Research Article

Phytochemical screening, FTIR and GCMS analysis of aqueous extract of *Caralluma bicolor* – An endangered plant

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Abstract

Background: Plants produce various chemicals to protect themselves; but recent studies proved that many phytochemicals can also protect humans against infectious diseases. **Objective:** The present study was aimed to analysis the phytochemicals through preliminary phytochemical screening, FTIR and GCMS analysis by aqueous extract of *Caralluma bicolor*. **Material and Methods:** Phytochemical analysis was carried out for aqueous extract as per the standard methods. FTIR analysis of the aqueous extract was carried out through equipped with Jasco FT/IR-6300. The chemical compositions of aqueous extract of *C. bicolor* was investigated using Perkin-Elmer Gas Chromatography- Mass Spectroscopy. **Results:** Preliminary phytochemical screening of aqueous extract of *C. bicolor* showed positive result for alkaloids, flavonoids and triterpenoids, saponins, glycosides, gum & mucilages and fixed oils. In quantitative analysis the total phenols (49.7 mg), tannin (7.6 mg), alkaloids (78 mg), total flavonoids (3.8 mg), saponins (24 mg), total terpenoids (2.1 mg) were determined. In FTIR analysis functional groups like phenols, aliphatic primary amine, alkanes, carboxylic acid, nitrile, aromatics, alcohol, aliphatic amines, alkyl halides and alkynes were identified. The GCMS analysis of *C. bicolor* plant aqueous extract exhibited the presence of 98 compounds with 10 known bioactive uses. **Conclusion:** From this work, it can be concluded that the species contain effective phytochemical compounds, needs further research on toxicological aspects to develop a safe drug.

Keywords: Preliminary phytochemical, FTIR, gas chromatography, *bicolor* and alkaloids.

Introduction

Traditional systems of medicine continue to be widely practiced on many accounts. Inadequate supply of drugs, increase in population, prohibitive cost of treatments, side effects of many synthetic drugs and development of resistance to present day drugs for infectious diseases have led to increased emphasis on the uses of plant based medicines for wide variety of human ailments. Plants produce bioactive molecules in a diverse range making them a rich source of different types of medicines (Jeeva and Johnson, 2012; Florence et al., 2014). Traditionally, herbal extracts were known to be effective against microorganisms as a result; plants form the basis of modern medicine. Plants produce phytochemicals to protect themselves; but recent studies indicate that many phytochemicals can also protect humans against infectious diseases (Florence et al., 2014; Domettila et al., 2013).

The FTIR spectrum is used to identify the functional groups of the plant extract based on the peak value ratio. The gas chromatography and mass spectroscopy (GCMS) studies have been more progressively useful for the investigation of most of the medicinal plant because this technique has established to be a precious method for the chemical analysis of non-polar components and volatile essential oil, fatty acids and lipids (Khare, 2007).

The family Asclepiadaceae comprises about 200 genera and 2500 species (Evans, 2002) with a global distribution and represented in all types of habitats. A total of 16 species and 8 varieties of *Caralluma* occur in India out of which 5 species and 5 varieties are solely endemic to Peninsular India (Jagtap and Singh, 1999). They grow in arid, rocky regions in the foot hills of Western Ghats and Eastern Ghats (Karuppusamy et al., 2013).

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The member of the genus is small plant, erect, fleshy (Prabu et al., 2013). The species of *Caralluma* found in India are edible and form the part of the traditional medicine system of the country (Yaha and Sattar, 2000). The species of this family possess significant anti-inflammatory, antitumor, anticancer, antiulcer activity and cytoprotective, analgesic, antioxidant, hypolipidemic, antihyperglycemic, antidiabetic, treating paralysis and joint pain and antipyretic properties (Vajha et al., 2010). The major constituents of *Caralluma* species is pregnane glycosides, saponin and flavonoids (Bauer et al., 1966). *Caralluma* is used in the treatment of Rheumatism, Leprosy, Antiseptics and Disinfectants (Jeeva and Johnson, 2012).

The phytochemistry of genus *Caralluma* is characterized by many pregnane glycosides and megastimane glycosides (Jayakar and Rajkapoor, 2004). *Caralluma* extracts have also been found to be appetite suppressant a property which is well known to Indian tribal and hunters (Sreelatha and Pullaiah, 2010).

Caralluma bicolor V.S. Ramach., S. Joseph, H.A. John & Sofiya. (Apocynaceae) is similar to *C. adscendens* and *C. sarkariae* but differs in size and branched, branch lets sagittate leaves, glabrous petals and large seeds. This species is endemic to the Eastern slopes of the Western Ghats of the Coimbatore district in Tamil Nadu (Anilkumar et al., 2013). The whole plant is used as vegetable by the tribals of Attappady. To our knowledge and literature survey, there is no report on phytochemical and biological studies. Hence realizing the importance the present study was carried out to preliminary phytochemical, quantitative analysis, FTIR and GCMS analysis of aqueous extract of *Caralluma bicolor*.

Materials and methods

Plant collection and authentication

The aerial part of *C. bicolor* was collected from Paalamalai hills, kovanur, Periyanaikenpalayam, Coimbatore, Tamilnadu, India authenticated by the Botanical Survey of India, Southern regional Centre, Coimbatore (BSI / SRC/ 5/23/2012-13/ tech 1268).

Extract preparation

The grinded aerial parts of 20 grams weighed using an electronic balance and were crushed in 100 ml of sterile water, boiled at 60°C for 30 minutes on water bath and filtered through Whatman No.1 filter paper. Then filtrate was centrifuged at 2500 rpm for 15 minutes and filtrate was stored in sterile bottles at 30 °C for further use (Harbone, 1973).

Preliminary phytochemical studies

Phytochemical qualitative analysis

The aqueous extract was subjected to preliminary phytochemical qualitative screening by various chemical test such as tests alkaloids (Ciulci, 1994), flavonoids (Sofowora, 1993), tannins (Ciulci, 1994), steroids (Ciulci, 1994), triterpenoids (Finar, 1986), saponins (Kokate, 1999), glycosides (Camporese et al., 2003), gum and mucilage (Whistler and BeMiller, 1993), fixed oil (Kokate, 1999) and anthraquinones (Sanker and Nahar, 2007) to determine the presence of secondary metabolites.

Determination of total phenolic content

The concentration of phenolic in plant extract was determined using spectrophotometric method. Folin-Ciocalteu assay method was used for the determination of the total phenol content. The reaction mixture consists of 1 ml of extract and 9 ml of distilled water was taken in a volumetric flask (25 ml). One millilitre of Folin-Ciocalteu phenol reagent was treated to the mixture and shaken well. After 5 minutes, 10 ml of 7% Sodium carbonate (Na_2CO_3) solution was treated to the mixture. The volume was made up to 25 ml. Incubated for 90 min at room temperature and the absorbance for test and standard solutions were determined against the reagent blank at 550 nm with an Ultraviolet (UV)/Visible spectrophotometer. A set of standard solutions of gallic acid (20, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. Total phenol content was expressed as mg of GAE/g of extract.

Determination of tannin Content

The tannins were determined by Folin - Ciocalteu method. About 0.1 ml of the sample extract was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu phenol reagent, 1 ml of 35% Na_2CO_3 solution and dilute to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of gallic acid (20, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. Absorbance for test and standard solutions were measured against the blank at 725 nm with an UV/Visible spectrophotometer. The tannin content was expressed in terms of mg of GAE /g of extract.

Determination of Alkaloid

The plant extract (1mg) was dissolved in dimethyl sulphoxide (DMSO), added 1ml of 2N HCl and filtered. This solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and collected in a 10 ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (20, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as

described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer. The total alkaloid content was expressed as mg of AE/g of extract.

Determination of Total flavonoid content

Total flavonoid content was measured by the aluminium chloride colorimetric assay. The reaction mixture consists of 1 ml of extract and 4 ml of distilled water was taken in a 10 ml volumetric flask. To the flask, 0.30 ml of 5% sodium nitrite was treated and after 5 minutes, 0.3 ml of 10% aluminium chloride was mixed. After 5 minutes, 2 ml of 1M Sodium hydroxide was treated and diluted to 10 ml with distilled water. A set of reference standard solutions of quercetin (20, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 510 nm with an UV/Visible spectrophotometer. The total flavonoid content was expressed as mg of QE/g of extract.

Estimation for Saponin

Total saponin determination was done using anisaldehyde reagent. Sample solution was prepared in water. Weigh 10 mg of diosgenin, dissolve in 16 mL of methanol, and add 4 mL of distilled water. Standard solutions of diosegenin (20, 40, 60, 80 and 100µg/ml) were prepared 80% aqueous methanol. Mixed thoroughly and start pipetting immediately. For total saponins estimation 500µl of sample, 500µl of 0.5% anisealdehyde reagent were mixed and kept aside for 10 min. Later, 2 ml of 50% sulphuric acid reagent was added. Tubes were kept in water bath with constant temperature of 60ºC. After 10 min tubes were cooled and absorbance was taken at 435 nm. Same method for standard also followed. The amount of saponins was calculated as saponin equivalent from the calibration curve of standard.

Total terpenoid determination

Total terpenoid content was determined by the method of Ghorai et al. (2012). To 1 mL of the plant extract, 3 mL of chloroform was added. The sample mixture was thoroughly vortexed and left for 3 min and then 200 µl of concentrated sulfuric acid (H_2SO_4) was added. Then it was incubated at room temperature for 1.5 h -2 h in dark condition and during incubation a reddish brown precipitate was formed. All supernatant of reaction mixture was decanted without disturbing the precipitation. 3 mL of 95% (v/v) methanol was added and vortexed thoroughly until all the precipitation dissolve in methanol completely. The absorbance was read at 538 nm using UV/visible spectrophotometer. The standard solutions of Linalool (20, 40, 60, 80 and 100 µg/ml) were prepared in the same manner. The total terpenoid content was calculated by calibration curve of Linalool.

FTIR analysis

FTIR analysis of the aqueous extract was carried out through the potassium bromide (KBr) pellet (FTIR grade) method in 1:100 ratio and spectrum was recorded using Jasco FT/IR-6300 Fourier transform infrared spectrometer equipped with JASCO IRT-7000 Intron Infrared Microscope using transmittance mode operating at a resolution of 4 cm^{-1} (JASCO, Tokyo, Japan).

GC-MS analysis

The Clarus 680 GC was used in the analysis employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250µm df) and the components were separated using Helium as carrier gas at a constant flow of 1 ml/min. The injector temperature was set at 260 °C during the chromatographic run. The 1µL of extract sample injected into the instrument the oven temperature was as follows: 60 °C (2 min); followed by 300 °C at the rate of 10 °C min^{-1}; and 300 °C, where it was held for 6 min. The mass detector conditions were: transfer line temperature 240 °C; ion source temperature 240 °C; and ionization mode electron impact at 70 eV, a scan time 0.2 sec and scan interval of 0.1 sec. The fragments from 40 to 600 Da. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS NIST (2008) library.

Results

Preliminary phytochemical screening of *C. bicolor*

Preliminary phytochemical screening of aqueous extract of *C. bicolor* was carried out using different methods in order to identify either the presence or absence of secondary metabolites such as alkaloids, flavonoids, tannins, steroids, terpenoids, saponins, glycosides, gum and mucilage, fixed oils and anthraquinones are presented in table 1 . Extract showed positive result for alkaloids, flavonoids and triterpenoids present in Salkowski's test. Saponins, glycosides, gum & mucilages and fixed oils were present in this extract. Tannins, steroids and anthraquinones were absent.

Quantitative analysis of *C. bicolor*

C. bicolor aqueous extract was evaluated the quantification of various secondary metabolites. Table 2 shows the amount of secondary metabolites presence in this extract. Alkaloids was observed presence of highest amount (78 mg/g) in aqueous extract. Other secondary metabolites quantity such as total phenolics (49.7 mg/g, saponins (24 mg/g) tannins (7.6 mg/g), total flavonoids (3.8 mg/g), and total terpenoids (2.1 mg/g) were noticed in *C. bicolor* aqueous extract.

FTIR analysis

Phytocompounds of *C. bicolor* aqueous extract was

Table 1. Preliminary phytochemical analysis of *C. bicolor* in plant aqueous extract

Category of chemical constituents	Tests	*C. bicolor* plant aqueous extract
Alkaloids	Draggendorff's test	+
	Mayer's test	-
	Wagner's test	-
	Hager's test	-
Flavonoids	10% HCl & 5% NaOH test	+
	Alkaline test	+
Tannins	5% FeCl test	-
Steroids	Libermann - Burchard's test	-
Triterpenoids	Libermann - Burchard's test	-
	Salkowski's test	+
Saponins	Foam test	+
Glycosides	Killer & Kilian test	+
Gum & Mucilages	Whistler & BeMiller test	+
Fixed oils	Spot test	+
Anthraquinones	NH_4OH test	-

Table 2. Quantitative determination of secondary metabolites in *C. bicolor*

Secondary metabolites	Amount of sample (mg/g)
Total Phenols	49.7 mg
Tannin	7.6 mg
Alkaloids	78 mg
Total flavonoids	3.8 mg
Saponins	24 mg
Total terpenoids	2.1 mg

analyzed by biomolecule functional groups. Eleven major peaks are 3452.58, 3302.13, 2931.80, 2376.30, 2229.71, 1585.49, 1419.61, 1076.28, 655.80, 617.22 and 428.20 cm^{-1} in the region between 500-4000 cm^{-1} (Figure 1). Functional groups like phenols, aliphatic primary amine, alkanes, carboxylic acid, nitrile, aromatics, alcohol, aliphatic amines, alkyl halides and alkynes were identified. The corresponding functional group are carbon dioxide (2341.58 cm^{-1}), isocyanate (2252.86 cm^{-1}), nitriles (2252.86 cm^{-1}), aromatic compound (1874.81 cm^{-1}), aromatic amines (1323.17 cm^{-1}), alkyl aryl ether (1276.88 cm^{-1}) and alkyl

Table 3. FTIR analysis of plant aqueous extract of *C. bicolor*

S. No	Frequency (Cm^{-1})	Bond	Functional group name
1	3714.90 (m)	N-H stretching	Amides
2	3452.58 (b)	H–bonded	Phenols
3	3302.13(b)	N-H stretching	Aliphatic primary amine
4	2931.80 (s)	C–H stretch	Alkanes
5	2376.30 (s)	O-H stretching	Carboxylic acid
6	2341.58 (s)	O=C=O stretching	Carbon dioxide
7	2276.00 (b)	N=C=O stretching	Isocyanate
8	2252.86 (b)	C≡N stretch	Nitriles
9	2229.71(b)	C≡N stretching	Nitrile
10	1874.81 (m)	C-H	Aromatic compound
11	1585.49 (s)	C–C stretch	Aromatics
12	1419.61 (s)	O-H	Alcohol
13	1323.17 (m)	C-N stretch	Aromatic amines
14	1276.88 (m)	C-O stretching	Alkyl aryl ether
15	1076.28 (s)	C–N stretch	Aliphatic amines
16	655.80 (m)	C–Br stretch	Alkyl halides
17	617.22 (m)	≡C-H bend	Alkynes
18	428.20 (m)	C-I stretch	Alkyl halides

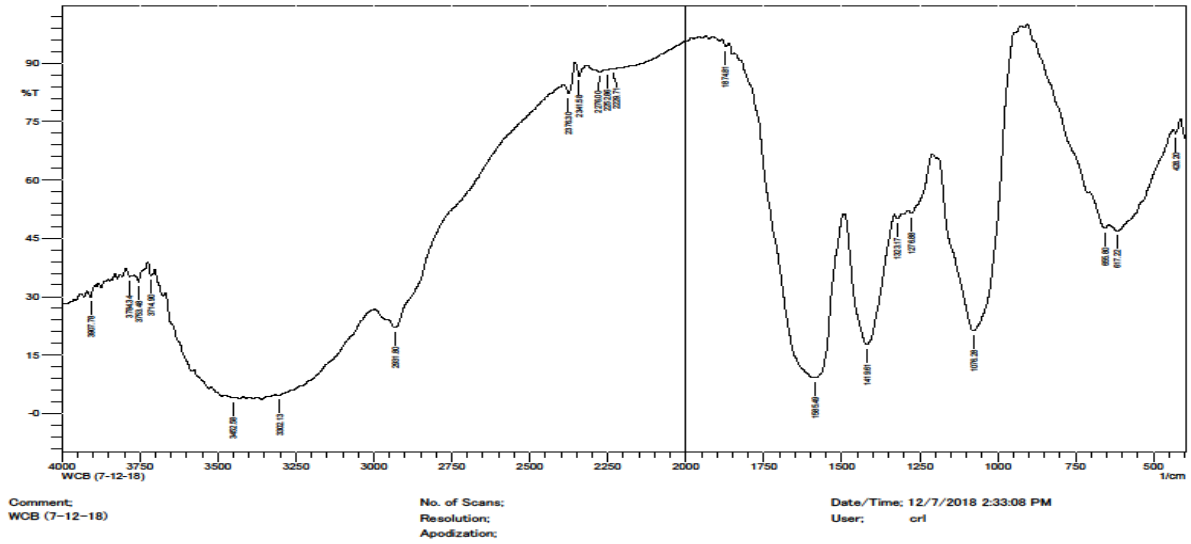

Figure 1. FTIR analysis of plant aqueous extract of *C. bicolor*

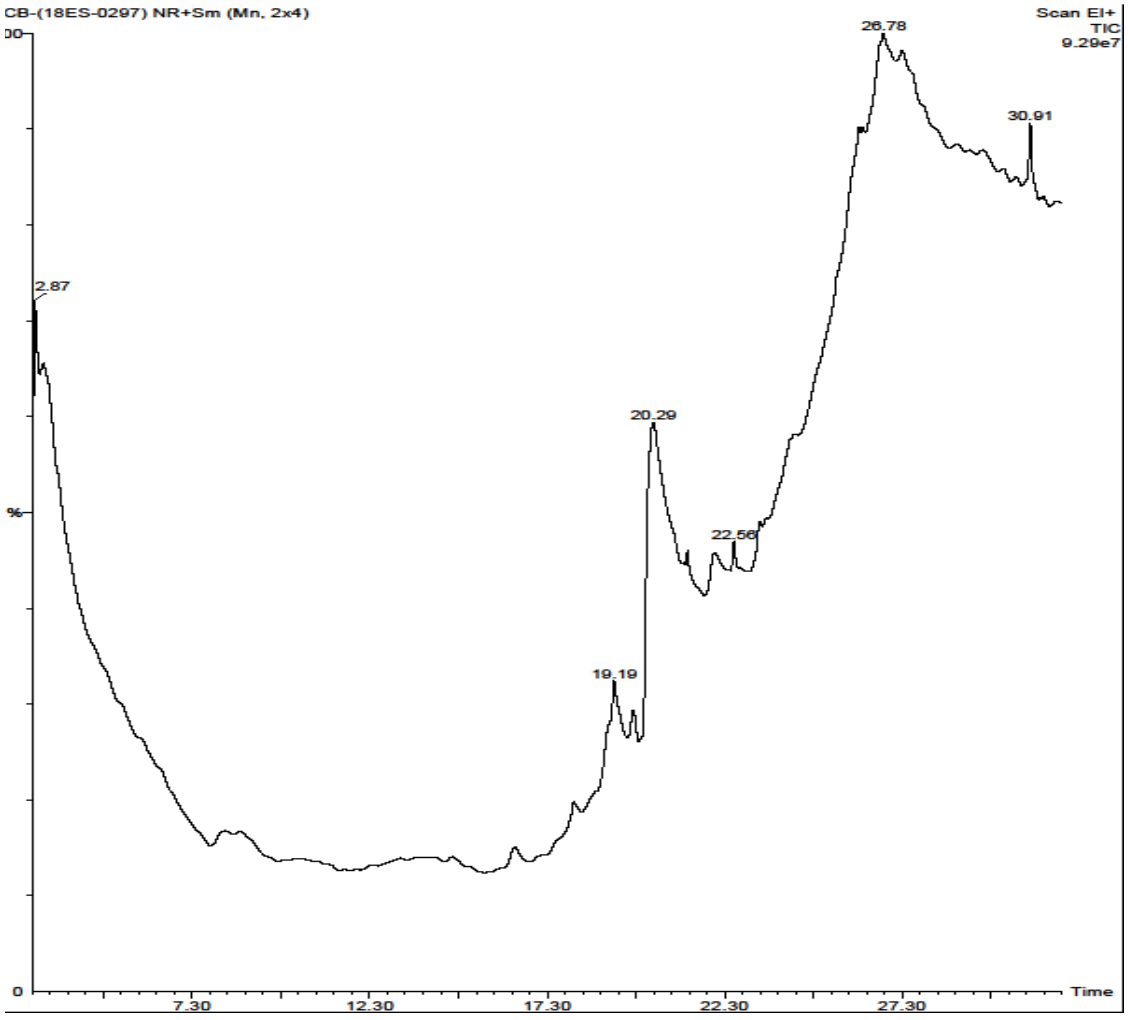

Figure 2. GCMS analysis plant aqueous extract of *C. bicolor*

halides (428.20 cm^{-1}) respectively (Table 3).

Gas Chromatography Mass Spectrometer (GCMS) analysis:

The GCMS analysis of *C. bicolor* plant aqueous extract exhibited the presence of 98 compounds (Figure 2) with 10 known bioactive principles (Table 4). These 10 bioactive

compounds namely 1-Octadecyne, 1-Hexadecyne, 9,12-Octadecadienoic Acid (Z,Z)-, Cis-9,10-Epoxyoctadecan-1-ol, 7-Heptadecyne, 1-Chloro-, 9-Octadecyne, 9,17-Octadecadienal, (Z)-, 9,12-Octadecadienoyl Chloride, (Z,Z)-, 9,12-Octadecadienoic Acid, methyl ester, 8,11,14-Eicosatrienoic Acid, (Z,Z,Z)- and Doconexent were present in aqueous extract. The identification of the phytochemical compounds was confirmed based on the retention time, peak area and molecular formula. The main properties of bioactive compounds are antimicrobial, anti-inflammatory, antioxidant, cancer preventive, antiandrogenic, antiarthritic and hepatoprotective activities.

Discussion

The medicinal and pharmacological actions of medicinal herbs are often depended on the presence of bioactive compounds, the secondary metabolites (Heinrich et al., 2004). Qualitative phytochemical analysis of this plant confirm the presence of various secondary metabolites like alkaloids, flavonoids, glycosides, saponins, triterpenes, gum & mucilage and fixed oils. The results suggest that the phytochemical properties for curing various ailments and possess potential anti-inflammatory, antimicrobial and antioxidant and leads to the isolation of new and novel compounds. Most alkaloids have a strong bitter taste and are very toxic, for these reasons they are used by plant to protect themselves against herbivory and attacks by microbial pathogens and invertebrate pests (Harbone, 1998).

Phenolic herb secondary metabolites are widely distributed

Table 4. Identified bioactive compounds in aqueous extract of *C. bicolor*

S. No	Compound Name	Molecular Formula	Molecular Weight	Bioactive uses
1	1-octadecyne	$C_{18}H_{34}$	250	Antimicrobial activity (Zahir Hussain and Kumaresan, 2013) , Anti-inflammatory agent, Antibacterial agent, Fragrance (Senthamarai Selvi and Anusha Basker, 2012)
2	1-Hexadecyne	$C_{18}H_{32}O_2$	222	Antibacterial (Senthamarai Selvi and Anusha Basker, 2012)
3	9,12-octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	Fatty acid ester, Anti-inflammatory, Antibacterial, Antiarthritic, Hepatoprotective, Anti -histaminic, Anticoronary (Olaleye et al., 2018). Hypocholesterolemic, nematicide, antiarthritic, hepatoprotective, anti - androgenic, hypocholesterolemic 5 -alpha reductase inhibitor, antihistaminic anticoronary, insectifuge, antieczemic, antiacne (Sudha et al., 2013)
4	7-Heptadecyne, 1-chloro-	$C_{17}H_{31}Cl$	270	Antioxidant, Nematicide, Pesicide (Pauline Fatima Mary, 2016)
5	9-octadecyne	$C_{18}H_{34}$	250	Antioxidant, antimicrobial (Akhilesh Upgade and Anusha Bhaskar, 2013)
6	9,17-octadecadienal, (z)-	$C_{18}H_{32}O$	264	antibacterial and antifungal (Suresh et al., 2011)
7	9,12-octadecadienoyl chloride, (Z,Z)-	$C_{18}H_{31}OCl$	298	Anti-inflammatory, cancer preventive, insectifuge, nematicide, anti - acne, antieczemic, anticoronary (Olaleye et al., 2018)
8	9,12-octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294	Antiinflammatory, Hypocholesterolemic Cancer preventive, Hepatoprotective, Nematicide Insectifuge, Antihistaminic Antieczemic, Antiacne, 5 -Alpha reductase inhibitor Antiandrogenic, Antiarthritic, Anticoronary, Insectifuge (Lalitha et al., 2015; Sunita Arora and Ganesh Kumar, 2017).
9	8,11,14-icosatrienoic acid, (Z,Z,Z)-	$C_{20}H_{34}O_2$	306	Cardio protective (Balamurugan et al., 2017)
10	Doconexent	$C_{22}H_{32}O_2$	328	Antiinflammatory, Hyperthermic, Anemia, Hypercholesterolemic, Hematotoxic, Pulmonary edema, Antineoplastic. (Babu et al., 2014)

in herbs and are responsible for colour development, pollination and protection against UV radiation and pathogens (Bruneton, 1999; Heinrich et al., 2004). Flavonoids have several proven medicinal properties such as anti-inflammatory, antioxidant, anticancer, antibacterial and antiviral properties (Cheynier, 2005; Manach et al., 2004). Terpenoids, also known as isoprenoids constitute the largest group of herbal secondary metabolites (Bruneton, 1999). Terpenoids are involved in defense wound scaling and thermo tolerance of plants as well as in the pollination of seed crops (Heinrich et al., 2004) and also used as an antibacterial, antifungal, antimalarial, antioxidant activity (Gurib-Fakim, 2006).

Medicinally important glycosides consist of anthraquinone glycosides, coumarins glycosides and steroidal (Cardiac) glycosides. Coumarins glycosides have been shown to have hemorrhagic, anti-fungicidal and antitumor activities (Bruneton, 1999). The aglycones of a coumarin glycoside dicumarol and its synthetic structural analog, warfarin are used in modern medicine as anticoagulants. Tannins are complex moieties produced by majority of plants as protective substances, they have wide pharmacological activities. They have been used since past as tanning agents and they possess astringent, anti-inflammatory, antidiarrheal, antioxidant and antimicrobial activities (Killedar and More, 2010).

FTIR spectrum is generally used tool in plant biological studies (Berthomieu and Hienerwadel, 2009). The FTIR analysis gives totally 21 peaks. In this eleven main and ten corresponding peaks are identified. Eleven main peaks observed through the spectrum of plant sample were 3452.58, 3302.13, 2931.80, 2376.30, 2229.71, 1585.49, 1419.61, 1076.28, 655.80, 617.22 and 428.20 cm^{-1} in the region between 500-4000 cm^{-1}. Generally alkyl halides and alkanes prevalent in FTIR studies of plant samples which were found more significant against microbes (Janakiraman et al., 2011).

The present study is the first report on the GC-MS analysis in *C. bicolor* aqueous extract. Totally ten compounds have been reported to possess interesting biological activities. The prevailing major compounds were 1-Octadecyne which acts as antimicrobial activity, anti-inflammatory agent, antibacterial agent, fragrance (Selvi and Basker, 2012). 1-Hexadecyne have antibacterial (Selvi and Basker 2012), similarly 9,12-Octadecadienoic acid (Z,Z)- Fatty acid ester showed the anti-inflammatory, antibacterial, antiarthritic, hepatoprotective, anti-histaminic and anticoronary (Olaleye et al., 2018). Another compound 7-Heptadecyne, 1-Chloro- has strong antioxidant, nematicide and pesicide (Mary, 2016). The 9-Octadecyne compound has antioxidant, antimicrobial (Upgade and Bhaskar, 2013).

The 9,17-Octadecadienal, (Z)- showed antibacterial and antifungal (Suresh et al., 2011). Similarly 9,12-Octadecadienoyl Chloride, (Z,Z)- has anti-inflammatory, cancer preventive, insectifuge, nematicide, anti-acne, antieczemic, anticoronary etc. (Olaleye et al., 2018). The compound 9,12-Octadecadienoic acid methyl ester reported to have, anti-inflammatory, hypocholesterolemic cancer preventive, hepatoprotective, nematicide insectifuge, antihistaminic antieczemic, antiacane, 5-Alpha reductase inhibitor antiandrogenic, antiarthritic, anticoronary and insectifuge (Lalitha et al., 2015; Arora and Kumar, 2017). Similarly another compound 8,11,14-Eicosatrienoic acid, (Z,Z,Z)- showed the cardio protective (Balamurugan et al., 2017) at last Doconexent showed the anti-inflammatory, hyperthermic, anemia, hypercholesterolemic, hematotoxic, pulmonary edema and antineoplastic (Babu et al., 2014).

Conclusion

The result concludes that the major compound possess antioxidant, anti-inflammatory, anticancer and antimicrobial properties. In the present study the aqueous extract could have been produced number of active constituents responsible for many biological activities. However further studies will need to be undertaken its bioactivity and toxicity profile.

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