# Pharmacognostical and Phytochemical Analysis and Identification of Bioactive Compounds Using GC-MS Technique of Croton bonplandianus Baill

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***Abstract*—Croton bonplandianus Baill. (Euphorbiaceae), popularly known as Ban Tulsi, is a widely distributed medicinal weed traditionally used for wounds, skin infections, inflammation and gastrointestinal disorders, yet its phytochemical profile remains incompletely standardised. The present study was undertaken to carry out the pharmacognostical and phytochemical evaluation of the leaves of C. bonplandianus and to identify their bioactive constituents using Gas Chromatography-Mass Spectrometry (GC-MS). The powdered leaves were successively extracted with water, ethanol and petroleum ether by the Soxhlet method. Macroscopic and microscopic (transverse-section) evaluation of the petiole and leaf was carried out for pharmacognostic standardisation, and each extract was subjected to qualitative phytochemical screening followed by GC-MS analysis. The aqueous extract showed the highest percentage yield (22%), followed by petroleum ether (18.5%) and ethanol (4.87%). Microscopic evaluation revealed diagnostic features including collateral vascular bundles, rosette crystals, oil globules and abundant phenolic content. Phytochemical screening showed that the ethanolic extract possessed the widest range of secondary metabolites, including alkaloids, triterpenoids, flavonoids, quinones, tannins and diterpenes. GC-MS analysis identified numerous bioactive constituents across the three extracts, predominantly phenolic acids, fatty acids, terpenoids, aromatic esters and nitrogen-containing derivatives, many of which are reported to possess antioxidant, antimicrobial, anti-inflammatory and cytotoxic properties. These findings provide a chemical fingerprint that supports the traditional medicinal use of C. bonplandianus and lay a scientific foundation for its future standardisation, quality control and bioassay-guided isolation of individual bioactive compounds.**

***Index Terms*—Croton bonplandianus; Pharmacognosy; Phytochemical screening; GC-MS; Soxhlet extraction; Bioactive compounds**

## I. INTRODUCTION

Croton bonplandianus Baill., a member of the family Euphorbiaceae, is an annual herbaceous plant widely distributed in tropical and subtropical regions, especially in India, where it commonly grows as a weed along roadsides, wastelands and agricultural fields. The plant is popularly known as “Ban Tulsi” or “Jungle Tulsi” due to its superficial resemblance to Ocimum sanctum. It is an erect, branched herb, 30–100 cm in height, with simple, alternate, lanceolate to ovate-lanceolate leaves bearing serrated margins, and small greenish-white flowers arranged in terminal or axillary inflorescences.

Traditional healers have long used the leaves, stem, root, seed and latex of C. bonplandianus for the treatment of wounds, skin infections, fever, inflammation, digestive disorders, cough and microbial infections. Phytochemical investigations have identified the presence of alkaloids, flavonoids, tannins, phenolic compounds, terpenoids, glycosides, steroids, saponins and essential oils in the plant, and pharmacological studies have demonstrated antimicrobial, antioxidant, antihyperglycaemic, antihelminthic and cytotoxic activities for extracts of different plant parts.

Dutta, Dey and Chaudhuri [6] carried out a phytochemical investigation of the stem of C. bonplandianus and reported considerable alkaloid, flavonoid and phenolic content. Joshi [13] analysed the essential oil of the aerial parts by GC-MS and identified 37 compounds, dominated by β-caryophyllene, germacrene D and borneol, demonstrating the value of GC-MS for characterising

this species. Sivagnanam and Valliammai [25] analysed the chloroform extract of the aerial parts by GC-MS and reported 59 bioactive components, while a further study by the same authors [26] carried out preliminary phytochemical screening of different extracts and highlighted the influence of solvent polarity on the extraction and detection of secondary metabolites. Dutta et al. [4] evaluated the hepatoprotective potential of an antioxidant-rich leaf extract against CCl4-induced liver injury and used GC-MS coupled with molecular docking to identify α-amyrin as a promising bioactive compound. More recently, Javed et al. [11] investigated the phytochemical characterisation and anti-arthritic potential of leaf extracts using combined in-vivo and in-silico approaches, and Jawhari et al. [12] applied GC-MS and molecular docking to identify nematicidal compounds from the leaf extract.

Despite this considerable body of work, the phytochemical profile of C. bonplandianus is not fully standardised, and the specific bioactive constituents remain incompletely characterised across geographical regions and plant parts. GC-MS analysis extends beyond simple compound identification: it enables comparison of phytochemical profiles across different growing regions, seasons and extraction methods, helping to optimise extraction procedures and identify markers for quality control. The present study was therefore designed to carry out a systematic pharmacognostical and phytochemical evaluation of the leaves of C. bonplandianus collected from Dharmapuri, Tamil Nadu, and to identify their bioactive constituents using GC-MS across three solvents of increasing polarity, with the aim of generating a chemical fingerprint that supports the traditional medicinal use of the plant and provides a basis for its future standardisation.

## II. MATERIALS AND METHODS

### A. Plant Material

The aerial parts (leaves) of Croton bonplandianus Baill. were collected from Karimangalam, Dharmapuri district, Tamil Nadu, India. The plant material was authenticated and transported to the laboratory in clean, labelled bags. The collected leaves were washed under running water to remove dust and debris, shade-dried for several days to prevent loss of volatile constituents, and then ground to a coarse powder using a mechanical grinder. The powdered material was stored in an airtight, labelled container away from light and moisture until further use.

### B. Selection of Solvents

Three solvents of increasing polarity were selected for successive extraction: petroleum ether (non-polar, boiling range 40–60°C), ethanol (moderately polar, boiling point 78.4°C) and water (highly polar, boiling point 100°C). This gradient of polarity was chosen to enable extraction of a broad spectrum of phytoconstituents, ranging from non-polar lipids and waxes to highly polar carbohydrates, tannins and glycosides.

### C. Extraction Procedure

A weighed quantity of dried, powdered plant material was subjected to continuous hot extraction using a Soxhlet apparatus. The powder was placed in the thimble of the Soxhlet chamber and the round-bottom flask was filled with the selected solvent. The apparatus was assembled with a condenser, and the solvent was heated to reflux for 6–10 hours per solvent until the siphoning solvent ran clear, indicating exhaustive extraction. The apparatus was then cooled, and the extract was collected and concentrated for further analysis. The percentage yield of each extract was calculated using the formula:

Percentage yield (%) = (Weight of dried extract / Weight of dried plant material) × 100

### D. Pharmacognostical (Macro- and Microscopic) Evaluation

Macroscopic characters of the plant were recorded by direct observation. For microscopy, samples of the petiole and leaf were preserved in FAA fixative for more than 48 hours. Thin transverse sections were cut using a sharp blade and stained with 0.8% safranin and 0.5% astra blue. The stained sections were photographed using an Axiolab5 trinocular microscope fitted with a Zeiss Axiocam208 colour digital camera under bright-field illumination, with magnification indicated by a scale bar.

### E. Qualitative Phytochemical Screening

Each of the three extracts (aqueous, ethanolic and petroleum ether) was subjected to standard qualitative chemical tests to detect the presence of alkaloids, glycosides, triterpenoids, carbohydrates, proteins,

anthraquinones, flavonoids, quinones, tannins, cholesterol, diterpenes, terpenoids, saponins, fats and sterols, following standard pharmacognostic procedures (e.g., Mayer's, Wagner's, Fehling's, Salkowski's, ferric chloride and Liebermann–Burchard tests).

F. Gas Chromatography–Mass Spectrometry (GC-MS) Analysis

GC-MS analysis was carried out to identify and characterise the bioactive constituents of each extract by combining the separation capability of gas chromatography with the detection power of mass spectrometry. For sample preparation, 1 g of each homogenised extract (petroleum ether, ethanol and water) was taken in a 15 mL plastic tube, 5 mL of ethyl acetate was added, and the mixture was vortexed for 5 minutes. An aliquot was then transferred to a 1 mL vial, and 1 µL of the sample was injected into the GC-MS system. Compounds were tentatively identified by comparing their mass spectra with the NIST spectral library, and the relative concentration of each constituent was expressed as percentage peak area.

## III. RESULTS

A. Percentage Yield

The percentage yield obtained for each solvent extract is summarised in Table I.

TABLE I. PERCENTAGE YIELD OF SUCCESSIVE SOXHLET EXTRACTS

| S.No. | Solvent used | Weight of plant material used (g) | Weight of dried extract obtained (g) | % Yield |
|---|---|---|---|---|
| 1 | Water | 100 | 22 | 22.0% |
| 2 | Petroleum ether | 100 | 18.5 | 18.5% |
| 3 | Ethanol | 100 | 4.8 | 4.87% |

B. Qualitative Phytochemical Screening

The results of qualitative phytochemical screening of the three extracts are presented in Table II ('+' indicates presence and '–' indicates absence).

TABLE II. QUALITATIVE PHYTOCHEMICAL SCREENING OF C. BONPLANDIANUS LEAF EXTRACTS

| S.No. | Phytochemical | Water | Petroleum ether | Ethanol |
|---|---|---|---|---|
| 1 | Alkaloids | – | – | + |
| 2 | Glycosides | + | – | – |
| 3 | Triterpenoids | – | + | + |
| 4 | Carbohydrates | + | – | – |
| 5 | Proteins | + | – | – |
| 6 | Anthraquinones | – | – | – |
| 7 | Flavonoids | + | – | + |
| 8 | Quinones | + | – | + |
| 9 | Tannins | + | – | + |
| 10 | Cholesterol | + | + | + |
| 11 | Diterpenes | + | – | + |
| 12 | Terpenoids | – | + | + |
| 13 | Saponins | + | + | – |
| 14 | Sterols | – | + | + |
| 15 | Fats | – | + | – |

C. GC-MS Analysis

GC-MS analysis of the aqueous extract revealed approximately 60 phytoconstituents, predominantly phenolic acids, nitrogen-containing (hydrazide/heterocyclic) compounds and fatty acids. Representative major compounds are listed in Table III.

TABLE III. REPRESENTATIVE BIOACTIVE COMPOUNDS IDENTIFIED IN THE AQUEOUS LEAF EXTRACT BY GC-MS

| Peak No. | Compound name | RT (min) | Area % | Chemical class | Reported bioactivity |
|---|---|---|---|---|---|
| 20 | 2,4-Di-tert-butylphenol | 13.007 | 0.35 | Phenolic | Antioxidant, antimicrobial |
| 36 | 3,5-Di-tert-butyl-4- | 18.187 | 0.65 | Phenolic | Antioxidant / |

| Peak No. | Compound name | RT (min) | Area % | Chemical class | Reported bioactivity |
|---|---|---|---|---|---|
| | hydroxyphenylpropionic acid | | | | free-radical scavenging |
| 35 | n-Hexadecanoic acid (Palmitic acid) | 17.970 | 3.43 | Fatty acid | Antioxidant, antimicrobial, cytotoxic |
| 43 | Octadecanoic acid (Stearic acid) | 19.876 | 1.29 | Fatty acid | Antioxidant / cytoprotective |
| 44 | 1,2-Hydrazinedicarboxamide | 20.085 | 0.26 | Hydrazide | Antimicrobial |
| 37 | (3,5-Dimethylpyrazol-1-yl)methyl(4-methylfuryl)... | 18.360 | 0.15 | N-heterocycle (pyrazole) | Antimicrobial, anti-inflammatory |
| 48 | N-ω-Nitro-L-arginine | 21.358 | 0.26 | Amino-acid derivative | Nitric-oxide synthase inhibitor |

The petroleum ether extract yielded predominantly phenolic compounds, aromatic alcohols, aromatic esters and hydrocarbons. Representative compounds are listed in Table IV.

TABLE IV. REPRESENTATIVE BIOACTIVE COMPOUNDS IDENTIFIED IN THE PETROLEUM ETHER LEAF EXTRACT BY GC-MS

| Peak No. | Compound name | RT (min) | Area % | Chemical class | Reported bioactivity |
|---|---|---|---|---|---|
| 17 | 2,4-Di-tert-butylphenol | 13.333 | 0.24 | Phenolic | Antioxidant, antibacterial, antifungal |
| 39 | Benzyl benzoate | 16.825 | 2.09 | Aromatic ester | Scabicidal, pediculicidal |
| 18 | Bibenzyl | 13.616 | 3.43 | Aromatic hydrocarbon | Antioxidant, antibacterial, anti-inflammatory, antitumour |
| 54 | 1-Nonadecene | 19.252 | 4.20 | Long-chain alkene | Antimicrobial, antioxidant, anti-gastritic |
| 65 | Octacosanol | 22.882 | 1.98 | Fatty alcohol | Antioxidant, anti-inflammatory, anti-fatigue |
| 80 | 1-Hydroxy-4-(p-toluidino)anthraquinone | 28.801 | 0.57 | Anthraquinone | Antibacterial, antioxidant, anti-inflammatory, cytotoxic |
| 4 | Benzyl alcohol | 5.369 | 0.19 | Aromatic alcohol | Antimicrobial, pediculicidal, local anaesthetic |

The ethanolic extract profile revealed phenolic compounds, benzoxazinoids, terpenoids, steroid-like

constituents, hydrocarbons and ketones, including the bioactive terpenoid neophytadiene. Representative compounds are listed in Table V.

TABLE V. REPRESENTATIVE BIOACTIVE COMPOUNDS IDENTIFIED IN THE ETHANOLIC LEAF EXTRACT BY GC-MS

| Peak No. | Compound name | RT (min) | Area % | Chemical class | Reported bioactivity |
|---|---|---|---|---|---|
| 65 | 3,5-Di-tert-butylphenol | 13.369 | 0.55 | Phenolic | Antibiofilm, antioxidant, antimicrobial |
| 42 | 2H-1,4-Benzoxazin-3(4H)-one glucoside derivative | 10.060 | 0.19 | Benzoxazinoid | Antifeedant, insecticidal, antimicrobial, allelopathic |
| 106 | Neophytadiene | 17.600 | 2.60 | Terpenoid | Antioxidant, anti-inflammatory, antimicrobial, analgesic, neuroprotective |
| 56 | D-Homo-24-nor-17-oxachola-20,22-diene-3-... | 12.181 | 0.74 | Steroid-like / limonoid | Not established (requires confirmation) |
| 79 | Hexadecane | 14.716 | 0.95 | Aliphatic hydrocarbon | Plant-associated volatile |
| 102 | (E)-5-Eicosene | 17.116 | 0.86 | Aliphatic hydrocarbon (alkene) | No established use |
| 109 | 3,5-Octanedione, 2,2,7-trimethyl- | 18.005 | 0.97 | Aliphatic ketone | Not established |

Note: Peak numbers, retention times and area percentages correspond to the original GC-MS chromatograms of each extract; complete peak lists (approx. 50–60 compounds per extract) are available in the authors' full project report.

## IV. DISCUSSION

The present study evaluated the extractive yield, pharmacognostic characters, preliminary phytochemical constituents and GC-MS profile of C. bonplandianus leaves across three solvent systems. The percentage yield was highest with water (22%), followed by petroleum ether (18.5%) and ethanol (4.87%), suggesting that the majority of extractable constituents in this plant are polar to moderately polar in nature. Microscopic evaluation of the petiole and leaf revealed characteristic diagnostic features, including collenchymatous hypodermis, collateral vascular bundles, rosette crystals, oil globules and abundant phenolic content, which can serve as useful pharmacognostic markers for identification and standardisation of the crude drug.

Qualitative phytochemical screening showed that the ethanolic extract possessed the widest range of secondary metabolites, including alkaloids, triterpenoids, flavonoids, quinones, tannins and diterpenes, indicating ethanol as the most efficient solvent for extracting bioactive constituents from this plant. The aqueous extract was comparatively richer in glycosides, carbohydrates and proteins, while the petroleum ether extract predominantly yielded non-polar constituents such as triterpenoids, sterols and fats.

GC-MS analysis of the aqueous extract identified about 60 compounds, dominated by phenolic acids, fatty acids and nitrogen-containing hydrazide/heterocyclic derivatives, many of which are reported to possess antioxidant and antimicrobial properties. The petroleum ether extract was

characterised by aromatic esters, hydrocarbons and long-chain alkenes, with bibenzyl and benzyl benzoate notable for their reported antioxidant and scabicidal/pediculicidal activities respectively. The ethanolic extract profile revealed phenolic compounds, benzoxazinoids and terpenoids such as neophytadiene, several of which have documented anti-inflammatory, antimicrobial and neuroprotective potential in preclinical literature. A few detected compounds, such as certain phthalate esters, may represent contaminants and require further confirmation before being attributed to the plant itself.

## V. CONCLUSION

The present study was undertaken to carry out the pharmacognostical and phytochemical evaluation of Croton bonplandianus Baill. leaves, a widely distributed medicinal weed belonging to the family Euphorbiaceae. Successive Soxhlet extraction of the powdered plant material using solvents of varying polarity — water, ethanol and petroleum ether — showed the highest yield with the aqueous extract (22%), followed by petroleum ether (18.5%) and ethanol (4.87%). Microscopical evaluation of the petiole and leaf revealed characteristic anatomical features, including collenchymatous hypodermis, collateral vascular bundles, rosette crystals, oil globules and abundant phenolic content, useful as diagnostic markers for identification of the plant. Preliminary qualitative phytochemical screening confirmed the presence of major secondary metabolites such as alkaloids, flavonoids, glycosides, tannins, saponins, terpenoids, diterpenes, sterols and cholesterol, with the ethanolic extract showing the widest range of positive phytochemical tests. GC-MS analysis of the three extracts revealed a diverse array of phenolic acids, alkaloids, fatty acids, benzoxazinoids, terpenoids, steroids, hydrocarbons and ketones, including the bioactive constituent neophytadiene, and aromatic compounds such as 2,4-di-tert-butylphenol and bibenzyl. Many of the identified compounds are reported in the literature to possess antioxidant, antimicrobial, anti-inflammatory, antifungal and antitumour properties, supporting the traditional medicinal use of this plant.

The combined pharmacognostical, phytochemical and GC-MS findings provide a valuable chemical fingerprint for the standardisation and quality control of Croton bonplandianus and validate its traditional use in the treatment of wounds, infections and inflammatory conditions. However, a few detected compounds, such as certain phthalate esters, may represent contaminants and require further confirmation. The study establishes a strong phytochemical basis for the reported pharmacological activities of C. bonplandianus leaves and highlights the need for isolation and characterisation of individual bioactive compounds in future work. Bioassay-guided fractionation and in-vivo pharmacological evaluation are recommended as the next steps.

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