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Short Communication

PHYTOCHEMICAL STUDIES AND GCMS ANALYSIS OF *CAESALPINIA BONDUC* STEM BARK

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ABSTRACT

Caesalpinia bonduc (Lin.) Roxb. (Family: Fabacea) is well known for its medicinal and therapeutic values in Ayurveda. The leaves, root bark, seeds, seed kernel of the plant are used as medicine. This plant is used as febrifuge, expectorant, anthelmintic and used for diabetes, leprosy, piles, phantom tumour etc. The present study investigated extraction and physicochemical studies of stem bark of *C. bonduc* which include extractive and ash values. Preliminary phytochemical studies and thin layer chromatographic studies were conducted using the extracts. GC-MS analysis of the ethanolic extract was also carried out. The preliminary phytochemical studies indicate the presence of phenolics, flavonoids, triterpenoids, saponins and carbohydrates in abundant quantities in the stem bark. The GC-MS analysis of the TEE identified 12 components. The major compounds identified were 1-hentetracontanol, 1, 1, 3 triethoxy propane, Tetracosa methyl cyclododecasiloxane and Octadecamethyl cyclononasiloxane. The studies suggest further phyto-chemical studies on the bark to isolate chemical constituents.

Keywords: *Caesalpinia bonduc*, Chromatography, GC-MS analysis.

1. INTRODUCTION

Phytochemistry is concerned with the enormous variety of organic substances that are elaborated and accumulated by plants and deals with the chemical structure of these substances, their biosynthesis, turnover and metabolism, their natural distribution and their biological function [1]. Herbal drugs constitute a diverse range of plant materials such as herbs, roots, bark, leaves, flowers, seeds, and other plant parts. The major metabolites in herbal drugs which exhibit medicinal properties are alkaloids, flavonoids, terpenoids, coumarins, steroids, polysaccharides, glycosides etc. [2]. Compared to synthetic drugs, herbal drugs have fewer side effects. Hence traditional medicine practitioners and scientists are turning towards medicinal plants to treat various ailments.

Caesalpinia bonduc (Family: Fabacea) is a medicinal plant predominantly distributed in the tropics and subtropics. In India, it is distributed in the plains on waste lands and coastal areas [3], especially all over Bengal, Bombay [4] and to an altitude of 1,000 m in the Himalayas; it is also found in the deltaic regions of Western, Eastern and Southern India. The root bark, leaves, seeds and bark of this plant have medicinal properties [3, 4]. This plant is mainly used as anthelmintic, emmenagogue, febrifuge, bitter, astringent, acrid, thermogenic, anodyne, anti-inflammatory, digestive, stomachic, liver tonic, depurative, expectorant, contraceptive, antipyretic, aphrodisiac and tonic [4, 5]. C. *bonduc* has been the subject of several chemical investigations, wherein a number of Diterpenoids [6], Cassane Furanoditerpenes [7-10] Cassane Diterpenes [11-15], Homoisoflavonoids [16], Cassane butenolide hemiketal diterpenes [17] have been isolated. Numerous studies conducted in this plant have proven that these isolated compounds have Cytotoxic [6], Antifungal and GST inhibitory [16], insecticidal [18] properties. We have reported the anti-inflammatory and anticancer activities of the bark extract [19]. Due to the diversity of chemical constituents and medicinal importance of this plant, the present study deals with the phytochemical studies and the GC MS analysis.

2. MATERIAL AND MEHODS

2.1. Collection of Plant material

C. bonduc stem bark used in the present study were collected from Athani, Kerala and authenticated by the Botanist, Mr. Joby Paul, Department of Environmental Science, M.G University, Athirampuzha, Kottayam, Kerala. A voucher specimen was been deposited in the herbarium of University College of Pharmacy under number (SES, M.G UTY No. 1505).

2.2. Preparation of extract

Fresh stem barks were collected from the tree and dried at room temperature to remove moisture, and size was reduced. The dried powdered stem bark of *C. bonduc* was kept for cold maceration in a 1000 ml round bottom flask for 3 d. Total 475g of dried powder was soaked in 2.1L ethanol (95%) for 3 d for cold maceration. Extraction of the stem bark of *C. bonduc* was done using hot continuous percolation in a Soxhlet apparatus using ethanol (95%) as solvent for 5 h. The extract obtained is denoted as TEE (total ethanolic extract).

2.3. Physicochemical evaluation

Physicochemical evaluation of the stem bark was done by the determination of Ash values and Extractive values.

2.3.1. Fractionation of extract

The extract TEE was fractionated using petroleum ether (PEE), chloroform (CHE), ethyl acetate (EAE) and water (AQE) in increasing order of their polarity.

2.3.2. Determination of Ash values

The Total ash was determined by incinerating the ground drug (2-3g) in a silica dish at a temperature not exceeding 450°C until free from carbon, cooled and weighed the percentage [20].

The Acid insoluble ash was determined by boiling the Total ash with 25 ml of dil. HCl for 5 minutes; the insoluble matter was collected in a Gooch crucible, washed with hot water and ignited to constant weight, calculated the percentage [20].

The water insoluble ash was determined by boiling the Total ash with 25 ml water for 5 minutes; the insoluble matter was collected in a Gooch crucible, washed with hot water, and ignited for 15 minutes at a temperature not exceeding 450°C. The difference in weight of insoluble matter and the weight of the ash represents the water-soluble ash [20].

2.4. Preliminary Phytochemical screening

The Total ethanolic extract, Pet ether extract and aqueous extracts were subjected to preliminary phytochemical screening for Alkaloids, Glycosides, Flavonoids, Tannins (Phenolic compounds), saponin glycosides, Sterols, Carbohydrates, Proteins and amino acids [21].

2.5. Thin Layer Chromatographic Studies:

The pet ether extract, Chloroform extract and the Ethyl acetate extract of *C. bonduc* stem bark were subjected to Thin Layer Chromatographic Studies.

The stationary phase prepared using silica gel slurry, activated by keeping it in oven at 110°C for 20 min. Small quantities of each extracts were dissolved in their respective solvents used for extraction and were taken in capillary tubes separately, spotted on the pre coated TLC plates at a distance of 2cm above from the base of the plates. Chromatogram was developed by placing the spotted plates in the saturated mobile phase chambers. When the mobile phase reached $3/4^{th}$ of the plates, plates were removed, dried in air, then visualized under UV and examined for the presence of spots. The Rf values were calculated [22].

2.6. GC-MS Analysis

GC-MS is a direct and fast analytical approach for the identification of phytoconstituents. The Total Ethanolic Extract of stem bark of *Caesalpinia* was subjected to GC-MS Analysis. Analysis was performed using a Varian GC MS (Saturn 2200). Helium gas (99.999%) was used as carrier gas at a constant flow of 1 ml/min and an injection volume of 1 µl sample was employed; injector temperature 250°C; the oven temperature was programmed from 100-270°C at the rate of 5°C; total GC running time was 63 minutes. The components were identified on the basis of retention time (RT) for GC and interpretation of mass spectrum was done by comparing spectral fragmentation obtained, to the database provided by Wiley Library and National Institute Standard and Technology (NIST 14 LIB).

3. RESULTS AND DISCUSSION

3.1. Physicochemical analysis

The extractive values are useful to evaluate the chemical constituent present in the crude drug, the estimation of specific constituents soluble in a particular solvent and the nature of chemical constituents. The amount of extracts in a given solvent gives an approximate measure of certain constituents or group of related constituents the drug contains. The results indicate that relatively more chemical constituents are present in total ethanolic extract.

Ash values are used to determine the quality and purity of the crude drug. The ash of any organic material is composed of their nonvolatile inorganic components. A high ash value is indicative of contamination, substitution, adulteration or carelessness in preparing the crude drug for marketing.

The ash values determined in the present study (Total ash, Acid insoluble ash, Water soluble ash) are significant in the determination of excess calcium oxalate or calcium

carbonate crystal, contamination with silicious material and previous extraction of the water-soluble salts in the drug or incorrect preparation respectively.

The Extractive values are useful to evaluate the nature of chemical constituents present in extracts. The higher alcoholic extractive value indicates the presence of polar phytoconstituents such as phenols, steroids, glycosides etc.

Table 1: Physicochemical studies of *C. bonduc* stem bark

Physicochemical parameters	Yield (% w/w)
Ash values	
Total Ash	8.62±0.268
Acid insoluble Ash	2.39±0.324
Water soluble Ash	1.03±0.201
Extracts and fractions	
TEE	42.2
PEE	0.97
CHE	1.93
EAE	4.34

TEE- Total ethanolic extract, PEE- Petroleum ether fraction, CHE- Chloroform fraction, EAE- Ethyl acetate fraction.

3.2. Preliminary phytochemical evaluation

The preliminary phytochemical screening helps to find out the phytoconstituents present in the plant extracts, which further leads to the isolation of pharmacologically active constituents.

The phytochemical constituents of *Caesalpinia bonducella* stem bark extracted by different solvents were analyzed and identified as multiple medicinally active components (Table 2). The Total Ethanolic Extract revealed the presence of Tannins (phenolic compounds) and Triterpenoids in high amounts whereas saponin glycosides and carbohydrates in low amounts. The Petroleum Ether fraction revealed the presence of Tannins (phenolic compounds), Triterpenoids and Carbohydrates in high amounts whereas flavonoids in low amounts. The ethyl acetate fraction revealed the presence of triterpenoids and flavanoids in low amounts only. The results of preliminary phytochemical screening showed that more phytoconstituents are present in TEE. Hence TEE is selected for GC-MS Analysis.

Thin layer chromatographic studies were carried out on the extract and fractions to find out the best separating solvent system. Results are depicted in table 3, indicating hexane : ethyl acetate (1:2) as the best separating solvent system for CHE and EAE.

3.3. GC-MS Analysis

GC-MS Analysis of TEE of *C. bonduc* shows the presence of 12 compounds. Fig. 1 represents the GC-MS spectra of the extract. The compounds enlisted along with their retention time, molecular formula and percentage are presented in table 4.

Table 2: Preliminary phytochemical evaluation of *C. bonduc*

S. No.	Phytoconstituents	TEE	PEE	EAE
1.	Tannins (phenolic compounds)	++	++	-
2.	Saponin glycosides	+	-	-
3.	Triterpenoids	++	++	+
4.	Carbohydrates	+	++	-
5.	Flavonoids	-	+	+

++ indicates active constituents in high amount, + indicates active constituents in low amount, - indicates absence of active constituents.

Table 3: Thin layer chromatography pattern of the different extracts of *C. bonduc*

Solvent systems	PEE		CHE		EAE	
	No. of spots	Rf value	No. of spots	Rf value	No. of spots	Rf value
Hexane: ethyl acetate (2:1)	1 spot	0.18	4 spots	0.22, 0.5, 0.56, 0.74	3 spots	0.18, 0.5, 0.74
Toluene : ethyl acetate: formic acid (36:12:5)	-	-	2 spots	0.42, 0.25	1 spot	0.5
Toluene : acetone: formic acid (38:10:5)	1 spot	0.52	2 spots	0.43, 0.23	1 spot	0.52
Pet-ether: acetone: formic acid(35:10:5)	-	-	1 spot	0.91	-	-

1-hentetracontanol, 1,1,3 triethoxy propane, Tetracosa methyl cyclododecasiloxane and Octadecamethyl cyclononasiloxane were the major compounds. Tritetracontane, Piperonylic acid, 5β-Cholestan-3-one ethylene acetal, N-Depropionyl-N-acetyl-aspidoalbin, Tetrapentacontane 1, 54-dibromo, 2-ethyl hexyl phtha-late, Dimethoxylycopene, Cyclopropa [3, 4] benz [1,2-e] azulen-5-on are the other bioactive compounds present in the TEE.

GC-MS analysis resulted in isolation of 26 peaks, of which 12 were identified using Wiley online library and enlisted in table 4.

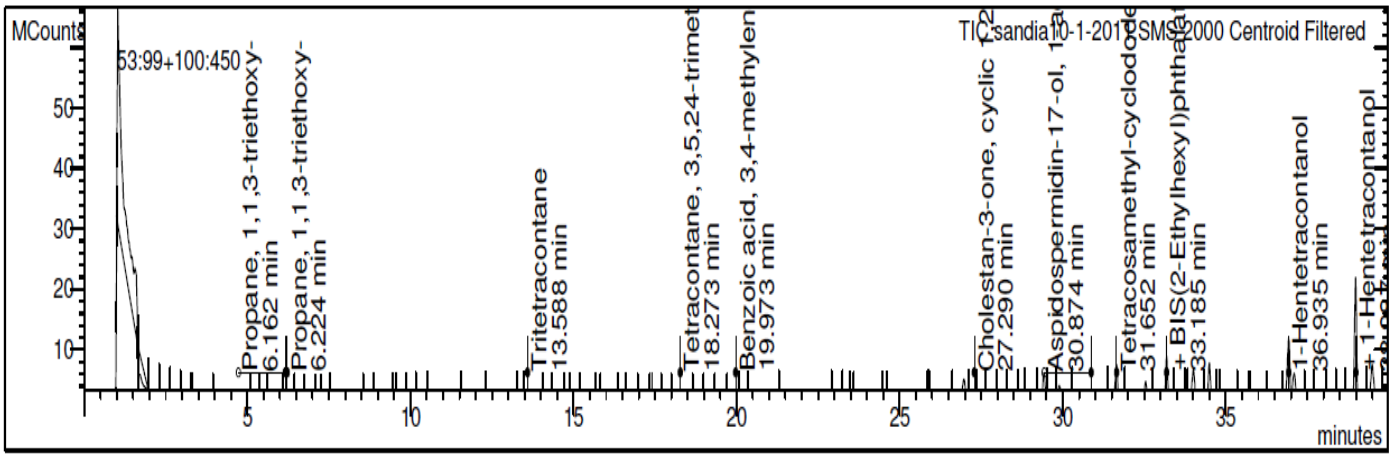

Fig. 1: GC MS analysis of TEE of *C. bonduc*

Table 4: GC MS values of TEE of *C. bonduc*

S. No.	Retention time	Compound	Percentage	Molecular formula
1	6.162	1,1,3 triethoxy propane	9.27	$C_7H_{16}O_3$
2	13.588	Tritetracontane	1.72	$C_{43}H_{88}$
3	19.973	Piperonylic acid	1.09	$C_8H_6O_4$
4	26.968	Octadecamethyl cyclononasiloxane	5.48	$C_{18}H_{54}O_9Si_9$
5	27.29	5β-Cholestan-3-one ethylene acetal	0.31	$C_{29}H_{50}O_2$
6	30.874	N-Depropionyl-N-acetyl-aspidoalbin	0.95	$C_8H_{15}NO_5$
7	31.652	Tetracosa methyl cyclododecasiloxane	8.41	$C_{24}H_{72}O_{12}Si_{12}$
8	32.55	Tetrapentacontane 1,54-dibromo	1.63	$C_{54}H_{108}Br_2$
9	33.185	2-ethyl hexyl phthalate	2.89	$C_{24}H_{38}O_4$
10	36.935	1-hentetracontanol	18.55	$C_{41}H_{84}O$
11	39.154	Dimethoxylycopene	0.24	$C_{40}H_{56}$
12	39.9	Cyclopropa [3,4] benz [1,2-e] azulen-5-on	0.38	$C_{45}H_{50}O_8$

4. CONCLUSION

Physicochemical parameters like Ash values help to identify the crude drug and thereby adulteration can be prevented. The preliminary phytochemical analysis revealed the presence of phenolics, flavonoids, triterpenoids in high amount in PEE and TEE. These phytoconstituents can be responsible for the anti-inflammatory and anticancer activities of the *Caesalpaenia bonduc* that is already reported. GC-MS analysis and chromatographic studies shows that *C. bonduc* stem bark contains several phytoconstituents, suggesting further isolation of active constituents.

Conflict of interest

Authors declare that we do not have any conflict of interest.

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