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Analysis of Bioactive Compounds in Latex of *Croton bonplandianum* (Baill.) Using GC-MS

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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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ABSTRACT

Background: *Croton bonplandianum* Baill. belongs to the family of Euphorbiaceae and commonly known as 'Bantulsi'. Traditionally, the latex of this plant is used for cuts and wounds.
Objective: The present investigation was designed to determine the phytochemical constituents of latex of *C. bonplandianum* by GC-MS. In the present study, the mass spectrum of the compounds found in the ethanolic extract of latex was matched with the National Institute of Standards and Technology (NIST) library.
Place and Duration of Study: Post Graduate and Research Department of Biochemistry at Government Arts College (Autonomous), Kumbakonam and Centre for Advanced Research in Indian System of Medicine (CARISM), SASTRA University, Tirumalaisamudram, Thanjavur, Tamilnadu, India, during the months between October to December, 2015.
Methodology: 10 ml of fresh latex was mixed with 10 ml of ethanol and kept at shaker for 3 hours.

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The sample was filtered and concentrated through nitrogen flushing to 1 ml. From this, 2 µl of prepared sample was injected into GC-MS instrument for phytochemical analysis.
Results: The GC-MS analysis of the ethanolic extract of latex of *C. bonplandianum* revealed that the presence of twenty three phytocompounds including the anti-inflammatory, antioxidant and antimicrobial compounds but not all of them is bioactive compounds. The major chemical constituents present in the latex are 2-C-methylmyoinositol (32.4), mequinol (0.74), 4-methylphenol (6.86), 1,2,3-benzenetriol (3.54), 3-methylquinoline, (0.44), n-hexadecanoic acid [palmitic acid] (5.36) and octadecanoic acid [stearic acid] (1.95).
Conclusion: Further study is needed to isolate, identify, characterize and elucidate the structure of bioactive compounds responsible for therapeutic values of latex of *C. bonplandianum*.

Keywords: Croton bonplandianum; latex; GC-MS; ethanolic extract; Bantulsi.

1. INTRODUCTION

Medicinal plants have been used by human being since ages in traditional medicine due to their therapeutic potential. The search on medicinal plants has led to the discovery of novel drug candidates used against diverse diseases [1]. According to the World Health Organization [2], more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. Phytochemicals are natural bioactive compounds found in plants such as vegetables, fruits, medicinal plants, flowers, leaves and roots that act among these as a defense system against diseases [3]. Screening of biologically active medicinal compounds has been conducted on well-known species of plants used in traditional medicines [4]. In the recent years GC-MS has been applied to unambiguously identify the structures of different phytoconstituents in plant extracts and biological samples with great success [5,6]. GC-MS is one of the best techniques to identify the constituents of volatile matter such as long and branched chain hydrocarbons, alcohols, acids, esters etc [7]. The phytochemical screening by GC-MS analysis proved that the plants are pharmaceutically important due to presence of different medicinally important phytochemical compounds and secondary plant metabolites. The GC-MS analysis of the plants showed that the presence of important bioactive compounds.

The plant latex is a natural polymer composed of 320 to 35,000 isoprene molecules and it is stored in specialized cells called laticifer [8,9]. The latex is known to be phytochemically diverse matrix that contains complex mixtures of terpenoids, phenolics, proteins and alkaloids [10], although, all parts of the plant have been used for medicinal purposes [11,12]. Comprehensive literature survey on the plant latex revealed that it contains wide diversities of defense chemicals and proteins, which displayed different biological activities [13,14]. Moreover, some compounds with wound healing properties were reportedly found in the plant latex [11]. *C. bonplandianum* belongs to the family of Euphorbiaceae. It is widely distributed throughout the plains of India. It is a small perennial herb, green and laticiferous growing upto 1-2 ft tall [15]. *C. bonplandianum* is also well known for its medicinal value. Seeds of *C. bonplandianum* are used for the treatment of jaundice, acute constipation, abdominal dropsy and internal abscesses [16]. Its ethanolic extract has been found to possess hypotensive, spasmolytic and antimicrobial activities [17,18]. The latex of *C. bonplandianum* is used to treat cuts and wounds traditionally [19,20]. The latex of *C. bonplandianum* (1:5 v/v in 50% acetone) showed antifungal activity [21]. Many pharmacological studies are available using latex of *C. bonplandianum*. But there is no study on the screening of bioactive compounds of latex of *C. bonplandianum*. Therefore, the present study was aimed to investigate the phytoconstituents of ethanolic extract of latex of *C. bonplandianum* by GC-MS.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

C. bonplandianum latex was collected from the waste lands in and around Mayiladuthurai, Nagapattinam District, Tamilnadu, India, where it was found naturally. The plant was identified by Rev. Dr. S. John Britto, Director, Rabinat Herbarium and Centre for Molecular Systematics, St. Joseph's College, Tiruchirappalli, Tamilnadu, India. 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. The collections of latex were made in the mornings [22]. 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℃ until the time of use.

2.2 Identification of Phytocompounds by GC-MS

2.2.1 Sample preparation

10 ml of fresh latex was mixed with 10 ml of ethanol and kept in the orbital shaker for overnight [23]. The sample was filtered and concentrated through nitrogen flushing to 1 ml. From this, 2 µl of sample was injected into GC-MS instrument for phytochemical analysis.

2.2.2 Equipment

GC-MS analysis was carried out by using a Perkin Elmer Clarus 500 for the screening of phytocompounds of *C. bonplandianum* [24]. The data were obtained on 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℃. GC oven temperature started at 110℃ and holding for 2 min and it was raised to 200℃ at the rate of 10℃/min without holding. Holding was allowed at 280℃ for 9 min with program rate of 5℃/min. The injector and detector temperatures were set at 250℃ and 280℃, respectively. GC interface and ion source temperature was maintained at 200℃. The mass spectrum of compounds in the sample 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 were used for identifying the phytocomponents 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 *C. bonplandianum* were ascertained.

3. RESULTS AND DISCUSSION

Nowadays the search on the phytocompounds of medicinal plants with their activity has increased. The combination of the best separation technique (GC) with the best identification technique (MS) made GC–MS and it is an ideal technique for screening of volatile and semi-volatile bioactive compounds [25]. In the present study, twenty three bioactive chemical compounds in the latex of *C. bonplandianum* were identified and their names, retention time (RT), peak area percentage and molecular weight are represented in Table 1. The highest peak area percentage was obtained by 2-C-methylmyoinositol (32.4%) with the retention time of 10.48 and the lowest peak area percentage was obtained by N-ethyl-4-methoxy-1,3,5-Triazin-2-amine (0.07%) with the retention time of 23.31. The most abundant components were found in the latex are 2-C-methylmyoinositol (42.40%), 13-octadecenal (23.31%), 2-methoxy-4-methylbenzoic acid (10.90%), D-allose (10.63%) and 4-methylphenol (6.86%).

In our previous study, 10 ml of latex was mixed with 90 ml of ethanol and prepared the extract as mentioned in the section 2.2.1 and then carried out GC-MS analysis. In the previous study, the latex extract of *C. bonplandianum* showed that the presence of only one compound 2-C-methylmyoinositol (30.8%) [26]. But in the present study, the latex extract of *C. bonplandianum* showed that the presence of twenty three compounds including 2-C-methylmyoinositol (32.4%). This variation may be due to the dilution of the latex with ethanol because in present study the latex was mixed with equal volume of ethanol, but in the previous study the latex was mixed with 9 fold volume of ethanol for extract preparation. So, the present study confirmed that the dilution of the latex play a main role in the presence of phytocompounds and appearance of peaks in the GC-MS chromatogram.

The biological activities of identified phytocompounds in the latex extract of *C. bonplandianum* were predicted based on Dr. Duke`s phytochemical and ethnobotanical databases [27]. Among the identified phytocompounds in the latex of *C. bonplandianum*, a few compounds like 2-C-methylmyoinositol (32.4%), 3-methylquinoline (0.44%), 4-amino-6-methylpiperidin-2-one (5.24%), 1,2,3-benzenetriol (3.54%), 2-methoxy-4-methylbenzoic acid (10.90%) and n-

hexadecanoic acid (5.36%) possess antimicrobial activity. The phytocompound 1,2,3-benzenetriol (pyrogallol) is an important allelochemical belongs to phenolic compound present in the plants and it is used in controlling bacterial diseases [28].

The phytocompounds mequinol (0.74%), 4-methylphenol (6.86%) and 3-methylquinoline (0.44%) have anti-inflammatory activity. Mequinol is a common active ingredient in topical drugs used for skin depigmentation and it is a derivative of hydroquinone (4-hydroxyanisole, hydroquinone monomethyl ether). The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites. A number of studies have focused on the phytocompounds of biological properties such as antiapoptosis, anti-ageing, anticarcinogen, anti-inflammation, anti-artherosclerosis, cardiovascular protection and improvement of the endothelial functions, as well as inhibition of angiogenesis and cell proliferation [29]. Phenolic compounds have been extensively used in disinfections and as bacteriocides [30]. Quinoline compounds have excellent antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* [31]. Based on the Dr. Duke's phytochemical and ethnobotonical databases, the 2-methoxy-4-methylphenol (9.55%), D-allose (10.63%), 6-methoxy-1H-indole-3-carbaldehyde (5.98%) and 2-methoxy-4-methylbenzoic acid (10.90%) have antioxidant activity. The n-hexadecanoic acid (5.36%) and 2-methoxy-1-naphthylthiocyanate (2.58%) have antitumour activity and 3-methylquinoline (0.44%) has antimalarial activity.

The antioxidant properties of phenolic compounds are due their redox properties, ability to chelate metals and quenching of singlet oxygen [32]. Natural phenolic compounds have great potential as natural antimicrobials and food preservatives [33]. D-allose is a novel antitumor monosaccharide, which causes cell growth inhibition on cancer cells by inducing the tumor suppressor gene [34]. The n-hexadecanoic acid has anti-tumor activity against human leukemic cells as well as murine cells [35]. The fatty acids exhibit cytotoxicity against HeLa cells and retard tumor growth [36]. The n-hexadecanoic acid and octadecanoic acid were showed significant cytotoxicity against oral cancer (KB), breast cancer (MCF-7) and lung cancer (NCI-H187) [37].

The peak area of 1.54% was obtained by benzyl alcohol and it occurs naturally in many plants and it has strong activity against gram positive bacteria and has weak activity against gram negative bacteria, yeasts and moulds [38]. Benzyl alcohol has mild local anesthetic and anti-spasmodic properties [39].

In addition, the results of the GC-MS profile of latex of *C. bonplandianum* may be used as pharmacognostical tool for the identification of bioactive compounds. The presence of various bioactive compounds may be responsible for the therapeutic value of latex of *C. bonplandianum* to treat various ailments by traditional practitioners. However, further study is needed to isolate the individual phytochemical compounds from the latex of *C. bonplandianum* and it may be helpful to find a novel drug.

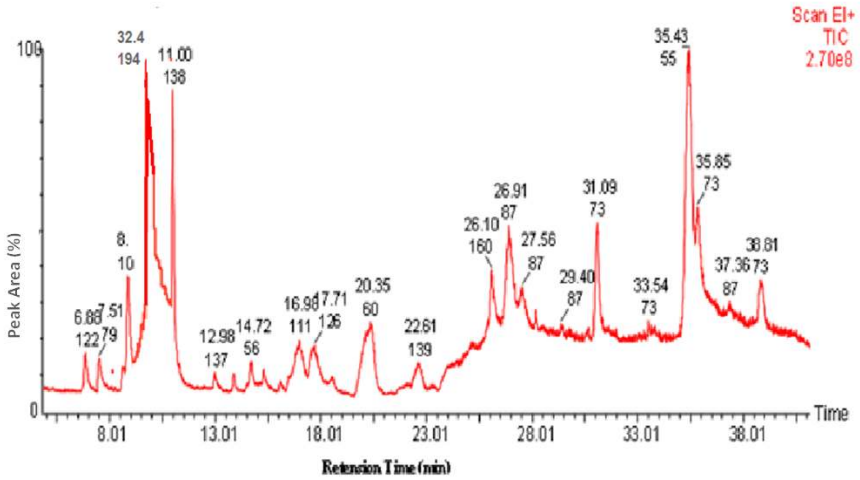

Fig. 1. GC-MS chromatogram of ethanolic extract of latex of *C. bonplandianum*

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

Sl. No.	RT	Name of the compound	Molecular formula	MW	Peak area %	Nature of compound	**Activity
1.	6.88	1-Methoxy-1,3,5-cycloheptatriene	$C_8H_{10}O$	122	1.52	Alkene ether compound	No activity reported
2.	7.51	Benzyl alcohol	C_7H_8O	108	1.54	Alcoholic compound	Antibacterial
3.	8.62	Mequinol	$C_7H_8O_2$	124	0.74	Phenolic compound	Anti-inflammatory
4.	8.87	4-Methylphenol	C_7H_8O	108	6.86	Phenolic compound	Anti-inflammatory
5.	10.48	2-C-Methylmyoinositol	$C_7H_{17}O_6$	194	32.4	Sugar derivative	Antimicrobial
6.	11.00	2-Methoxy-4-methylphenol	$C_8H_{10}O_2$	138	9.55	Phenolic compound	Antioxidant Cytotoxicity
7.	12.98	4-Ethyl-2-methoxyphenol	$C_9H_{12}O_2$	152	0.77	Phenolic compound	No activity reported
8.	13.90	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150	0.54	Phenolic compound	No activity reported
9.	14.72	4,4-Dimethyl-1,3-cyclopentanedione	$C_7H_{10}O_2$	126	1.01	Ketone compound	Herbicidal activity
10.	15.33	3-Methylquinoline	$C_{10}H_9N$	143	0.44	Alkaloid compound	Antimalarial Antibacterial Antifungal Anti-inflammatory
11.	16.98	4-Amino-6-methypiperidin-2-one	$C_6H_{12}N_2O$	128	5.24	Alkaloid compound	Antimicrobial
12.	17.71	1,2,3-Benzenetriol	$C_6H_6O_3$	126	3.54	Phenolic compound	Antimicrobial
13.	18.55	1H-Indol-5-ol	C_8H_7NO	133	0.35	Alkaloid compound	No activity reported
14.	20.34	D-Allose	$C_6H_{12}O_6$	180	10.63	Sugar compound	Antiproliferative, Antioxidant Endocytic
15.	22.61	3-Acetyl-2,5-dimethythiophene	$C_8H_{10}OS$	154	2.45	Thiophene derivative	No activity reported
16.	23.31	N-Ethyl-4-methoxy-1,3,5-Triazin-2-amine	$C_6H_{10}N_4O$	154	0.07	Amide compound	No activity reported
17.	26.10	6-Methoxy-1H-Indole-3-carbaldehyde	$C_{10}H_9NO_2$	175	5.98	Indole aldehyde compound	Antioxidant
18.	26.91	2-Methoxy-4-methylbenzoic acid	$C_9H_{10}O_3$	166	10.90	Benzoic acid derivative	Antimicrobial Antioxidant
19.	27.56	5-Methoxy-1H-Indole-3-carboxaldehyde	$C_{10}H_9NO_2$	175	4.20	Indole aldehyde compound	Antioxidant
20.	31.09	n-Hexadecanoic acid (palmitic acid)	$C_{16}H_{32}O_2$	256	5.36	Fatty acid	Antitumor Antibacterial Antifungal
21.	35.43	13-Octadecenal (stearaldehyde)	$C_{18}H_{34}O$	266	23.31	Fatty aldehyde compound	No activity reported
22.	35.84	Octadecanoic acid (stearic acid)	$C_{18}H_{36}O_2$	284	1.95	Fatty acid	Antibacterial
23.	38.81	2-Methoxy-1-naphthylthiocyanate	$C_{12}H_9NOS$	215	2.58	Naphthyl derivative	Antitumor Antifungal

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

4. CONCLUSION

In the present study, the GC-MS analysis of ethanolic extract of latex of *C. bonplandianum* revealed that the presence of twenty three phytocompounds. According to the Dr. Duke's phytochemical and ethnobotonical databases, eight compounds have antimicrobial, three compounds have antiinflammatory, five compounds have antioxidant, two compounds have antitumour and one compound has antimalarial activities. Thus, the GC-MS analysis is the first step towards understanding the nature of active principles in the latex of *C. bonplandianum* and this study may also be helpful to further research related to phytopharmacology.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that there are no competing interests exist.

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