

# Extraction and Chromatographic Separation of Bioactive Compounds from *Croton Bonplandianum*: A Green Chemistry Approach

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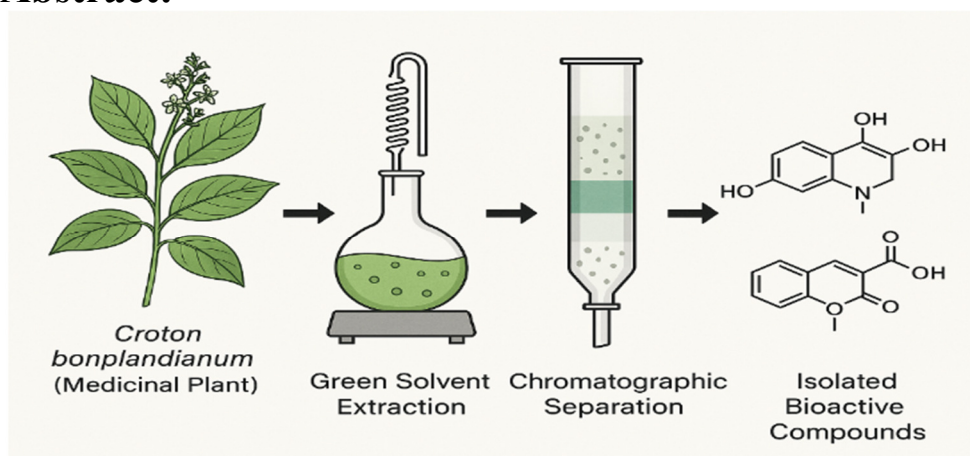
## Abstract:

This study focuses on the green extraction and purification of bioactive compounds from *Croton bonplandianum*, collected from the banks of River Yamuna near Naini, Allahabad. Ethanol, a sustainable solvent, was employed for extraction, followed by concentration using rotary evaporation. Subsequent separation and identification of organic constituents were performed using Column Chromatography and Thin Layer Chromatography (TLC). This methodology emphasizes the retention of bioactive compounds and solvent recovery, aligning with green chemistry principles.

**Keywords:** *Croton bonplandianum*, bioactive compound extraction, rotary evaporation, chromatography, green chemistry, ethanol extract.

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## Graphical Abstract:



## 1. INTRODUCTION

*Croton bonplandianum* (See fig. 1), a species prevalent in tropical regions, has a rich history in traditional medicine due to its diverse phytochemical profile. The plant is known to contain triterpenoids such as lupeol, oleanolic acid, and ursolic acid, along with steroids like  $\beta$ -sitosterol. These compounds exhibit various pharmacological activities, including antimicrobial and anti-inflammatory properties. This

study aims to employ green extraction techniques using ethanol, a polar solvent recognized for its efficiency and environmental friendliness, to isolate and purify these bioactive compounds. [1]



Fig. 1 *Croton bonplandianum* or Ban Tulsi

## MATERIALS AND METHODS

**Plant Material Collection and Preparation:** Fresh shoots of *Croton bonplandianum* were collected from the banks of River Yamuna in Naini, Allahabad. Approximately 2 kg of plant material was harvested and shade-dried for two days to reduce moisture content and concentrate on phytochemicals.

**Preparation of Ethanolic Extract:** The dried plant material was placed in a 10 L round bottom flask containing 5 L of ethanol. The mixture underwent reflux using a hot water bath, with a motorized bucket system cooling the condenser to maintain optimal reflux conditions. (See Fig. 2)



Fig. 2 Experimental work (left) steam condensation process & (right) Rota-vapor instrumentation

## CHROMATOGRAPHIC SEPARATION METHODS

**Column Chromatography:** The ethanolic extract was subjected to column chromatography for the separation and purification of organic components. A cylindrical glass column was packed with silica gel as the stationary phase. A filter (glass wool or cotton) was placed at the lower end to maintain the stationary phase, and a layer of sand was added on top to protect the bed during sample addition. Elution was carried out using appropriate solvents under gravity or gentle pressure. Fractions obtained were analyzed using UV-Vis spectroscopy and TLC.[2]

**Thin Layer Chromatography (TLC):** TLC was employed to monitor the fractions obtained from column chromatography. Plates were prepared using silica gel mixed with a binder (calcium sulfate), poured onto glass or plastic plates, and dried by heating to 110°C for 30 minutes. Small aliquots of the extracts or fractions were applied 1.5 cm above the baseline. The plate was placed in a chamber containing solvent vapors, ensuring the sample spots remained above the solvent level. As the solvent ascended via capillary action, different compounds migrated, forming distinct spot patterns. (See Fig. 3 below) After drying, the plate was examined under UV light or treated with staining reagents to visualize separated compounds.[3]



Fig. 3 TLC instrumentation

4. **RESULT AND DISCUSSION**

The extraction and chromatographic separation techniques effectively isolated bioactive compounds from *Croton bonplandianum*. Notably, triterpenoids such as lupeol, oleanolic acid, and ursolic acid were identified, consistent with previous studies. These compounds have demonstrated significant antimicrobial activity, underscoring the pharmacological potential of *C. bonplandianum*.

5. **CONCLUSION**

This study demonstrates the efficacy of green extraction and chromatographic techniques in isolating bioactive compounds from *Croton bonplandianum*. The use of ethanol as a solvent aligns with sustainable practices, ensuring the retention of bioactive compounds and solvent recovery. These findings provide a foundation for future phytochemical and pharmacological investigations into *C. bonplandianum*. (See Table below)

**CALCULATION OF Rf VALUE –**

**Rf value** = Distance moved by the solute / Distance moved by the solvent

Rf value obtained based on the order of polarity of solvent –

Solvent	Distance moved by solute (cm)	Distance moved by solvent (cm)	Rf value
n-Hexane	0	2.7	0
Benzene	1.3	5.5	0.23
Ethyl acetate	1.9	5.5	0.34
Chloroform	2.7	5.5	0.49
Methanol	3.2	5.5	0.58

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**REFERENCES**

[1.] Vennila, V., & Udayakumar, R. (2015). Antibacterial Activity of *Croton bonplandianum* (Bail.) Against Some Bacterial Isolates from Infected Wounds. *British Microbiology Research Journal*, 5(1), 83–93. <https://doi.org/10.9734/BMRJ/2015/12620>

[2.] Ghosh, A., Saha, B., Das, J., & Ghosh, P. (2014). *Phytochemical investigation of the toluene extract of the root of Croton bonplandianum Bail.* 10(5).

[3.] Shivasharana, C., & Kesti, S. S. (2012). Journal of Advanced Scientific Research. ... of *Advanced Scientific ...*, 1(2), 19–23. [http://www.sciensage.info/journal/1359303580JASR\\_3006121.pdf](http://www.sciensage.info/journal/1359303580JASR_3006121.pdf)