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## ***Croton bonplandianum* Baill. : A rich source of essential fatty acids, linoleic and linolenic acid**

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### **ABSTRACT**

*The plant *Croton bonplandianum* (Euphorbiaceae) is treat liver disorders, skin diseases including ring worm infection, to cure the swelling of body, bronchitis and asthma. The seeds are used for the treatment of jaundice, acute constipation, abdominal dropsy and internal abscesses. Since petroleum ether extract of *C. bonplandianum* revealed the presence of high content of (40 % w/w) fatty acids, hence the present study was carried out to evaluate the fatty acid composition of the petroleum ether extract from *C. bonplandianum*. The oily residue obtained from the column chromatography of petroleum ether extract was converted in the form of fatty acid methyl esters. The fatty acid methyl esters were identified by gas chromatography with flame ionization detector. Major fatty acids were found to be palmitic acid (27.2%), stearic acid (4.1%), oleic acid (6.6%), linoleic acid (16.8%), and linolenic acid (30.5%). Therefore the plant *C. bonplandianum* may be used as a rich source of essential fatty acids (linoleic acid and linolenic acid).*

**Keyword:** linolenic acid, linoleic acid, PUFA, fatty acid, GC-FID

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### **INTRODUCTION**

*Croton bonplandianum* (Euphorbiaceae), commonly known as Ban tulusi is a perennial herb found in waste lands and road side areas in India, Bangladesh and all other countries of South Asia [1]. This plant is native to the Southern Bolivia, Paraguay, South Western Brazil and Northern Argentina [2]. Flowering and fruiting time of this plant is from September to December [3]. *C. bonplandianum* is a lactiferous, green herb, growing up to 1-2 ft. long. Leaves of the plant are simple, petiolate, alternately arranged, 3-5 cm long, lance shaped with toothed margin. Flowers are small, white, unisexual; contain 5 sepals, 5 petals and numerous long stamens protruding out. Fruits are deciduous with two valved cocci, 5 mm oblong capsule having warty surface. Seeds are small, smooth and albuminous [4].

Traditionally, this plant is used to treat liver disorders, skin diseases including ring worm infection, to cure the swelling of body, bronchitis and asthma [5,6]. Bark and roots of *C. bonplandianum* are alterative and chologogue [7,8]. Leaves of this plant are medicinally used for the treatment of cut and wounds, venereal sores and cholera [9,10]. The seeds are used for the treatment of jaundice, acute constipation, abdominal dropsy and internal abscesses [3]. Fresh juice of the plant is used in headache [11,12]. Pharmacological activities reported for this plant are antimicrobial [11,13], antioxidant, cytotoxic [14] and wound healing [15].

The plant *C. bonplandianum* has been reported to contain secondary metabolites including alkaloids, terpenoids and toxic component like phorbol esters [16, 17]. 3 $\alpha$ -Hydroxy-urs-12, 15-dien, oleanolic acid, ursolic acid,  $\beta$ -sitosterol [13], norcinoacutine and a new alkaloid 3-methoxy-4, 6-dihydroxymorphinandien-7-one [18] have been reported from *C. bonplandianum*.

Column chromatography of the petroleum ether extract of *C. bonplandianum* revealed the presence of 40 % w/w of fatty acids. Present study was aimed to evaluate the fatty acid composition of the petroleum ether extract from *C. bonplandianum*.

## MATERIALS AND METHODS

### Plant material

The whole plant of *C. bonplandianum* was collected during the month of October-November, 2012, from the Rajiv Gandhi South Campus, Banaras Hindu University, Mirzapur, Uttar Pradesh, India. The plant material was authenticated at the Botanical Survey of India, Howrah, West Bengal, India (plant identification letter No. CNH/104/2012/Tech. II/950). A voucher specimen (No. PRL-04) of the whole plant has been deposited for the further reference at the Department of Medicinal Chemistry, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, Varanasi.

### Preparation of plant extract

The fresh plant material of *C. bonplandianum* was washed thoroughly under running tap water and shade dried for two weeks. The whole plant material was pulverized to coarse powder with the help of mechanical grinder and passed through sieve (20 #). The coarse powdered drug (1.6 Kg) was extracted in Soxhlet apparatus for 72 h with petroleum ether (60-80°C, 5L). Petroleum ether extract obtained was concentrated under reduced pressure in rotatory evaporator below 60°C temperature to get semisolid sticky residue (285 g)

### Column chromatography

Petroleum ether extract of the whole plant of *C. bonplandianum* (10 g) was subjected to column chromatography using silica gel (80-120 #) as adsorbent and eluted with the mixture of petroleum ether: ethyl acetate in gradient manner. Petroleum ether: ethyl acetate (95: 5) fraction yielded dark yellow color liquid (mixture of fatty acids, 4 g).

### Preparation of fatty acids methyl ester (FAME)

Methyl esters of Fatty acids were prepared according to the method reported by Grifin, (1960) [19]. 5.0 mg of oily residue was taken in a reaction tube and added with BF<sub>3</sub>: CH<sub>3</sub>OH reagent (5 mL). The mixture was boiled for 5 min, cooled and added with n-hexane (5 mL) and boiled again for 1 min. After cooling the tube, a saturated solution of salt was added and vortexed. The upper layer containing methyl esters was transferred to a vial containing anhydrous sodium sulphate at the bottom. The mixture of esters were filtered through syringe filter and transferred into a small vial (2 mL).

### Gas chromatography of FAME

The fatty acid methyl esters were identified by gas chromatography (Chemito 8610) equipped with DB-23 capillary column (30m  $\times$  0.25 mm) and flame ionization detector. Nitrogen gas was used as carrier gas at a flow rate of 1 mL/min. The detector and injector were kept at constant temperature 245°C. The oven was started from 75°C and heated up to 245°C with a heating rate of 10°C /min. Each fatty acid methyl ester (FAME) in the oil sample was identified by comparing retention times with the standard FAME.

## RESULTS

Fatty acids in the oil sample obtained from column chromatography of petroleum ether extract of *C. bonplandianum* were identified on the basis of retention time of fatty acid methyl esters by GC-FID. Major fatty acids were found to be palmitic acid (27.2%), stearic acid (4.1%), oleic acid (6.6%), linoleic acid (16.8%), and linolenic acid (30.5%) (Figure 1, Table 1).

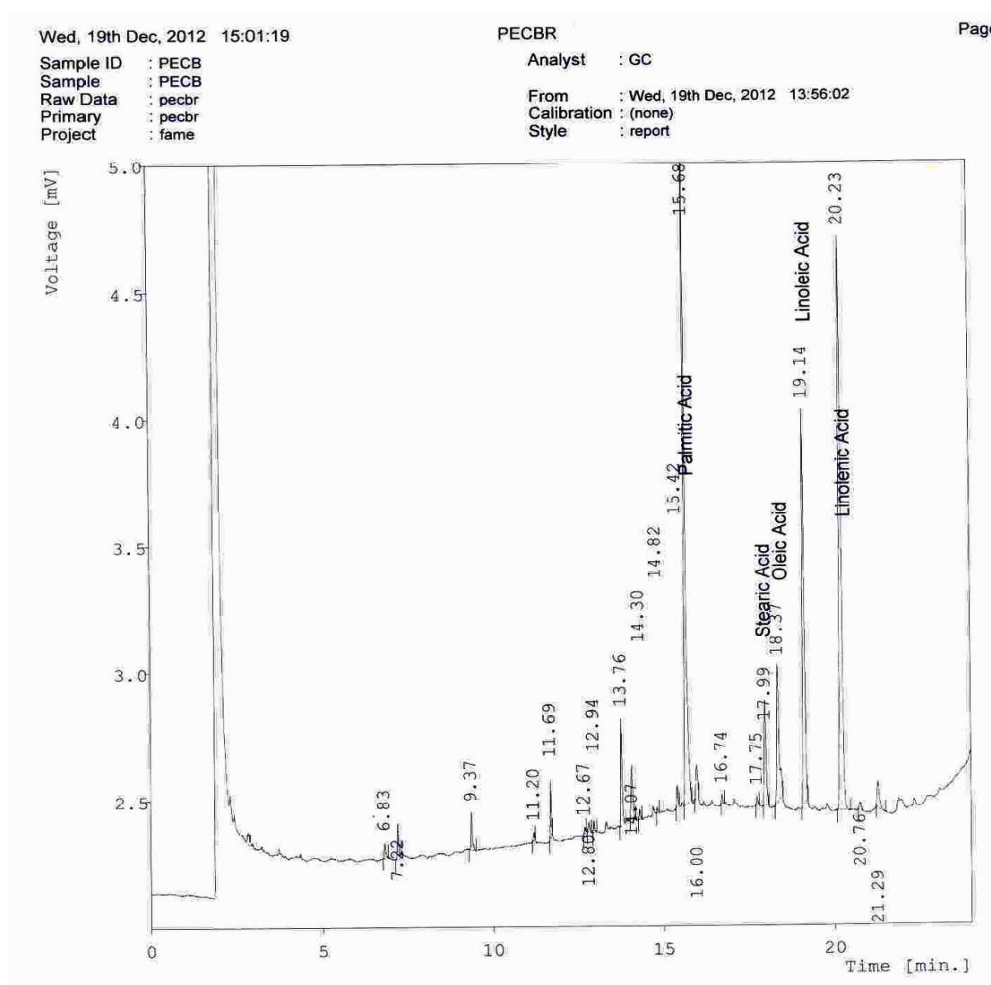


Figure 1: GC-FID spectrum of fatty acids mixture obtained from *C. bonplandianum*.

Table 1: Fatty acid composition of the fixed oil obtained from *C. bonplandianum*

Peak No.	Fatty acid	Molecular formula	Retention time (min)	Nature of fatty acid
14	Palmitic acid	$C_{16}H_{32}O_2$	15.68	Saturated
18	Stearic acid	$C_{18}H_{36}O_2$	17.98	Saturated
19	Oleic acid	$C_{18}H_{34}O_2$	18.36	Unsaturated
20	Linoleic acid	$C_{18}H_{32}O_2$	19.14	Unsaturated
21	Linolenic acid	$C_{18}H_{30}O_2$	20.23	Unsaturated

## DISCUSSION

Several higher plants are reported to contain polyunsaturated fatty acids (PUFA). There are two principle families of PUFA,  $\omega$ -6 and  $\omega$ -3 fatty acids, derived biosynthetically by the plants from linoleic acid and linolenic acids respectively. Both linoleic acid and linolenic acid cannot be synthesized by animal tissues and has to be taken in the diet, therefore called essential fatty acids [20-22]. PUFA ( $\omega$ -6 and  $\omega$ -3 fatty acids) are required in the body for growth, reproduction, good health and for the prevention of cardiovascular diseases [23, 24]. Column chromatography of petroleum ether extract of *C. bonplandianum* yielded 40 % w/w fatty acids. GC- FID spectroscopy revealed the presence of palmitic acid (27.2%), stearic acid (4.1%), oleic acid (6.6%), linoleic acid (16.8%), and linolenic acid (30.5%). It has been reported that *C. bonplandianum* contains toxic components like phorbol esters. In order to use as a source of essential fatty acids, these toxic components must be removed from the plant. Recently we have reported the method development for the removal of phorbol esters from the plant *C. tiglium* by Ayurvedic process *Shodhana* [25].

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**CONCLUSION**

Results of the present study revealed that the plant *C. bonplandianum* may be used as a rich source of essential fatty acids (linoleic acid and linolenic acid) after the removal of toxic components like phorbol esters.

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