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GC–MS and FTIR analysis of the essential oil from flowers of *Chonemorpha fragrans*

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Abstract
The study was aimed to investigate the chemical composition of *Chonemorpha fragrans* flowers growing in Karnataka, India. The oil was extracted by cold extraction and steam distillation methods. The chemical composition was determined by GC – MS analysis and 21 compounds were identified. The major compounds were 2, 3-epoxy-2, 3- dihydrosqualene (27.92%), squalene (18.76%) and 1, 2 - benzenediol (20.92%) from fresh flower volatiles. The dried flower volatiles showed predominantly β - sitosterol (43.91%), sitostenone (15.09%), and methyl commate D (8.29%). The FTIR analysis confirmed the presence of N-H, O-H, C=C, C-H, C-O, PO_3 and halogen stretch functional groups. Comparison between the volatile compounds of fresh and dried flowers from different extraction methods showed differences in the volatile components. GC-MS and FTIR analysis reveals the various phytochemical constituents. This study will be helpful for the identification, quantification and isolation of phytocompounds.

Keywords: apocynaceae, β – sitosterol, flower oil, steam distillation, volatile compounds

Introduction
The *Chonemorpha fragrans* (*C. fragrans*) is widely distributed in dense evergreen forests of Southeast Asia namely India, China, Indonesia, Malaysia, Myanmar, Sri Lanka and Thailand. In Karnataka, it is distributed in Chikkamagalur, Hassan, Kodagu, Shimoga, Udupi and Uttara Kannada [1, 2]. It is found in dense mountain forests, often clinging to trees at 400 - 1800 m. It is commonly known as the frangipani vine, a member of the family Apocynaceae. The plant is scandent, with a rich milky latex, takes several years to mature and flowering. The blooming of the flowers seen during June - September.

The flowers are terminal cymes with white colour and yellow in the centre, the petals have salver-shaped lobes with rich fragrans. The plant is used in several ayurvedic drugs and in the indigenous system of medicine. The roots of *Chonemorpha* possess several medicinal properties used to treat fever, stomach disorders, skin diseases, leprosy, scabies, syphilis, dyspepsia, hyperdipsia, cardiac debility, diabetes, jaundice, bronchitis and intermittent fevers [3]. Extraction of essential oil is valuable; it can be obtained from different parts of the plants such as roots, stem, leaves, flowers, seeds, wood etc. The flowers of *C. fragrans* exhibit a pleasant aroma during blooming and the oil glands were active in the morning [4]. There are limited studies on the extraction of essential oil and identification of chemical constituents *C. fragrans* flowers. Therefore, our study was aimed to extract the essential oil from fresh and dry flowers and identify the volatile chemical compounds through GC – MS and FTIR analysis.

Materials and Methods
Collection of flowers
C. fragrans flowers were collected from wild plants (Joida, Karnataka) in the month of July. Healthy flowers were plucked in the morning and were collected in zip lock covers. The bracts, sepals, were separated and weight was recorded.

Extraction of oil from flowers
The oil was extracted by following methods

a. **Cold extraction:** The oil extraction from fresh flowers (CF1) was done by the cold extraction method [5]. It is suitable for delicate and thermally sensitive materials like flowers. 200 g of fresh flowers were taken and chopped into small pieces, then soaked with 200 ml of petroleum ether and covered with aluminium film. Shake the contents in 3 - 4 hrs interval for 24 - 48 hrs, decant the solvent and the residue is left in a hot air oven at 35 - 40 ºC and stored at room temperature for further analysis.
b. **Steam distillation:** The oil extraction of dried flowers (CF2) was done by steam distillation [6]. 20 g of dried flowers were powdered and subjected to steam distillation in Clevenger apparatus for 12 - 16 hrs. 50 ml of hexane was added to the mixture and mixed well in the separating funnel; two layers of the liquids get separated. Hydrosol was removed, the hexane with oil was collected in a beaker and kept on a hot plate for evaporation. The oil was collected in a narrow test tube and a pinch of anhydrous sodium sulphate was added to remove the water content. The oil was recovered with the help of a micropipette and stored in screw cap glass vial at room temperature in dark.

The percentage of oil yield was calculated by the equation [7]
Oil yield (% v/w) = Volume of the extracted oil / weight of the flowers

GC – MS analysis
The Gas Chromatography - Mass Spectrometry (GC – MS) was performed to determine the phytoconstituents present in the flowers of *C. fragrans*. The Shimadzu GC - MS analyser (Model No. QP2010S) was used to examine the phytocompounds. The column specifications are Elite - 5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 0.25 µm thickness) and the compounds were

separated through helium as a gas carrier at a constant flow of 1 ml/min. Initially the GC's oven temperature was programmed at 80 °C for 1 min and the injector temperature was set at 260 °C during the chromatographic run. 1 µl of extract sample inserted into the instrument, the oven temperature was 60 °C (2 min); followed by 300 °C at the rate of 10 °C /min. The mass spectra range was 50 – 500 m/z and recorded with EI (electron ionization) mode at 70 eV, a scan interval of 0.5 sec and 0.2 sec scan time. The chromatograms were plotted using the software GC – MS solutions, the spectra of each compound compared with the NIST - 11 and WILEY – 8 libraries [8].

FTIR analysis

Fourier Transform Infrared Spectrophotometer (FTIR) is a spectroscopic tool to detect the compound functional groups. The wavelength of light absorbed showed the characteristics of specific chemical bonds that appeared on the spectrum. Based on the infrared spectrum absorption, it can be detecting the chemical bonds in a compound. FTIR analysis of the oil samples was carried out by Perkin Elmer spectrometer (Spectrum 1000) using Attenuated total reflectance (ATR) mode in the range 4000 – 512 /cm with the resolution 0.1 /cm [9].

Results and Discussion

There are more than 200 constituents present in pure essential oils which includes, terpenes, and phenylpropanoid derivatives. The constituents of essential oils are broadly classified as volatile and non-volatile fractions. The overall chemical composition of the volatile fraction of aromatic oil includes mono- and sesquiterpene components, and several oxygenated derivatives along with alcohols, aliphatic aldehydes, and esters [6].

Essential oils are the concentrated hydrophobic volatile liquid compounds of different plant parts extracted by using various distillation methods. An essential oil may contain number of chemical compounds; this composite mixture may contain volatile and non-volatile compounds; these provides the distinctive flavours and fragrance to the oil [10]. The essential oils from *C. fragrans* flowers were extracted by two methods. The fresh flowers from cold extraction method (CF1) yielded 0.05% of brown coloured semisolid oil. The dry flowers from steam distillation method (CF2) yielded 0.03% of pale-yellow coloured liquid oil (Figure 1 a & b).

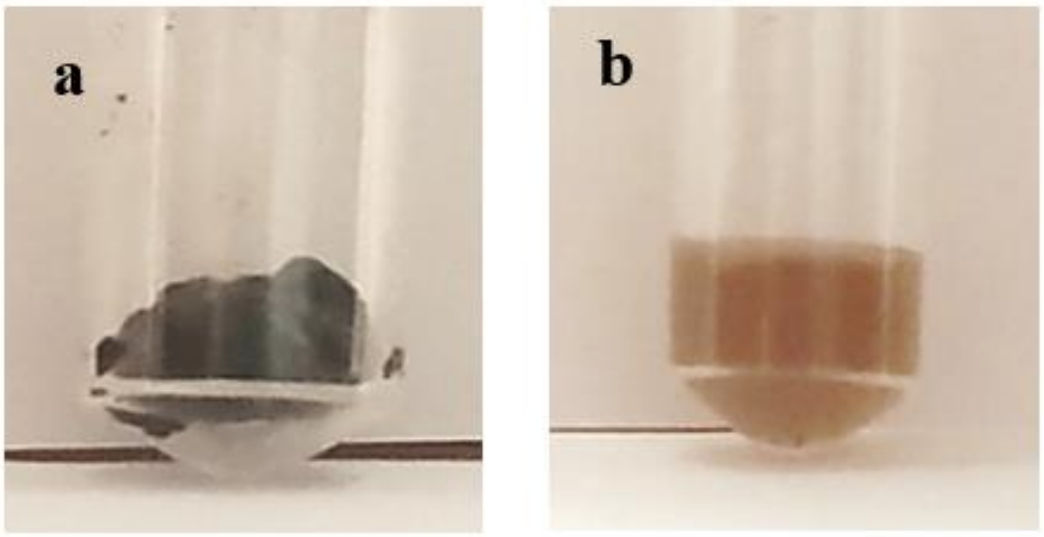


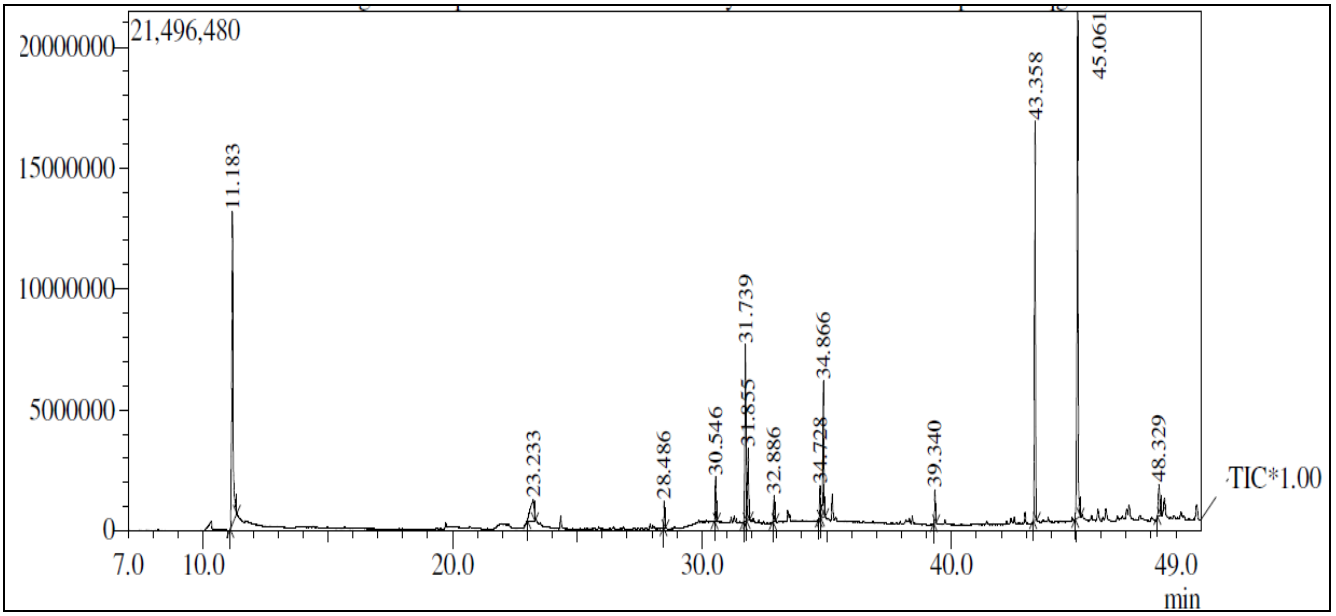
Fig 1: Solvent extraction from a) CF1 b) CF2

GC – MS analysis

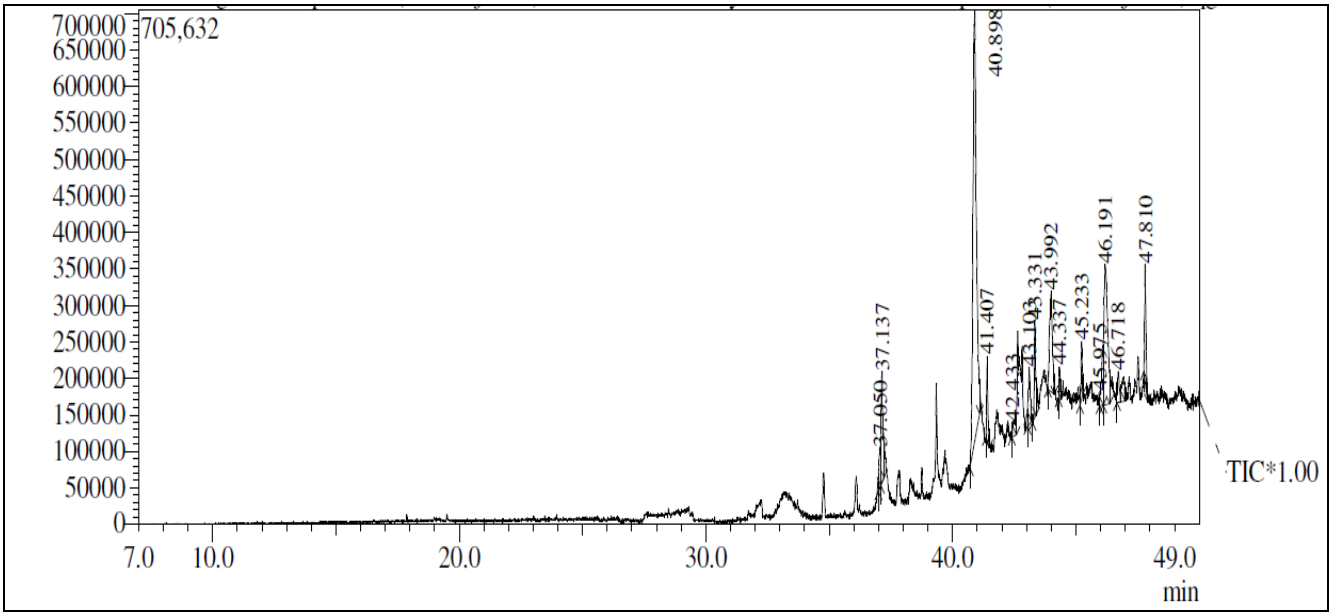
GC - MS is a simple and efficient method used for determining chemical constituents present in essential oils. It affords reproducible and precise readings of m/z (mass/charge), retention time and peak area percentage of volatile compounds. In this analysis the scan mode was performed for the non-specific groups of target analytes [11]. Based on the GC peak areas the relative percentage of the individual components were calculated.

The chromatograms of GC – MS analysis revealed 21 phytochemicals from fresh and dried flower extracts (Table 1 & 2). The fresh flower extracts showed the presence of 12 constituents. The major constituents of fresh flowers are 2, 3-Epoxy-2, 3-dihydrosqualene (27.92%), 1, 2-Benzenediol (20.92%) and squalene (18.76%) along with the other nine compounds mentioned in the Table 1; Figure 2 a. The detected compounds in the extract were mainly terpenes, triterpenes, sesquiterpenes, phenols, and fatty acid esters. Predominantly 47.75% of the compounds belongs to terpenoids and their derivatives. These were involved in the synthesis of the cell membrane sterols. Squalene is a unsaturated isoprenoid hydrocarbon which acts as strong antioxidant, natural antibiotic and also exhibit anticancer properties [12]. 1, 2-Benzenediol belongs to the phenol and acts as a precursor for the synthesis of fragrans and flavours in *Kirika acuminata* [13].

The chromatogram of the dried flower oil reveals the presence of 9 compounds (Table 2 Figure 2 b), out of which β - sitosterol (43.91%), sitostenone (15.09%) and methyl commate D (8.29%) are the major compounds identified. The flower oil expressed prime amount of phytosterols like stigmasterol, β – sitosterol and sesquiterpenoids such as calarene epoxide, sitostenone. These phytochemical compounds were responsible for various pharmacological activities such as antimicrobial, antiviral, anticancer, antifertility, antioxidant, antidiabetic and immunomodulatory properties [14, 15, 16].



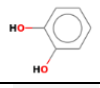
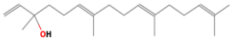
A



B

Fig 2: GC-MS chromatograms of volatile oil extracts of *C. fragrans* a) CF1 b) CF2.

Table 1: Chemical composition of essential oil from fresh flowers

Sl. No.	Name of the compound	Molecular formula	Molecular weight g/mol	Peak area in (%)	Retention time	Molecular structure	Therapeutic activity
1.	1,2-Benzenediol	$C_6H_6O_2$	110.11	20.92	11.183		Antioxidant, anti-cancer and pesticides (Choudhary *et al*., 2019) [17]
2.	Mome inositol	$C_6H_{12}O_6$	180.16	3.76	23.233		anti-alopecic, anti-cirrhotic and anti-neuropathic (Das, 2014) [18]
3.	Methylpalmitate	$C_{17}H_{34}O_2$	270.5	1.07	28.486		Anti-inflammatory, anti-helminthic (Adnan *et al*., 2019) [19]
4.	Geranyl linalool isomer B	$C_{20}H_{34}O$	290.5	1.92	30.546		Antimicrobial & anti infalmmatory (Gnanapriyanka *et* al., 2018) [20]
5.	Octacosane	$C_{28}H_{58}$	394.8	8.95	31.739		Antioxidant, anti inflamatory (Tanod *et al*., 2019) [21]

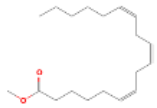
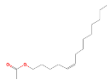
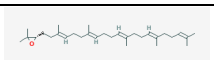
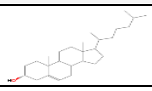
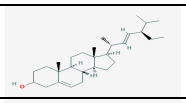
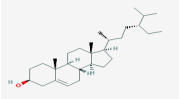
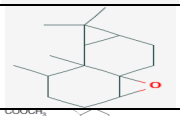
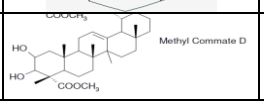
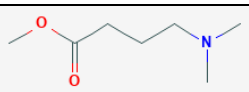
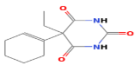
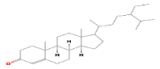
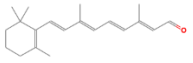
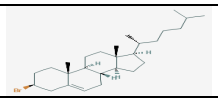
6.	Linolenic acid, methyl ester	$C_{19}H_{32}O_2$	292.5	3.91	31.855		Anti-inflammatory and cancer preventive (Krishnamoorthy *et al*., 2014) [22]
7.	9,12-octadecadien-1-ol	$C_{18}H_{34}O$	266.5	1.17	32.886		Antioxidant & antibacterial (Kim *et al*., 2020) [23]
8.	5-Tetradecen-1-ol, acetate, (Z)	$C_{16}H_{30}O_2$	254.41	1.41	34.728		No activity reported
9.	1,2-Benzenedicarboxylic acid	$C_8H_6O_4$	166.13	1.48	39.340		Cytotoxic activity (Krishnan *et al*., 2014) [24]
10.	Squalene	$C_{30}H_{50}$	410.73	18.76	43.358		Antioxidant, anti aging and antitumour (Huang *et al*., 2009) [25]
11.	2,3-Epoxy-2,3-dihydrosqualene	$C_{30}H_{50}O$	426.7	27.92	45.061		Anti-inflammmatory (Majumdar *et al*., 2020) [26]
12.	Cholesterol	$C_{27}H_{46}O$	386.654	2.38	48.329		Antiarthritic (Gomathi *et al*., 2015) [27]

Table 2: Chemical composition of essential oil from dry flowers

Sl. No.	Name of the compound	Molecular formula	Molecular weight g/mol	Peak area in (%)	Retention time	Molecular structure	Therapeutic activity
1.	Stigmasterol	$C_{29}H_{48}O$	412.69	2.18	37.050		Anti-inflammatory (Gabaey *et al*., 2009) [28]
2.	β-Sitosterol	$C_{29}H_{50}O$	414.71	43.91	40.898		Anticancerous (Ambavade *et al*., 2014) [14]
3.	Calarene epoxide	$C_{15}H_{24}O$	220.35	1.45	42.433		Anti-inflammatory & antioxidant (Sushma *et al*., 2017) [29]
4.	Methyl commate D	$C_{31}H_{50}O_3$	470.7	8.29	43.992		Antimicrobial (Venkataraman *et al*., 2012) [30]
5.	2-(dimethylamino)-1-phenylpropyl 4-(2-methyl-3-oxocyclohexyl) butanoate	$C_7H_{15}NO_2$	145.2	1.08	44.337		Not reported
6.	Cavonyl	$C_{12}H_{16}N_2O_3$	236.26	3.17	45.975		Antioxidant (Reffaei *et al*., 2015) [31]
7.	Sitostenone	$C_{29}H_{48}O$	412.7	15.09	46.191		Antimicrobial (Jain *et al*., 2018) [32]
8.	Retinal	$C_{20}H_{28}O$	284.4	3.36	46.718		Vitamin A (George 1934) [33]
9.	Cholesteryl bromide	$C_{27}H_{45}Br$	449.55	4.27	47.810		Cytotoxic (Albuquerque *et al*., 2018) [34]

FTIR analysis

FTIR spectrum was useful for the detecting functional groups of the active components present in the plant extracts based on peak values obtained in the IR radiation region [35, 36]. Functional groups of the *C. fragrans* flower oil extracts were explored in the FTIR spectra. The CF1 sample spectrum showed high broad peak at 3325 cm^{-1}, medium sharp peak at 1638 cm^{-1} and 660 cm^{-1} with alcohols (O-H), alkenes (C=C) and aliphatic bromocompounds (C-Br) respectively (Table 3; Figure 3 a). The spectrum CF2 showed the wider peak at 3261 cm^{-1} indicates the presence of alcohols & phenols (O-H). Sharp and narrow peaks obtained at 1592 cm^{-1} & 1061 cm^{-1} showed the presence of amides (N-H) and esters (C-O) respectively. Moderate peaks were noticed at 1380 cm^{-1}, 1251 cm^{-1}, and 662 cm^{-1} corresponds to alkanes (C-C), aromatic acid esters (C-O-C) and halogen groups (C-I/Cl) respectively (Table 4; Figure 3 b). The results exhibited the presence of different phytocompounds in essential oils of flowers of *C. fragrans*. These results were correlated with the studies of Hemalakshmi *et al.* [37], wherein FTIR analysis of *Erythrina variegate* flowers showed a very strong absorption bands appearing in the region 3942 - 802 cm^{-1} and indicated the presence of carboxylic, alcoholic, carbonyl, alkane and amide groups. The prominent peaks for C-O, O-H, C=C, C-H and N-H, were reported in the rhizome extracts of *Curcuma caesia* by Pakkirisamy *et al*. [38].

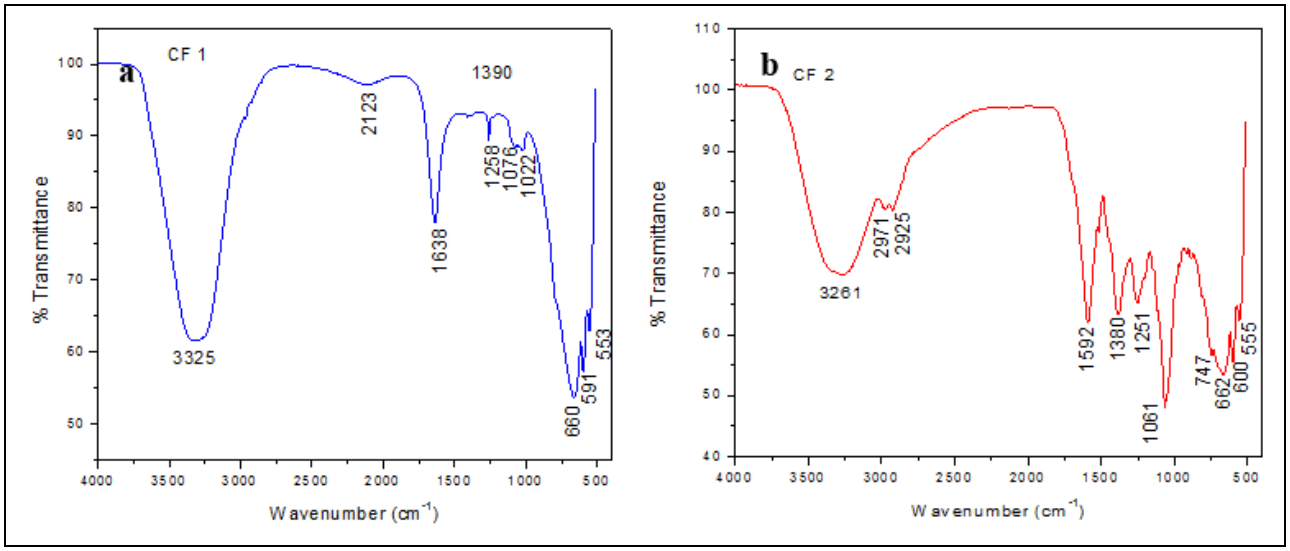


Fig 3: FTIR spectrum of flowers of *C. fragrans* a) CF1 & b) CF2

Table 3: FTIR spectral analysis of fresh flowers (CF1) of *C. fragrans*.

Sl. No.	Frequency of the peak value (cm^{-1})	Functional groups	Phytocompounds
1.	3325	O - H stretch	Alcohols, Phenols
2.	2123	C ≡ C bond	Alkynes
3.	1638	C = C bond	Alkenes
4.	1390	O-H bend	Alcoholic group
5.	1258	C - O stretch	Esters, ethers
6.	1076	C - O stretch	Esters, ethers, carboxylic group
7.	1022	PO_3 stretch	Phosphate ion
8.	660	C-Br stretch	Aliphatic bromocompounds
9.	591	C - I, C - Cl	Halogen group
10.	553	C - I, C - Cl	Halogen group

Table 4: FTIR spectral analysis of dry flowers (CF2) of *C. fragrans.*

Sl. No.	Frequency of the peak value (cm^{-1})	Functional groups	Phytocompounds
1.	3261	O - H stretch	Alcohols, Phenols
2.	2971	C - H stretch	Hydroxyl group
3.	2925	C - H stretch	Saturated aliphatic compound, lipid
4.	1592	N - H bending	Amides
5.	1380	C - C bond	Alkanes
6.	1251	C - O stretch	Esters, ethers, carboxylic acids
7.	1061	C - O stretch	Esters, ethers, carboxylic group
8.	747	C - Br stretch	Aliphatic bromocompounds
9.	662	C - I, C - Cl	Halogen group
10.	600	C - I, C - Cl	Halogen group
11.	555	C - I, C - Cl	Halogen group

Conclusion

In the present study, the GC-MS analysis revealed the phytochemical composition of flower extracts of *C. fragrans*. Our results varied the chemical composition between fresh and dry flower extracts. The flowers of *C. fragrans* showed the major important bioactive compounds like β-sitosterol, 2, 3-epoxy dihydrosqualene and 1, 2-benzenediol. FTIR analysis also confirmed the presence of phenols, hydroxyl compounds, saturated aliphatic amides, esters, ethers, alcohols, carboxylic acids, phosphate and halogen group of compounds in the extracted oils. However, future studies have to be carried out to understand the therapeutic potentials of oils for nutraceutical and pharmaceutical purposes.

Conflict of Interest

The authors declared that they have no conflicts of interest.

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