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GC-MS Analysis of Leaf, Fruits and Latex of *Croton bonplandianum* Baill

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Authors' contributions

This work was carried out in collaboration between both authors. The first author VV performed the research work and wrote the initial draft of manuscript. The corresponding author RU designed the research problem and corrected the final format of manuscript. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: To characterize the phytocompounds of different parts of *Croton bonplandianum* using GC-MS.
Study Design: GC-MS analysis of bioactive constituents of *C. bonplandianum.*
Place and Duration of Study: Post Graduate and Research Department of Biochemistry at Government Arts College (Autonomous), Kumbakonam and Food Safety and Quality Testing Laboratory, Indian Institute of Crop Processing Technology, Thanjavur, Tamilnadu, India, between May, 2011 to June, 2012.
Methodology: The *C. bonplandianum* leaf and fruit (25 grams) powder was soaked in 60 ml of ethanol each and kept at room temperature for 12 hours and the fresh latex of 10 ml was mixed

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with 90 ml of ethanol and kept at shaker for 3 hours. The samples were filtered and concentrated. Each sample was subjected to phytochemical analysis using GC-MS.
Results: The GC-MS analysis showed peaks of twenty one phytocompounds from different parts of *C. bonplandianum*. Out of which five compounds were found in leaf, one compound in latex and fifteen compounds in fruits. The highest peak area of 88.69% for 16-Hexadecanoyl hydrazide and the lowest peak area of 1.39% for Phytol were obtained in leaf extract. The latex of *C. bonplandianum* showed that the presence of Myo-Inositol, 2-C-methyl with the peak area of 30.8%. The fruits of *C. bonplandianum* showed that the presence of 9, 12, 15-Octadecatrienoic acid, methyl ester (z,z,z)- with the highest peak area of 41.81% and 2-Hexen-1-ol, 2-ethyl with the lowest peak area of 0.69%.
Conclusion: The phytochemical constituents of *C. bonplandianum* have been screened and the isolation of individual bioactive compounds from *C. bonplandianum* will be helpful to find new drugs.

Keywords: GC-MS analysis; Croton bonplandianum; bioactive compounds; leaf; fruits; latex.

1. INTRODUCTION

Medicinal plants are the richest bio-resources of folk medicines, traditional systems of medicine, food supplements, nutraceuticals, pharmaceutical industries and chemical entities for synthetic drugs [1]. Modern medicine has evolved from folk medicine and traditional system only after through chemical and pharmaceutical screening [2]. India is the birth place of renewed system of indigenous medicine such as Siddha, Ayurvedha and Unani [3]. Traditional systems of medicines are prepared from a single plant or combinations of a number of plants. The efficacy depends on the use of proper plant part and its biological potency which in turn depends upon the presence of required quantity and nature of secondary metabolite in a raw drug [4,5]. There is growing awareness in correlating the phytochemical constituents of a medicinal plant with its pharmacological activity [6]. Screening of active compounds from plants has lead to the invention of new medicinal drugs and they have an efficient protection against various diseases including cancer [7] and Alzheimer's diseases [8].

Phytochemicals are responsible for medicinal activity of plants [9]. These are non-nutritive chemicals that have protected human from various diseases [3]. Phytochemicals are basically divided into two groups that is primary and secondary metabolites based on the function in plant metabolism. Primary metabolites are comprise common carbohydrates, amino acids, proteins and chlorophylls while secondary metabolites consist of alkaloids, saponins, steroids, flavonoids, tannins and phenolic compounds [10,11]. Phytochemical constituents are the basic source for the establishment of several pharmaceutical industries. The phytochemical constituents are playing a significant role in the identification of crude drugs [8].

In recent years, the interest for the study of organic compounds from the plants and their activity has increased [12]. A knowledge of the chemical constituents of plant is desirable not only for the discovery of therapeutic agents, but also helps in disclosing new sources of economic phytocompounds for the synthesis of complex chemical substances and for discovering the actual significance of folkloric remedies [13]. There is an increasing interest on the phytochemical compounds, which could be relevant to their nutritional incidence and their role in health and disease [14]. The combination of an ideal separation technique - Gas Chromatography (GC) with the best identification technique - Mass Spectrum (MS) made GC-MS, which is an ideal technique for qualitative and quantitative analysis of volatile and semi volatile compounds. This technique has proved to be a valuable method for the analysis of non polar components and volatile oils, fatty acids, lipids and alkaloids [15]. *Croton bonplandianum* Baill. belongs to the family of Euphorbiaceae and it is distributed in Southern Bolivia, Paraguay, South Western Brazil, Northern Argentina and India. *C. bonplandianum* was reported to have many medicinal uses including the repellent property against the insects [16] and showed antibacterial [17], antifungal [18], antioxidant [19], analgesic [20], nematicide [21], anticoronary [22,23,24], hepatoprotective [25] and wound healing activities [26,27]. Local people in the remote area of West Bengal in India are using its root against snake bite and leaf extract against high fever [28]. The methanol extract of *C. bonplandianum*

has been found to exhibit antitumour properties in plants caused by *Agrobacterium tumefaciens* [29] and larvicidal activities [16]. Although the plant is used in Ayurvedic medicine for the treatment of various ailments but there are no detailed reports on the analysis of phytoconstituents of different parts of *C. bonplandianum*. With this background, the present study was aimed to identify the phytoconstituents present in leaf, fruit and latex of *C. bonplandianum* using GC-MS analysis.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

C. bonplandianum was collected from the waste lands in and around Mayiladuthurai, Tamilnadu, India, where it was found naturally. The plant was identified by Rev.Dr.John Britto, The Directer of the Rabinat herbarium and Centre for Molecular Systematics, St.Joseph's College, Tiruchirappalli, Tamilnadu, India. The leaves and fruits were separated and washed thoroughly in running tap water to remove soil particles and other adhered debris and then finally washed with sterile distilled water. The whole plants were shade dried and ground well into fine powder. The powdered materials were stored in air tight container until the time of use [30].

The latex of *C. bonplandianum* was obtained as exudates by and plucking of fresh leaves from actively growing plants. The latex was collected into sterile, plastic containers by pressing and squeezing in between fingers, the apex of the leaves to release as much as possible latex into the containers. After collection, the latex was centrifuged with 5000 rpm for 15 minutes to remove any solid particles present in it. The latex was decanted off into containers and plugged with cotton and stored at 4°C until required for use. Collections were made in the mornings during the days of each analysis [31].

2.2 Identification of Phytocompounds by GC-MS

2.2.1 Sample Preparation

25 grams of *C. bonplandianum* leaf and fruit powder were soaked in 60 ml of ethanol separately and kept at room temperature for 12 hours and 10 ml of fresh latex was mixed with 90 ml of ethanol and kept at shaker for 3 hours [30,32]. The samples were filtered and concentrated separately through nitrogen flushing to 1 ml. From this 2 µl of each prepared sample was injected into the GC-MS instrument for phytochemical analysis.

2.2.2 Equipment

The GC-MS analysis was carried out using a Perkin Elmer Clarus 500 for the analysis of phytocompounds of *C. bonplandianum* [33]. The data were obtained on a Capillary Column Elite-5MS (5% phenyl 95% dimethyl poly siloxane). Helium was used as the carrier gas with a flow rate of 1ml/min in the split mode (10:1). An aliquot of 2 µl of ethanol solution of the sample was injected into the column with the injector temperature at 250°C. GC oven temperature started at 110°C and holding for 2 min and it was raised to 200°C at the rate of 10°C /min without holding. Holding was allowed at 280°C for 9 min with program rate of 5°C /min. The injector and detector temperatures were set at 250°C and 280°C respectively. GC interface and Ion source temperature was maintained at 200°C. The mass spectrum of compounds in the samples was obtained by electron ionization at 70 eV and the detector was operated in scan mode from 40-450 amu (atomic mass units). A scan interval of 0.5 second and fragments from 40 to 450 Da were maintained. The total running time was 36 minutes.

2.2.3 Interpretation of Mass Spectrum (MS)

In the MS Programme, NIST Version 2.0 library database of National Institute of Standard and Technology (NIST) having more than 2,00,000 patterns was used for identifying the phytochemical components of *C. bonplandianum*. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

3. RESULTS

The name of identified phytocompounds of ethanolic extract of different parts like leaf, fruit and latex of *C. bonplandianum* with their retention time (RT), peak area percentage, molecular weight and their biological activities are given in (Tables 1-3).The phytocompound prediction is based on NIST Version 2.0 library database of National Institute of Standard and Technology (NIST). The GC-MS results showed that the presence of twenty one major

compounds in different parts of *C. bonplandianum.* Among these five phytocompounds such as 16-Hexadecanoyl hydrazide (88.69%), 1,2-Benzenedicarboxylic acid, diisooctyl ester (5.56%), 2-Piperidinone, N-[4-bromo-n-butyl] (2.56%), Phthalic acid, bis (7-methyloctyl) ester (1.80%) and Phytol (1.39%) were found in leaf. The latex of *C. bonplandianum* showed the presence only one phytocompound Myo-Inositol, 2-C-methyl (30.8%). The fruits of *C. bonplandianum* showed the presence of fifteen major phytocompounds including 9,12,15-Octadecatrienoic acid, methyl ester (z,z,z)- (41.81%), Diazoprogesterone (19.03%), Decanoic acid, ethyl ester (4.86%), 1-Propene, 2- nitro-3-(1-cyclooctenyl) (4.58%) and 6,9,12- Octadecatrienoic acid, 13-Tetradece-11-yn-1-ol (3.47%).

4. DISCUSSION

In the present study twenty one major phytocompounds were identified in different parts like leaf, fruit and latex of *C. bonplandianum*. The biological activities of identified phytocompounds in *C. bonplandianum* were predicted based on Dr. Duke`s phytochemical and Ethnobotanical Databases. In terms of peak area percentage of 16-Hexadecanoyl hydrazide was predominent in ethanolic leaf extract where as 9, 12, 15-Octadecatrienoic acid, methyl ester (z,z,z)- and Diazoprogesterone were predominent in fruits of *C. bonplandianum*. The compound like Myo-Inositol, 2-C-methyl was identified in latex of *C. bonplandianum*. The leaf of *C. bonplandianum* extract showed five major compounds, among these phytol has antimicrobial, anti-inflammatory, anticancer and diuretic activities, 1, 2-Benzenedicarboxylic acid, diisooctyl ester and Phthalic acid, bis (7-methyloctyl) ester compounds have antimicrobial and antifouling activities, 2-Piperidinone, N-[4-bromo-n-butyl] has antimicrobial and anti-inflammatory activities and 16-Hexadecanoyl hydrazide has antimicrobial activity (Table 1). Latex showed that the phytocompound of Myo-Inositol, 2-C-methyl and it has antimicrobial activity (Table 2). The *C. bonplandianum* fruit extract exhibits the phytocompounds like Decanoic acid, ethyl ester has insecticide activity, where as 9, 12, 15-Octadecatrienoic acid, methyl ester, (Z,Z,Z) and 6, 9, 12-Octadecatrienoic acid, phenylmethyl ester, (Z,Z,Z) compounds have anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insecticide, antihistaminic, antieczemic, antiacne, 5-alpha reductase inhibitor, antiandrogenic, anticoronary and antiarthritic activities, 2-Hexen-1-ol, 2-ethyl and 1-Propene, 2-nitro-3-(1-cyclooctenyl) compounds have antimicrobial activity, Pseudoephedrine has analgesic, antimicrobial and anti-inflammatory activities and Diazoprogesterone has antimicrobial, anti-inflammatory, anticancer, antiarthritic, antiasthma and diuretic activities (Table 3).

There is growing awareness in correlating the phytochemical components and their biological activities [34,35,36]. (Fig. 1) showed the highest peak area 88.69% for the phytocompound of 16-Hexadecanoyl hydrazide and lowest peak area 1.39% for the compound of phytol in leaf extract. Phytol is one among the five compounds of the leaf extract were observed in the present study. Similarly, the researchers reported that the presence of phytol in the leaf of other plants like *Lantana camara, Mimosa pudica* and *Erythropalum scandens* [37-40]. The antibacterial activity of phytol against *Staphylococcous aureus* was reported and it cause damage the cell membrane as a result there is a leakage of potassium ions from bacterial cells [41]. Phytol constitute a promising novel class of pharmaceuticals for the treatment of rheumatoid arthritis and possibly other chronic inflammatory diseases [42]. Phytol is an acyclic diterpene alcohol and it is also a precursor for vitamins E and K1. Different solvent extracts of leaf of *C. bonplandianum* showed effective inhibition activity against bacteria and fungi [43,44]. *In vitro* anti-inflammatory activity *of* ethanolic leaf extract of *C. bonplandianum* was reported [45]. It is also used as antiseptic and antidote [22,23,24]. Tumour formation by *Agrobacterium* in potato plants was distinctly inhibited by the methanolic leaf extract of *C. bonplandianum* [29].

As shown in (Fig. 2), the peak area 30.8% for Myo-Inositol, 2-C-methyl was observed in the latex extract of *C. bonplandianum* and it posses antimicrobial activity was recorded in Dr.Duke's phytochemical and ethnobotanical database. Similarly, the presence of Myo-Inositol, 2-C-methyl was reported in *Prosopis spicigera* [46]. The antifungal activity of latex of *C. bonplandianum* against *Microsporium gypsum* and *Trichophyron mentagrophyt* was reported [18]. The medicinal plants have healing effect on wounds and cuts [25,26] and it may be due to their antimicrobial activity which seems to be responsible for wound contraction and increased rate of epithelialisation [47].

(Fig. 3) showed that the highest peak area 41.81% for the 9,12,15-Octadecatrienoic acid, methyl ester (z,z,z)- in fruit extract which is an

Omega-3-fatty acid and this compound have the anti-inflammatory and antiarthritic properties [48,49]. Similarly 9, 12, 15-Octadecatrienoic acid methyl ester (z,z,z)- identified in the ethanol leaf extract of *Aloe vera* and *Vitex negundo* [50,51]. Omega-3-fatty acids have been found to be essential for normal growth and development. They play an important role in the prevention and treatment of coronary artery disease, hypertension, diabetes, arthritis, inflammation, autoimmune disorders and cancer [52]. The hepatoprotective compounds like 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- and 6,9,12-Octadecatrienoic acid, phenyl methyl ester, (Z,Z,Z)- were observed in the fruits of *C. bonplandianum*. *C. bonplandianum* is used for the treatment of jaundice [20] and liver diseases [44]. The plant has hypotensive effect [53] and also used in the treatment of high blood pressure [22,23,24]. The extracts of this plant showed antimicrobial and antitumour activities [54]. *C. bonplandianum* is also considered as chologogue and purgative [55]. The fresh juice of *C. bonplandianum* is used in the treatment of headache by ethnic groups [21]. *C. bonplandianum* has hepatoprotective and antihelmenthic properties [21,22,23] and also posses repellent property against the insects [21,56]. The lowest peak area 0.69% was observed for 2-Hexen-1-ol, 2-ethyl in the fruit extract of *C. bonplandianum*. It is used as Ayurvedic medicine, however there are no reports on phytochemical analysis in fruits of *C. bonplandianum* using GC-MS. The researchers were reported eleven phytocompounds in chloroform leaf extract and sixteen phytocompounds in ethanolic leaf extract of *C. bonplandianum* [57,58]. But in the present study, we found only five compounds in the ethanolic leaf extract, among these the compound phytol is commonly reported in both studies remaining four compounds are newly reported. Fifteen compounds in fruits extract and one compound in the latex extract were newly reported in this study. In the present study, we screened totally twenty one phytocompounds from different parts like leaf, fruit and latex of *C. bonplandianum* by GC-MS analysis. Up to our knowledge, this study may be a first report on the analysis of phytocompounds in different parts especially fruits and latex of *C. bonplandianum* using GC-MS analysis. So, the present study is the first step to find out the nature of active principles in different parts of *C. bonplandianum* and this study will also be helpful for further research.

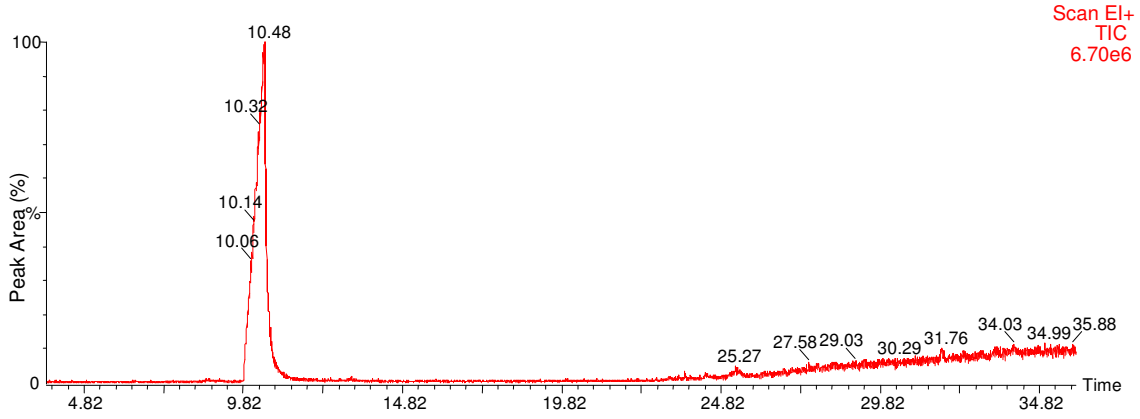

Fig. 1. GC-MS chromatogram of ethanolic leaf extract of *C. bonplandianum*

Table 1. Screening of the phytocompounds of ethanolic leaf extract of *C. bonplandianum* by GC-MS

Sl. no.	RT	Name of the compound	Molecular formula	MW	Peak area %	Nature of compound	**Activity
1.	14.97	Phytol	$C_{20}H_{40}O$	296	1.39	Diterpene compound	Antimicrobial Antiinflammatory Anticancer Diuretic
2.	20.89	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	5.56	Plasticizer compound	Antimicrobial Antifouling
3.	24.42	2-Piperidinone, N-[4-bromo-n-butyl]-	$C_9H_{16}BrNO$	233	2.56	Alkaloid compound	Antimicrobial Anti-inflammatory
4.	24.79	Phthalic acid, bis(7-methyloctyl) ester	$C_{26}H_{42}O_4$	418	1.8	Plasticizer compound	Antimicrobial Antifouling
5.	30.33	16-Hexadecanoyl hydrazide	$C_{16}H_{34}N_2O$	270	88.69	Nitrogen compound	Antimicrobial

*RT – Retention Time, MW – Molecular Weight, **Dr.Duke`s phytochemical and Ethnobotanical Databases*

Table 2. Screening of the phytocompounds of ethanolic latex extract of *C. bonplandianum* by GC-MS

Sl. no.	RT	Name of the compound	Molecular formula	MW	Peak area %	Nature of compound	**Activity
1.	10.48	Myo-Inositol, 2-C-methyl	$C_7H_{17}O_6$	194	30.8	Inositol compound	Antimicrobial

*RT – Retention Time, MW – Molecular Weight,**Dr.Duke`s phytochemical and Ethnobotanical Databases*

Table 3. Screening of the phytocompounds of ethanolic fruit extract of *C. bonplandianum* by GC-MS

Sl. no.	RT	Name of the compound	Molecular formula	MW	Peak area %	Nature of compound	**Activity
1.	13.42	Decanoic acid, ethyl ester	$C_{12}H_{24}O_2$	200	4.86	Fatty acid ester	Insecticide compound
2.	15.68	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	292	41.81	Linolenic acid ester	Antiinflammatory Hypocholesterolemic Cancer preventive Hepatoprotective Nematicide Insectifuge Antihistaminic Antieczemic Antiacne 5-Alpha reductase inhibitor Antiandrogenic Antiarthritic

							Anticoronary
3.	16.03	Decanoic acid, ethyl ester	$C_{12}H_{24}O_2$	200	2.78	Fatty acid ester	Insecticide compound
4.	18.46	1,2:4,5:9,10-Triepoxydecane	$C_{10}H_{16}O_3$	184	2.92	Epoxy compound	No activity reported
5.	19.35	1-Octadecyne	$C_{18}H_{34}$	250	1.25	Alkene compound	No activity reported
6.	20.79	1-Tridecyne	$C_{13}H_{24}$	180	1.25	Alkene compound	No activity reported
7.	23.09	2-Decen-1-ol	$C_{10}H_{20}O$	156	8.33	Alcoholic compound	No activity reported
8.	24.63	6,11-Dimethyl-2,6,10-dodecatrien-1-ol	$C_{14}H_{24}O$	208	2.22	Alcoholic compound	No activity reported
9.	25.8	2-Hexen-1-ol, 2-ethyl-	$C_8H_{16}O$	128	0.69	Alcoholic compound	Antimicrobial
10.	27.07	Pseudoephedrine, (+)-	$C_{10}H_{15}NO$	165	2.64	Alkaloid compound	Antimicrobial Antiinflammatory Analgesic compound
11.	27.76	Cyclohexane, (2-ethyl-1-methylbutylidene)-	$C_{13}H_{24}$	180	1.94	Alkene compound	No activity reported
12.	30.5	13-Tetradece-11-yn-1-ol	$C_{14}H_{24}O$	208	3.47	Alcoholic compound	No activity reported
13.	30.99	1-Propene, 2-nitro-3-(1-cyclooctenyl)	$C_{11}H_{17}NO_2$	195	4.58	Nitrogen compound	Antimicrobial
14.	32.25	Diazoprogesterone	$C_{21}H_{30}N_4$	338	19.03	Steroid compound	Antimicrobial Antiinflammatory Anticancer Antiarthritic Antiasthma Diuretic
15.	33.74	6,9,12-Octadecatrienoic acid, phenyl methyl ester, (Z,Z,Z)-	$C_{25}H_{36}O_2$	368	2.22	Linolenic acid ester compound	Antiinflammatory Hypocholesterolemic Cancer preventive Hepatoprotective Nematicide Insectifuge Antihistaminic Antieczemic Antiacne 5-Alpha reductase inhibitor Antiandrogenic Antiarthritic Anticoronary

*RT – Retention Time, MW – Molecular Weight, **Dr.Duke`s phytochemical and Ethnobotanical Databases*

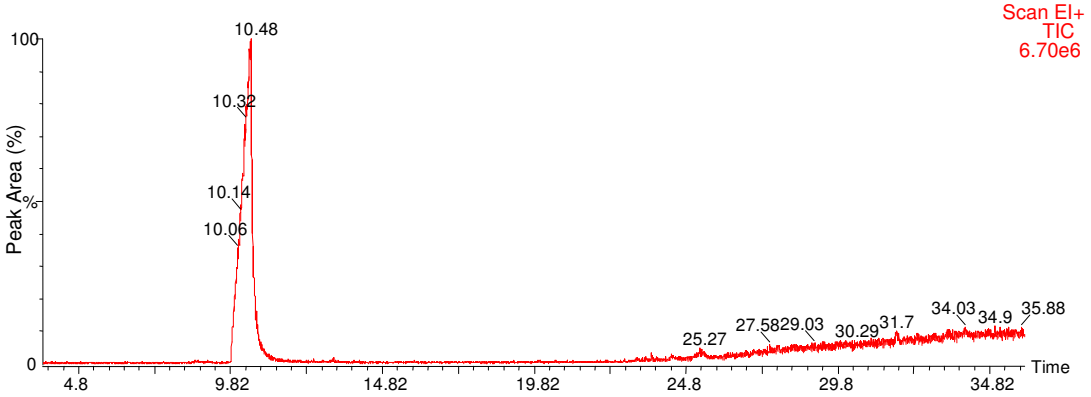

Fig. 2. GC-MS chromatogram of ethanolic latex extract of *C. bonplandianum*

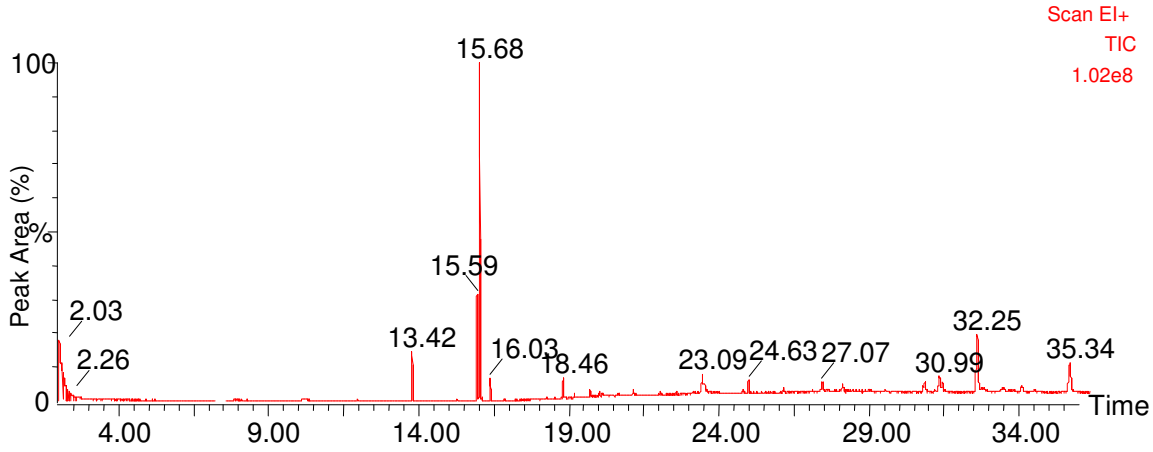

Fig. 3. GC-MS chromatogram of ethanolic fruit extract of *C. bonplandianum*

5. CONCLUSION

The medicinal plant *C. bonplandianum* could be a potential source for useful drugs like anti-inflammatory, anticancer, antimicrobial, insectifuge, nematicide, anticoronary, wound healing, hepatoprotective activities. So, further research is needed to isolate, identify, characterize and elucidate the structure of these bioactive compounds responsible for medicinal values of *C. bonplandianum*.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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