29 and 33.61. The fractions' maximum height ranged from 26.1 to 185.4, which indirectly indicates the oil's presence of major components (Table 2). Geethika and Sunojkumar[20] carried the HPTLC fingerprint profile of *Leucas stelligera*, *Leucas eriostoma*, and *Leucas ciliate*.

**Table 2:** HPTLC profile of Eo from *P. benghalensis*

| Peak | Start Rf | Start height | Max Rf | Max height | Max% | End Rf | End height | Area | Area% |
|------|----------|--------------|--------|------------|------|--------|------------|------|-------|
| 1 | 0.04 | 79.1 | 0.07 | 105.6 | 21.4 | 0.08 | 1 | 927.5 | 6.98 |
| 2 | 0.12 | 0.3 | 0.17 | 45.7 | 6.17 | 0.18 | 13.9 | 1,001.2 | 4.01 |
| 3 | 0.19 | 2.3 | 0.25 | 28.6 | 5.26 | 0.26 | 12.8 | 501.2 | 2.29 |
| 4 | 0.26 | 9.1 | 0.29 | 26.1 | 3.45 | 0.31 | 3.7 | 547.3 | 2.54 |
| 5 | 0.33 | 0.5 | 0.38 | 27.1 | 3.94 | 0.38 | 4.1 | 521.8 | 2.41 |
| 6 | 0.38 | 0.5 | 0.43 | 32.7 | 3.89 | 0.43 | 0.8 | 510.7 | 2.32 |
| 7 | 0.76 | 5.9 | 0.84 | 185.4 | 23.4 | 0.89 | 2.6 | 7,240.1 | 33.61 |
| 8 | 0.93 | 43.1 | 0.97 | 67.5 | 10.2 | 0.97 | 1.5 | 569.4 | 2.84 |

The Rf values ranged between 0.06 and 0.98 for the species. The fingerprint analysis gives the idea for the authentication of the herbal extracts and its constituents and provides the parameters for the quality of herbal formulations. Gomathi *et al.*[21] effectively discriminated compounds by HPTLC fingerprinting analysis of *Evolvulus alsinoides*. Iwin *et al.*[22] reported HPTLC analysis of the alkaloid profile of stem bark extracts of *Terminalia chebula*. Vinatha *et al.*[23] screened the phytochemicals by HPTLC fingerprint analysis of leaf extract from *Bridelia montana*. Bobby *et al.*[24] analyzed the crude powder and dry ethanolic extracts of *Albizia lebbeck* leaves by FTIR and recorded alcohols, phenols, alkanes, carboxylic acids, aromatics, ketones, and alkyl halides compounds corresponding to the peaks at 3,370.19, 2,955.65, 2,925.68, 2,853.4, 1,739.72, 1,463.02, and 506.57 cm$^{-1}$, respectively. Similarly, Thangarajan *et al.*[25] analyzed and documented the compounds in *Ichnocarpus frutescens*. Rao *et al.*[26] carried HPTLC analysis of the essential oil from *Pimenta dioica* leaf extracts.

The methanol extract of *Limonia acidissima* fruit contained alcohols, phenols, alkanes, amino acids, α, β-unsaturated esters, alkenes, nitro compounds, aromatics, aliphatic amines, carboxylic acid, alkenes, and alkyl halides compounds,[27] while the ethanolic extract of *Ipomoea obscura* showed the presence of most of the secondary metabolites in the plant leaves.[28] Similarly, *Cayratia trifolia* plant stem ethanolic extract holds more phytochemicals and bioactive compounds that were confirmed using FTIR.[29] Jayashree and Ramesh[30] analyzed the methanolic extract of fruit pulp of *Feronia limonia* and documented the presence of a phenolic, aromatic, and aliphatic functional group.

## GC-MS Analysis

GC-MS was a reliable tool used to identify and quantify the volatile compounds present in the Eos with the help of peak area, retention time, and comparison with authentic known standards in the library. In the present study, the essential leaf oils from *P. benghalensis* revealed 41 volatile compounds representing 97.43% of the total oil. The oil contained mainly terpenoids and its derivatives, *viz.*, monoterpene hydrocarbons, oxygenated monoterpenes,

sesquiterpene hydrocarbons, oxygenated sesquiterpenes, and other compounds (Table 3). The major fraction of Eo of *P. benghalensis* was oxygenated sesquiterpenes (48.41%), followed by sesquiterpene hydrocarbons (35.31%), oxygenated monoterpene (9.61%), monoterpene hydrocarbons (3.5%), and other volatiles (0.65%). The major compound identified from the GC-MS chromatogram of *P. benghalensis* was α-cadinol comprised 35.78% of the oil.

In addition to the major compounds, 1,8 cineole (7.14%), aromadendrene (4.16%), þ-cymene (1.6%), bornyl acetae (2.15%), longicyclene (2.74%), β–elemene (1.56%), longifolene (1.18%), α-caryophyllene (1.08%), β-caryophyllene (1.14%), trans-β-farnesene (1.56%), α-patchoulene (2.39%), gurjunene (2.86%), valencene (2.21%), epi-cubedol (1.03%), bicyclogermacrene (2.78%), trans-β-guaiene(1.26%), α-bisabolene (1.16%), ɗ-cadinene (2.45%), elemol (1.01%), spathulenol (1.16%), caryophyllene oxide (1.15%), guaiol (1.79%), isolongifol (1.20%), cubenol-1-epi (1.56%), α-murolol (1.45%), bulsenol (1.51%), and cadalene-8,9-epoxide (2.95%) were identified as remarkable fractions of Eo of *P. benghalensis.*

Total volatile products of *Mentha piperita* leaves were 58.61%, as revealed by the GC-MS analysis, and the yield was 1.02%. Thirty volatile compounds were identified and the major constituent in the leaf was menthone (29.01%), followed by menthol (5.58%), menthyl acetate (3.34%), menthofuran (3.01%), 1, 8-cineole (2.40%), isomenthone (2.12%), limonene (2.10%), α-pinene (1.56%), germacrene-D (1.50%), β-pinene (1.25%), sabinene (1.13%), and pulegone (1.12%).[31] The investigation on the Eo of *Leucas virgata* led to the identification of 43 constituents, comprising 93.9% of the total oil. The Eos of *L. virgata* was characterized by a high content of oxygenated monoterpenes (50.8%), followed by oxygenated sesquiterpenes (21%). Camphor (20.5%), β-eudesmol (6.1%), caryophyllene oxide (5.1%) exo-fenchol (3.4%), fenchon (5.4%), and borneol (3.1%) were identified as the main components.[32]

The chemical composition of the hydro-distilled essential oil from the flowering aerial parts of *Leucas indica* indicated the presence of fifty-six

**Table 3:** GC-MS profile of *P. benghalensis*

| No. | Compound | LRI (Cal.) | LRI (Lit.) | P. benghalensis |
|-----|----------|-----------|-----------|-----------------|
| 1 | α-pinene | 925 | 932 | 0.42 |
| 2 | Camphene | 935 | 946 | 0.18 |
| 3 | α-terpene | 1,018 | 1,014 | 0.89 |
| 4 | þ-cymene | 1,021 | 1,020 | 1.6 |
| 5 | 1,8-cineole | 1,026 | 1,026 | 7.14 |
| 6 | γ-terpinene | 1,054 | 1,054 | 0.41 |
| 7 | Linalool | 1,095 | 1,095 | 0.32 |
| 8 | Bornylacetae | 1,288 | 1,287 | 2.15 |
| 9 | ɗ-elemene | 1,336 | 1,335 | 0.53 |
| 10 | Longicyclene | 1,373 | 1,371 | 2.74 |
| 11 | β-elemene | 1,386 | 1,389 | 1.56 |
| 12 | β-longipinene | 1,395 | 1,400 | 0.98 |
| 13 | Longifolene | 1,402 | 1,407 | 1.18 |
| 14 | α-caryophyllene | 1,403 | 1,408 | 1.08 |
| 15 | β-caryophyllene | 1,414 | 1,417 | 1.14 |
| 16 | Aromadendrene | 1,437 | 1,439 | 4.16 |
| 17 | Trans-β-farnesene | 1,452 | 1,454 | 1.56 |
| 18 | α-patchoulene | 1,456 | 1,454 | 2.39 |
| 19 | Alloaromadendre | 1,460 | 1,458 | 0.76 |
| 20 | Gurjunene | 1,473 | 1,475 | 2.86 |
| 21 | Valencene | 1,496 | 1,491 | 2.21 |
| 22 | Epicubedol | 1,489 | 1,493 | 1.03 |
| 23 | Bicyclogermacrene | 1,498 | 1,500 | 2.78 |
| 24 | Trans b guaiene | 1,500 | 1,502 | 1.26 |
| 25 | β-bisabolene | 1,507 | 1,505 | 1.16 |
| 26 | α-bulnesene | 1,508 | 1,509 | 0.47 |
| 27 | ɗ- cadinene | 1,524 | 1,522 | 2.45 |
| 28 | Elemol | 1,542 | 1,548 | 1.01 |
| 29 | Germacrene B | 1,550 | 1,559 | 0.84 |
| 30 | Spathulenol | 1,570 | 1,577 | 1.16 |
| 31 | Caryophyllene oxide | 1,577 | 1,582 | 1.15 |
| 32 | Guaiol | 1,594 | 1,600 | 1.79 |
| 33 | Isolongifol | 1,615 | 1,618 | 1.2 |
| 34 | Cubenol-1-epi | 1,618 | 1,627 | 1.56 |
| 35 | Epi-α--cadinol | 1,632 | 1,638 | 0.38 |
| 36 | α-murolol | 1,635 | 1,644 | 1.45 |
| 37 | α-cadinol | 1,656 | 1,652 | 35.78 |
| 38 | Allohimachalol | 1,670 | 1,661 | 0.64 |
| 39 | Bulsenol | 1,672 | 1,670 | 1.51 |
| 40 | Cadalene 8,9 epoxide | 1,681 | 1,675 | 2.95 |
| 41 | Isoamycinnamate | 1,732 | 1,740 | 0.6 |
| | Monoterpene hydrocarbon | | | 3.5 |
| | Oxygenated monoterpene | | | 9.61 |
| | Sesquiterpene hydrocarbon | | | 35.31 |
| | Oxygenated sesquiterpene | | | 48.41 |
| | Others | | | 0.6 |
| | Total | | | 97.43 |

compounds with 71.8% sesquiterpene hydrocarbons. The other components were β-caryophyllene (51.1%) and α-caryophyllene (10.2%).[33] GC-MS result of *Thymus capitatus* Eo comprised of 22 components. Thymol (51.22%), carvacrol (12.59%), and γ-terpinene (10.3%) were identified as the major components, i.e., 74.11% of the total composition of the oil.[34] The essential oil of *Salvia officinalis* seeds showed a higher percentage of oxygenated monoterpenes (50.14%) than sesquiterpenes (17.32%), followed by monoterpenes (5.93%) in the GC-MS profile. Further, caryophyllene oxide (7.28%), 13-epi-manool (5.61%), δ-elemene (3.97%), and β-eudesmol (3.76%) were identified as the major fraction.[35]

The GC-MS analysis of *Rosmarinus officinalis* oil collected from different loci dominated by α-pinene (13.5–37.7%), 1,8-cineole (16.1–29.3%), verbenone (0.8–16.9%), borneol (2.1–6.9%), camphor (0.7–7%), and racemic limonene (1.6–4.4%).[36] Kariminik *et al.*[37] studied the essential oil composition of the four medicinal plants from the Lamiaceae, including *Salvia macrosiphon*, *R. officinalis*, *Dracocephalum polychaetum*, and *Origanum vulgare*. *S. macrosiphon*, *R. officinalis*, *D. polychaetum*, and *O. vulgare* represented 11, 14, 5, and 20 compounds, respectively, in the GC-MS analysis. Linalool (54.8%) and manool (27.3%) were the principal components of *S. macrosiphon*, while α-pinene (15.5%), verbenone (14.3%), 1, 8-cineole (12.4%), camphor (11.9%), bornyl acetate (7.9%), borneol (6.5%), and camphene (5.8%) were the major components in *R. officinalis*. Cyclo geranate (69.9%) and limonene (19.9%) were revealed as the main components of *D. polychaetum* Eo, and other components were present only as trace amounts. *O. vulgare*, GC-MS analysis indicated the presence of thymol (27.4%), γ-terpinene (12.1%), terpinene-4-ol (11.6%), trans-sabinene hydrate (5.9%), and trans-anethole (3.6%). The composition of Eos in the above-published data of other species of Lamiaceae showed the remarkable difference when compared with the present study.

A rapid, simple, accurate, and specific FTIR, HPTLC, and GC-MS method were carried in *P. benghalensis*. The results of the present study showed the presence of alkanes, alkenes, alkynes, arene, alcohols, aromatics, terpenoids, and phenols as the major fractions. The chromatographic investigation of Eo of *P. benghalensis* revealed the presence of 41 compounds. The data could be used as a quality control standard. The method resulted in good peak shape and enabled good resolution from the constituents of the essential oils. The presence of various bioactive compounds present in the Eo justifies its importance in the pharmaceutical industry. The present study revealed the ecological and economic significance of Eo from plants as a prospective for possible application in different medicinal, cosmetic, and food products based on further investigations, such as, isolation of the individual phytochemical constituents and their efficacies.

## Conclusion

The present study indicated that the spectra obtained from the essential oil of *P. benghalensis* correspond to the presence or absence of a wide variety of functional groups in a molecule. The GC-MS analysis of the essential oil revealed that the chemical composition was characterized by high content of oxygenated sesquiterpenes (48.41%) followed by sesquiterpenes hydrocarbons (35.31%). α-cadinol, 1, 8–cineole, aromadendrene were identified as the main components. The chromatographic fingerprints supports the essential oil from *P. benghalensis* can be utilized for the manufacture of perfumery products, as well as, in the pharmaceutical industry.

## Acknowledgment

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