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PHYSICO-CHEMICAL STANDARDISATION AND PHARMACOGNOSTIC PROFILES OF CAESALPINIA BONDUC (L) ROXB.

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ABSTRACT

Caesalpinia bonduc (L) Roxb. (Family – Leguminosae) is commonly used in Siddha, Ayurveda, and other traditional systems of medicine. It is a perennial plant growing as a hedge plant up to 15 m in height and widely distributed all over the world. The physico-chemical parameters such as Loss on Drying at 105°C%, total ash, acid insoluble ash, water soluble extractives, alcohol soluble extractives and volatile oil of the leaves, bark and root of *C. bonduc* were determined and compared. HPTLC studies of the plant materials were carried out for the identification of its phytoconstituent profile using solvent system toluene: ethyl acetate (6: 1). The tests for arsenic & heavy metals were carried out and were found to be within the permissible limit which proves the safety of the drug material. HPTLC finger print scanned at wavelength 254, 366 and 575 nm for *C bonduc* leaf, bark and root showed the presence of so many phytoconstituents and corresponding ascending order of R_f values. *Conclusions*: This study attempted to standardize and trace out the presence of similar phytoconstituents of root, from leaves and bark of the plant that enables the sustainable utilization of this medicinal plant.

Keywords: *Caesalpinia bonduc*; *Kazharchi*; Physico-chemical; Traditional uses; Phytochemicals; HPTLC.

INTRODUCTION

Herbal medicines from plants contain a spectrum of natural compounds that can promote health and alleviate illness. Green medicines are safe and more dependable than the synthetic drugs which have so many adverse side effects. *Caesalpinia bonduc* (L) Roxb. (Commonly known as Ivy Gourd) belonging to the family Leguminosae is a prickly shrub widely distributed all over the world (Fig.1). The plant is reported to contain a number of secondary metabolites namely sitosterol, caesalpins, bonducin, caesane and flavon-

oids[1]. Reports show that seeds and kernels contain bonducin, phytosterinin and bonducillin[2]. Jayakrishnan et al.[3] reported high concentration of sterols, amino acids and alkaloids and moderate concentration of saponins in the seeds of *Caesalpinia bonduc.* It is an ingredient of `Ayush-64' an indigenous compound preparation used as an anti-malarial drug.

Fig.1: *Caesalpinia bonduc* plant

Botanical characters of *Caesalpinia bonduc*

Caesalpinia bonduc is a vine-like shrub that reaches a length of 6 m and scrambles over other vegetation. Stems are covered in curved spines. Its 2 cm gray seeds known as nickernuts are buoyant and durable enough to be dispersed by ocean currents. It is an extensive climber. Branches are armed with hooked and straight hard yellow prickles. Leaves are bipinnate, 30-60 cm long and petioles are prickly. Flowers are in dense terminal racemes with long peduncles and supraaxillary racemes which are close at the top and 15-25 cm long. Petals are oblanceolate, yellow, filaments declinate, flattened at the base, clothed with long white silky hairs. Pods are oblong, densely armed on the faces with wiry prickles. Seeds are oblong, lead-coloured and 1.3 cm long[4].

Traditional uses: Root, stem, leaves, bark, seeds and nuts of this plant are used in indigenous systems of medicine as antioxidant, antidiabetic, laxative, rheumatoid arthritis and immune system modulator[5]. The plant is widely used in our traditional system of medicine to treat diseases like rheumatoid arthritis and as antileprotic, antidiabetic, antiperiodic, antipyretic and immune system modulator.

Uses in Siddha system of medicine

Siddha system of medicine is a traditional treatment system generated from Tamil culture which is focused to Ashtamahasiddhis. *C. bonduc* is one of the most widely used traditional herbal drugs in the Siddha system of medicine. Leaves of *C. bonduc* have been used by Siddha healer of Malabar region for psoriasis treatment. The seed shows stypic, purgative and anthelmintic actions and is used for the treatment of inflammations, colic, malaria, hydrocele, skin diseases and leprosy[6,7,8]. Coconut flower mixed with bonduc leaves and fried with sashes oil is used to dress the inflammatory swellings and scrotal swellings. Bonduc kernals, dried ginger, dried arecanut, mixed with egg white and this mixture is made into paste which is used externally for filarial swelling. Seed powder mixed with asafoetida and taken internally with butter milk for abdominal diseases and good complexion to skin. Seeds powder, dried arecanut and vengaaram taken internally for tooth diseases. Bonduc roots are used for the preparation of kazharchi ennai.

Ethnomedical uses

The indigenous ethnomedical practitioners of various Indian tribes also effectively used this plant in their medicines. In Katra valley (Jammu and Kashmir), the plant-juice is taken for two weeks after meals to cure intermittent fever. In Sri Lanka, in the indigenous system of medicine, the plant is used for treatment of skeletal fractures. The seeds and leaves are reported to be used in skin diseases and rheumatism. Powdered roots, taken with honey, cure leucorrhoea. In Kangra (Himachal Pradesh), the roots are used in intermittent fevers and diabetes. In Jamaica, the bark is used as a rubefacient and as a local application for sores. The seed powder is given in menstrual disorders in tribal areas of Madhya Pradesh. In Homoeopathy the plant is considered an excellent remedy for chronic fever with headache etc. The seed powder forms a household remedy for treatment of diabetes in Nicobar Island. The leaves of the plant have the activities as liver and gastric tonic, fungicidal, anticonvulsant. The root-bark is good for tumours and for removing the placenta after child birth. Bark of root possesses many properties and useful in intestinal worms, amenorrhoea, cough, and acts as anthelmintic. In Jamaica, it

is used as rubifacient and as a local application for sores. Flowers are used in treating ascites and fruits in treating urinary disorders, leucorrhoea, piles and wounds. Leaf and twigs are traditionally used in the treatment of tumors, inflammation and liver disorders. They are also useful for treating toothache. Leaf juice has been used traditionally in elephantiasis and small-pox.

Pharmacological activities

The crude aqueous and ethanolic extracts of its seeds have been reported to possess *in vivo* hyperglycemic effect in alloxan and streptozitocin induced type 2 diabetes in a rat model at a dose of 25 mg/kg body weight[9]. The methanolic, ethyl acetate and water fractions of crude extracts exhibit *in vitro* activity against the growth of an array of pathogenic bacteria and fungi. This plant finds extensive use in folk medicines. *C. bonduc* is found to be used traditionally for various purposes viz. antioxidant, anti-inflammatory, diabetes, asthma, splenopathy and ulcer. The root bark is emmenagogue, febrigue expectorant and anthelmintic[10,11]; also evaluated the effect of aqueous and ethanolic extracts of *Caesalpinia bonduc* root on sexual behaviour of male wistar rats suggesting that the chemical groups of *C. bonduc* roots could potentially induce aphrodisiac activities.

In the present paper, a pharmacognostic study on *Caesalpinia bonduc* Linn. (leaves, bark and root) based on its physico-chemical, preliminary phytochemical and HPTLC studies were carried out to lay down the pharmacopoeial standards. Widespread use of the roots may cause substantial decrease in the diversity of *C.bonduc.* Hence this study aims to analyse the presence of similar phytochemicals that of roots in leaves and bark for the effective replacement of root with leaves/bark.

MATERIALS AND METHODS

Plant Materials: The leaves, bark and root of *C. bonduc* were collected from Siddha Medicinal Plant Garden, Mettur Dam and identified by experts. The plant materials were carefully washed under running tap water followed by sterile water and shade dried for 4-5 days. The dried plant materials were ground to coarse powder, stored in airtight containers and used for all experimental purposes.

Physico-chemical parameters: The physico-chemical parameters like determination of loss on drying at 105°C%, total ash, acid insoluble ash, water soluble extractives, alcohol soluble extractives and volatile oil; and test for Arsenic and Heavy metals were carried out by standard methods[12].

Preliminary phytochemical study: In order to examine the presence of different natural products in the plant, characteristic phytochemical tests for sugar, starch, poly phenols, saponin, mucilage, steroid, alkaloid and flavonoid were performed using different extracts of the plant materials[13,14].

Preparation of Plant Extract for HPTLC analysis

Plants secondary metabolites possess various biological activities and ethanol is capable of extracting a wide range of polar compounds such as alkaloids, sterols, flavonoids and carbohydrates due to its high polarity, therefore it was used for extraction. 10 g of powdered samples were soaked in conical flasks containing 100 ml of ethanol for 24 hrs. Conical flasks were allowed to stand for 30 mins in a water bath (at 100°C) with occasional shaking. Each extract was filtered through a sterilized Whatman No. 1 filter paper and finally concentrated to dryness under vacuum at 40°C using a rotary evaporator. The dried extracts, thus obtained were sterilized by overnight UV-irradiation and stored at 4°C in refrigerator for further use[15].

High performance thin layer chromatography (HPTLC): Instrumentation and chromatographic conditions

HPTLC was performed on 10 x 10 cm aluminium plates coated with silica gel 60 F_{254} (Merck, Germany). Each sample was applied as separate bands of width 10 mm from bottom edge of the same chromatographic plate by the use of a Camag (Switzerland) Autosampler applicator equipped with a 100 µL Hamilton (USA) Syringe. Ascending development to a distance of 80 mm was performed at room temperature with Toluene: Ethyl acetate (6:1) as mobile phase, in a Camag glass twin-trough chamber previously saturated with mobile phase vapour. After development, the plates were air dried, visualized and scanned at

UV short and long wavelength (254 nm and 366 nm) with a Camag TLC scanner with WINCAT software. Then the plates were derivatized in Vanillin sulphuric acid reagent and the plates were dried on a hot plate. Fingerprint analyses of the compounds were done by visualizing and scanning the derivatised plates at 575 nm and the results were documented.

RESULTS AND DISCUSSION

The analytical data of physico-chemical parameters of the leaves, bark and root of *C. bonduc* obtained are given in Table 1. Total ash value of the materials indicated the amount of minerals and earthy material attached to the plant materials. It was observed to be maximum in roots (i.e., 12.66%). Acid insoluble ash usually represents the amount of silica present as sand and dust. Acid insoluble ash values were found to be low for all the plant materials, which might be an indicative of purity and absence of silicaceous materials in the selected plant parts. Loss on drying at 105°C shows the amount of moisture content and volatile oil present in the drug. The alcohol and water soluble extractives were found to be higher in leaves than other parts of the plant, which shows the maximum concentration or number of the polar constituents such as sugar, plant acid, mucilage and glycosides present in leaves when compared to other plant parts. The arsenic and heavy metals - lead, cadmium and mercury - were found to be within the permissible limit which proves the safety of the plant materials.

Table 1: Physico-chemical parameters of *C. bonduc* Leaves, Bark and Root

Sl. No.	Parameter	Leaves	Bark	Root
1.	Loss on Drying at 105°C %	13.56	14.01	7.74
2.	Total Ash Content %	6.25	3.41	12.66
3.	Acid Insoluble Ash %	1.25	0.19	0.70
4.	Water Soluble Extractive %	21.69	9.04	14.22
5.	Alcohol Soluble Extractive %	16.94	11.04	14.19
6.	Volatile oil %	Nil	Nil	Nil

Phytochemical Analysis

The secondary metabolites of the plants are responsible for the bioactivities shown by plants. The preliminary phytochemical evaluation of the leaves of *C. bonduc* reveals the presence of carbohydrate, proteins, saponins, steroids and other major secondary metabolites (Table 2).

Table 2: Preliminary tests for the presence of phytochemicals (groups) in *C. bonduc* leaves, bark and root

Sl. No.	Natural products	Test performed	Leaves	Bark	Root
1	Carbohydrates	Molisch's test	+ve	+ve	+ve
3	Poly phenols	Neutral $FeCl_3$ test	+ve	+ve	+ve
4	Saponins	Foaming in water	+ve	-ve	+ve
5	Proteins	Millon's test	+ve	+ve	+ve
6	Steroids	Liebermann's test	+ve	+ve	-ve
7	Alkaloids	Mayer's reagent test	+ve	+ve	+ve
8	Flavonoids	Shinoda test	+ve	+ve	+ve
9	Oils and Fats	Filter paper test	+ve	-ve	+ve
10	Tannins	Lead acetate test	+ve	+ve	+ve

The presence of these biochemical compounds might be making *C. bonduc* medicinally important and nutritionally valuable. Adam et al., (2015) also attempted the phytochemical screening of *C. bonduc* roots powder which revealed the presence of various compounds including tannins, flavonoids, anthocyans, leucoanthocyans, mucillag, saponosids, anthracemic, heterosids, alkaloids and quinons. Adulteration and

substitution have become a major problem due to the absence of standards relating to genuineness of drug. Microscopic, macroscopic data and other physical values including HPTLC will help to identify the correct species of the plant.

HPTLC Profile

HPTLC fingerprint analysis can be used as a diagnostic tool for the correct identification of the plant and its phytoconstituents. HPTLC fingerprint studies confirmed the results of phytochemical screening by the presence of various coloured bands at different wavelengths with specific solvent systems, symbolizing the presence of particular phytocompounds.

In the present study *C. bonduc* showed best results in Toluene: Ethyl Acetate (6:1) solvent system for all the three extracts, after scanning and visualizing the plates in absorbance mode at 254 nm, 366 nm and visible light (575 nm after spraying with Vanillin sulphuric acid reagent). The HPTLC images shown in Fig. 2 indicate that all sample constituents were clearly separated without any tailing and diffuseness.

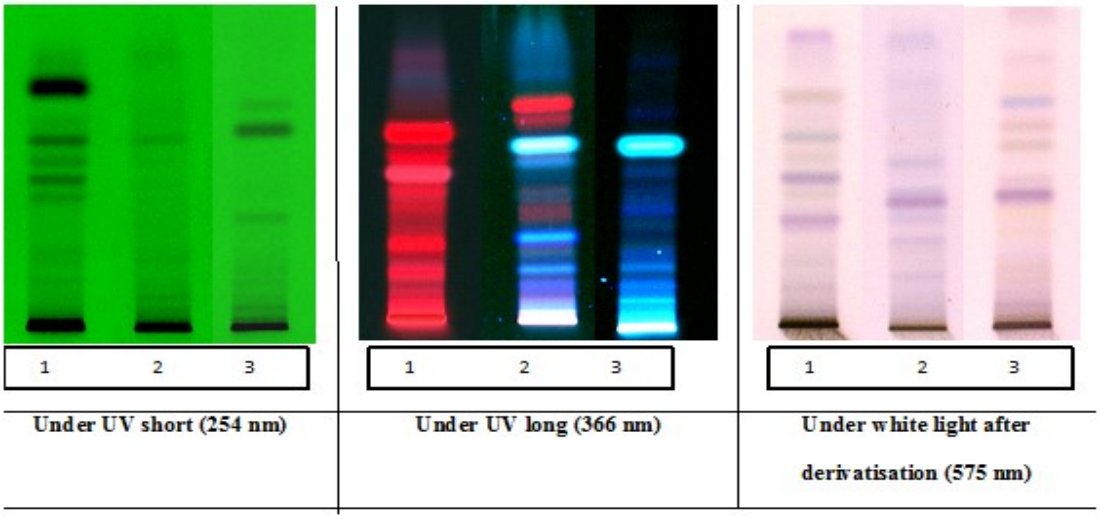
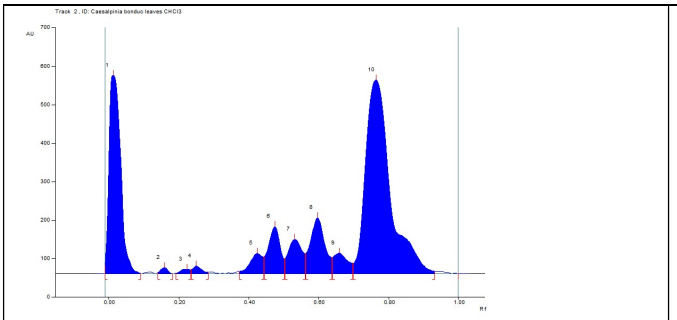
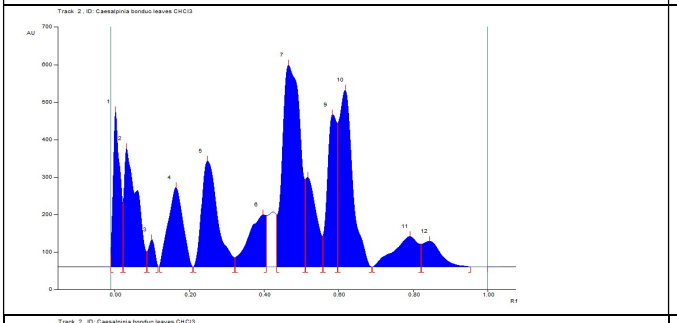


Fig. 2: HPTLC photodocumentation of *Caesalpinia bonduc (L) Roxb.* (Kalarci) Leaves Bark and Root under 254, 366 and 575nm (Track 1- 15 µl (leaf); Track 2 – 15 µl (bark); Track 3- 15 µl (root)).

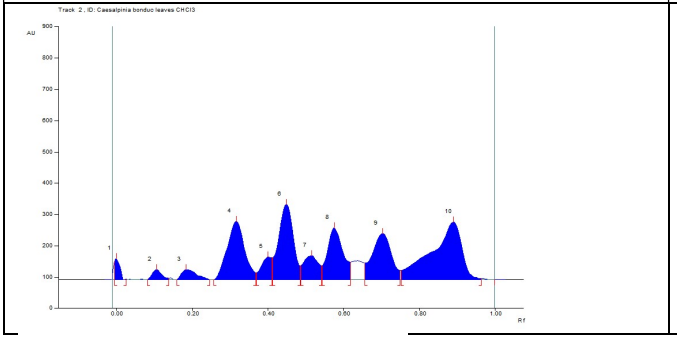
HPTLC finger print analysis showed prominent peaks in all the three plant parts ie., leaf, bark and root. The HPTLC profile of leaf showed the presence of ten to twelve spots at different wavelength of which 0.83 R_f at 254nm with maximum area of 49.46% was the highest one (Fig. 3). Similarly HPTLC finger print scanned at wavelength 575 nm for *C bonduc* bark also showed ten polyvalent phytoconstituents and corresponding ascending order of R_f values ranged from 0.06 to 0.98 in which highest concentration of the phytoconstituents was found to be 33.13 % and its corresponding R_f value was found to be 0.43 respectively (Fig. 4). HPTLC plate showed different colour phytoconstituents of *C bonduc* bark extract. Thus the developed chromatogram is specific with selected solvent system, R_f value and serve the better tool for standardization of the extract. The root extract in toluene ethyl acetate solvent system also showed twelve phytoconstituents (at 254nm) with R_f values ranging from 0.00 to 1.02. The highest % was observed by component with 0.68 R_f (i.e., 43.69%) and 0.62 R_f (76.48) at 254 and 366nm respectively (Fig. 5).



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.01 Rf	19.8 AU	0.01 Rf	515.2 AU	33.66 %	0.10 Rf	0.1 AU	13070.4 AU	24.20 %
2	0.15 Rf	3.0 AU	0.17 Rf	15.7 AU	1.03 %	0.20 Rf	0.0 AU	246.2 AU	0.46 %
3	0.21 Rf	1.2 AU	0.24 Rf	12.2 AU	0.80 %	0.25 Rf	10.6 AU	241.0 AU	0.45 %
4	0.25 Rf	10.9 AU	0.27 Rf	19.7 AU	1.29 %	0.31 Rf	3.6 AU	391.9 AU	0.73 %
5	0.41 Rf	6.1 AU	0.46 Rf	53.1 AU	3.47 %	0.48 Rf	43.6 AU	1426.8 AU	2.64 %
6	0.48 Rf	44.3 AU	0.51 Rf	122.5 AU	8.00 %	0.55 Rf	38.9 AU	3151.2 AU	5.83 %
7	0.55 Rf	39.5 AU	0.58 Rf	89.3 AU	5.84 %	0.61 Rf	52.2 AU	2691.9 AU	4.98 %
8	0.61 Rf	52.8 AU	0.65 Rf	145.5 AU	9.50 %	0.69 Rf	42.0 AU	4429.3 AU	8.20 %
9	0.69 Rf	42.1 AU	0.71 Rf	53.8 AU	3.51 %	0.76 Rf	27.1 AU	1651.1 AU	3.06 %
10	0.76 Rf	27.4 AU	0.83 Rf	503.7 AU	32.90 %	1.01 Rf	6.4 AU	26711.7 AU	49.46 %

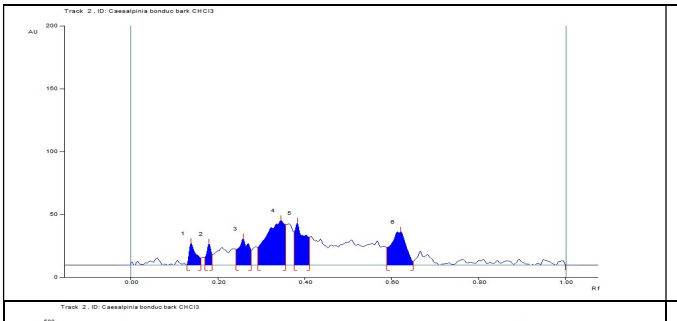


Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.01 Rf	34.1 AU	0.00 Rf	413.6 AU	12.78 %	0.02 Rf	68.2 AU	5578.9 AU	6.48 %
2	0.03 Rf	172.3 AU	0.04 Rf	315.4 AU	9.75 %	0.09 Rf	40.1 AU	7795.7 AU	9.06 %
3	0.09 Rf	40.4 AU	0.11 Rf	72.2 AU	2.23 %	0.13 Rf	1.3 AU	918.0 AU	1.07 %
4	0.13 Rf	2.6 AU	0.18 Rf	211.7 AU	6.54 %	0.23 Rf	0.5 AU	6247.2 AU	7.26 %
5	0.23 Rf	0.8 AU	0.27 Rf	281.5 AU	8.70 %	0.35 Rf	24.9 AU	8933.5 AU	10.38 %
6	0.35 Rf	25.2 AU	0.43 Rf	139.4 AU	4.31 %	0.44 Rf	36.7 AU	4930.5 AU	5.73 %
7	0.47 Rf	138.7 AU	0.50 Rf	537.6 AU	16.61 %	0.55 Rf	32.6 AU	19487.9 AU	22.65 %
8	0.56 Rf	233.5 AU	0.56 Rf	238.3 AU	7.37 %	0.61 Rf	82.3 AU	5236.2 AU	6.09 %
9	0.61 Rf	84.5 AU	0.63 Rf	405.7 AU	12.54 %	0.65 Rf	82.8 AU	7367.9 AU	8.56 %
10	0.65 Rf	383.0 AU	0.67 Rf	471.2 AU	14.56 %	0.75 Rf	0.3 AU	13009.3 AU	15.12 %
11	0.75 Rf	0.2 AU	0.86 Rf	80.9 AU	2.50 %	0.89 Rf	60.1 AU	3974.3 AU	4.62 %
12	0.89 Rf	60.3 AU	0.92 Rf	68.3 AU	2.11 %	1.03 Rf	0.1 AU	2561.3 AU	2.98 %

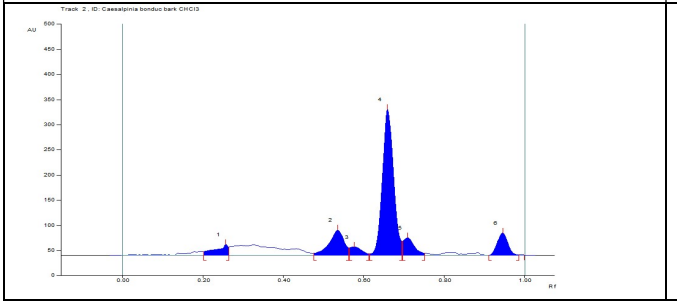


Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.00 Rf	58.0 AU	0.00 Rf	67.2 AU	5.59 %	0.03 Rf	1.5 AU	700.2 AU	1.70 %
2	0.09 Rf	0.2 AU	0.12 Rf	32.5 AU	2.70 %	0.15 Rf	4.4 AU	607.6 AU	1.47 %
3	0.17 Rf	0.1 AU	0.20 Rf	32.1 AU	2.67 %	0.27 Rf	0.3 AU	956.3 AU	2.32 %
4	0.28 Rf	0.0 AU	0.34 Rf	185.7 AU	15.43 %	0.40 Rf	21.9 AU	6507.2 AU	15.78 %
5	0.40 Rf	22.3 AU	0.44 Rf	72.2 AU	6.00 %	0.44 Rf	70.1 AU	1432.2 AU	3.47 %
6	0.45 Rf	70.3 AU	0.49 Rf	240.0 AU	19.95 %	0.53 Rf	43.9 AU	7076.6 AU	17.16 %
7	0.53 Rf	44.5 AU	0.56 Rf	76.7 AU	6.38 %	0.59 Rf	44.6 AU	2296.5 AU	5.57 %
8	0.59 Rf	45.0 AU	0.63 Rf	165.1 AU	13.72 %	0.67 Rf	56.4 AU	4968.6 AU	12.05 %
9	0.71 Rf	53.0 AU	0.76 Rf	147.7 AU	12.28 %	0.81 Rf	30.0 AU	5624.9 AU	13.64 %
10	0.82 Rf	30.2 AU	0.97 Rf	184.0 AU	15.29 %	1.04 Rf	3.0 AU	11058.2 AU	26.82 %

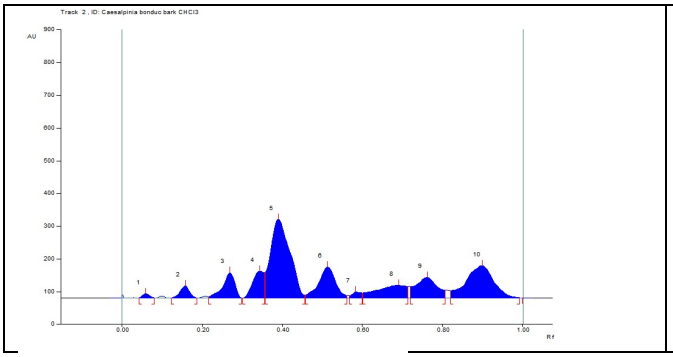
Fig. 3: HPTLC fingerprinting of *Caesalpinia bonduc (L) Roxb.* (Leaves) at 254, 366 and 575 nm



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.14 Rf	0.0 AU	0.15 Rf	17.2 AU	11.45 %	0.17 Rf	5.6 AU	204.6 AU	6.47 %
2	0.19 Rf	6.1 AU	0.19 Rf	17.1 AU	11.37 %	0.20 Rf	8.6 AU	136.3 AU	4.31 %
3	0.26 Rf	12.4 AU	0.28 Rf	21.1 AU	13.99 %	0.30 Rf	11.9 AU	374.0 AU	11.83 %
4	0.32 Rf	14.2 AU	0.38 Rf	35.3 AU	23.46 %	0.39 Rf	32.1 AU	1154.0 AU	36.51 %
5	0.41 Rf	26.0 AU	0.42 Rf	33.4 AU	22.15 %	0.44 Rf	22.2 AU	592.6 AU	18.75 %
6	0.64 Rf	14.0 AU	0.67 Rf	26.5 AU	17.58 %	0.70 Rf	2.9 AU	699.4 AU	22.13 %

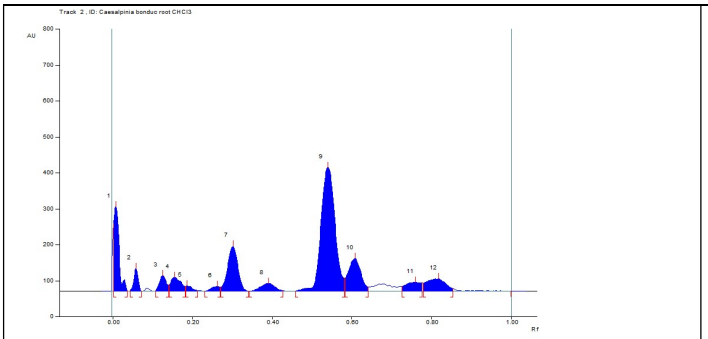


Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.22 Rf	7.6 AU	0.28 Rf	21.9 AU	4.78 %	0.29 Rf	16.6 AU	511.1 AU	5.13 %
2	0.52 Rf	3.8 AU	0.58 Rf	50.1 AU	10.95 %	0.61 Rf	14.9 AU	1393.0 AU	13.98 %
3	0.61 Rf	15.0 AU	0.63 Rf	16.5 AU	3.59 %	0.67 Rf	2.9 AU	359.3 AU	3.61 %
4	0.67 Rf	3.0 AU	0.71 Rf	290.4 AU	63.42 %	0.75 Rf	28.0 AU	6112.4 AU	61.34 %
5	0.76 Rf	28.2 AU	0.77 Rf	34.7 AU	7.58 %	0.82 Rf	3.7 AU	738.4 AU	7.41 %
6	0.99 Rf	0.3 AU	1.03 Rf	44.2 AU	9.66 %	1.07 Rf	1.1 AU	850.4 AU	8.53 %



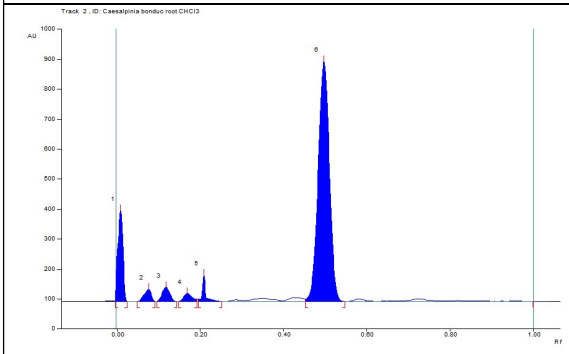
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.05 Rf	0.3 AU	0.06 Rf	12.8 AU	1.68 %	0.09 Rf	0.4 AU	168.3 AU	0.64 %
2	0.13 Rf	0.3 AU	0.17 Rf	37.4 AU	4.88 %	0.20 Rf	0.2 AU	662.8 AU	2.53 %
3	0.23 Rf	4.1 AU	0.29 Rf	77.3 AU	10.11 %	0.32 Rf	0.2 AU	1762.3 AU	6.73 %
4	0.33 Rf	0.0 AU	0.37 Rf	82.7 AU	10.81 %	0.39 Rf	77.3 AU	1769.9 AU	6.76 %
5	0.39 Rf	78.2 AU	0.43 Rf	240.7 AU	31.45 %	0.50 Rf	8.7 AU	8675.3 AU	33.13 %
6	0.50 Rf	8.8 AU	0.56 Rf	94.6 AU	12.36 %	0.61 Rf	7.9 AU	3020.8 AU	11.54 %
7	0.62 Rf	7.3 AU	0.63 Rf	19.2 AU	2.50 %	0.65 Rf	15.6 AU	309.3 AU	1.18 %
8	0.65 Rf	15.7 AU	0.75 Rf	38.5 AU	5.03 %	0.77 Rf	35.4 AU	2195.4 AU	8.38 %
9	0.78 Rf	35.4 AU	0.83 Rf	62.8 AU	8.21 %	0.88 Rf	23.5 AU	2480.1 AU	9.47 %
10	0.89 Rf	22.9 AU	0.98 Rf	99.2 AU	12.97 %	1.08 Rf	1.1 AU	5140.3 AU	19.63 %

Fig. 4: HPTLC fingerprinting of *Caesalpinia bonduc (L) Roxb.* (Bark) 254, 366 and 575 nm



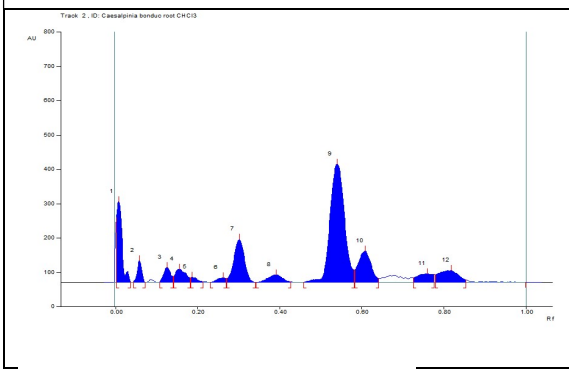
Track 2, ID: Caesalpinia bonduc root CHCl3

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	187.8 AU	0.01 Rf	235.2 AU	22.37 %	0.05 Rf	1.9 AU	2802.2 AU	11.68 %
2	0.05 Rf	0.9 AU	0.08 Rf	63.3 AU	6.02 %	0.09 Rf	0.0 AU	586.3 AU	2.44 %
3	0.14 Rf	0.1 AU	0.16 Rf	42.8 AU	4.07 %	0.18 Rf	17.0 AU	641.6 AU	2.67 %
4	0.18 Rf	17.4 AU	0.19 Rf	38.8 AU	3.69 %	0.23 Rf	12.8 AU	852.0 AU	3.55 %
5	0.23 Rf	13.0 AU	0.23 Rf	15.1 AU	1.44 %	0.27 Rf	1.0 AU	211.2 AU	0.88 %
6	0.29 Rf	0.0 AU	0.33 Rf	13.6 AU	1.30 %	0.34 Rf	12.0 AU	245.5 AU	1.02 %
7	0.34 Rf	12.1 AU	0.38 Rf	125.6 AU	11.94 %	0.43 Rf	0.0 AU	2782.7 AU	11.60 %
8	0.43 Rf	0.0 AU	0.49 Rf	22.1 AU	2.11 %	0.54 Rf	1.6 AU	666.8 AU	2.78 %
9	0.57 Rf	0.4 AU	0.68 Rf	344.5 AU	32.75 %	0.73 Rf	36.5 AU	10481.4 AU	43.69 %
10	0.73 Rf	36.8 AU	0.76 Rf	91.5 AU	8.70 %	0.80 Rf	11.6 AU	2466.3 AU	10.28 %
11	0.91 Rf	13.7 AU	0.95 Rf	24.6 AU	2.33 %	0.97 Rf	22.4 AU	828.2 AU	3.45 %
12	0.98 Rf	22.1 AU	1.02 Rf	34.7 AU	3.30 %	1.07 Rf	7.3 AU	1425.7 AU	5.94 %



Track 2, ID: Caesalpinia bonduc root CHCl3

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.00 Rf	0.0 AU	0.01 Rf	302.7 AU	23.14 %	0.03 Rf	0.3 AU	3304.1 AU	13.43 %
2	0.06 Rf	0.2 AU	0.10 Rf	40.8 AU	3.12 %	0.12 Rf	0.7 AU	614.8 AU	2.50 %
3	0.12 Rf	0.4 AU	0.15 Rf	47.1 AU	3.60 %	0.18 Rf	0.1 AU	760.7 AU	3.09 %
4	0.19 Rf	0.2 AU	0.21 Rf	26.8 AU	2.05 %	0.24 Rf	7.4 AU	481.1 AU	1.96 %
5	0.25 Rf	7.4 AU	0.26 Rf	89.5 AU	6.85 %	0.32 Rf	0.0 AU	624.9 AU	2.54 %
6	0.57 Rf	8.8 AU	0.62 Rf	801.0 AU	61.24 %	0.69 Rf	0.2 AU	18810.5 AU	76.48 %



Track 2, ID: Caesalpinia bonduc root CHCl3

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.00 Rf	124.1 AU	0.00 Rf	153.9 AU	14.66 %	0.02 Rf	0.9 AU	1455.7 AU	4.19 %
2	0.05 Rf	0.4 AU	0.07 Rf	21.4 AU	2.03 %	0.09 Rf	0.1 AU	299.9 AU	0.86 %
3	0.13 Rf	0.1 AU	0.15 Rf	12.5 AU	1.19 %	0.16 Rf	8.3 AU	170.1 AU	0.49 %
4	0.17 Rf	8.4 AU	0.19 Rf	22.5 AU	2.14 %	0.21 Rf	10.7 AU	476.0 AU	1.37 %
5	0.22 Rf	10.8 AU	0.23 Rf	22.3 AU	2.12 %	0.26 Rf	0.1 AU	360.0 AU	1.04 %
6	0.36 Rf	6.8 AU	0.44 Rf	283.0 AU	26.94 %	0.54 Rf	0.2 AU	11103.4 AU	31.97 %
7	0.54 Rf	0.5 AU	0.61 Rf	103.8 AU	9.88 %	0.64 Rf	43.5 AU	2946.9 AU	8.49 %
8	0.64 Rf	44.4 AU	0.67 Rf	102.3 AU	9.74 %	0.71 Rf	31.6 AU	2858.1 AU	8.23 %
9	0.71 Rf	31.9 AU	0.75 Rf	145.8 AU	13.88 %	0.84 Rf	31.4 AU	5619.0 AU	16.18 %
10	0.85 Rf	31.4 AU	0.90 Rf	76.9 AU	7.32 %	0.95 Rf	17.4 AU	2926.8 AU	8.43 %
11	0.95 Rf	17.4 AU	1.07 Rf	105.9 AU	10.08 %	1.15 Rf	7.2 AU	6510.4 AU	18.75 %

Fig. 5: HPTLC fingerprinting of *Caesalpinia bonduc (L) Roxb.* (Root) at 254, 366 and 575 nm

In order to trace out the presence of similar phytoconstituents in the selected plant parts an attempt was made to compare the R_f values obtained in leaves, bark and root. Interestingly it was noticed that some of the R_f values were shared with leaves, bark and root of

C. bonduc. R_f values 0.15, 0.19, 0.38, 0.68 of root (observed under the 254nm) was present in the bark. R_f values 0.01, 0.15, 0.23, 0.49 and 0.68 of root (observed under the 254nm) was present in the leaves. Similarly, R_f of 0.12, 0.18 and 0.69 observed in 366nm was shared between leaf and root. The spots with R_f values 0.28 and 0.69 was shared between root and bark. R_f of 0.44, 0.63, 0.75 and 0.98 was seen in leaf, bark and root after derivatising and observed under 575nm (Table 3).

Table 3: Comparison of R_f values of *C. bon*duc leaf, bark and root extract at different wavelengths

Wavelength	Leaf	Bark	Root
Under UV short (254 nm)	**0.01**, **0.19**, **0.24**, 0.27, 0.46, **0.51**, 0.58, **0.65**, 0.71, 0.83	**0.15**, **0.19**, 0.28, **0.38**, 0.42, **0.67**	**0.01**, 0.08, **0.15**, **0.19**, **0.23**, 0.33, **0.38**, **0.49**, **0.68**, 0.76, 0,95,
Under UV long (366 nm)	0.04, **0.12**, **0.18**, **0.28**, 0.43, 0.50, **0.58**, 0.63, **0.69**, 0.86, 0.92	**0.28**, **0.58**, **0.69**, 0.71, 0.77	0.01, **0.12**, **0.18**, **0.28**, 0.32, **0.69**
Under white light after derivatisation (575 nm)	0.12, 0.20, 0.34, **0.44**, 0.49, **0.56**, **0.63**, **0.75**, **0.98**	0.06, 0.17, 0.29, 0.37, **0.44**, **0.56**, **0.63**, **0.75**, 0.83, **0.98**	0.07, 0.15, 0.19, 0.23, **0.44**, **0.63**, 0.67, **0.75**, **0.98**

The results from the HPTLC study showed that there are so many identical spots which represent similar compounds. Hence leaves and bark can be suggested as an alternative to root as the presence of similar constituents in them was observed. The present study provides sufficient information about phytoconstituents present in the leaf, bark and root extracts of *C bonduc* and also in the identification, standardization and quality control of this medicinal plant. After further advanced assays and purification procedures and also *in vivo* analysis on animal models these hypothesis can be effectively proven with substantial evidence. The sustainable use of this plant by substituting the root by the other parts, stem or leaves needs more research work.

CONCLUSION

C. bonduc is a herb reported in Ayurveda and Siddha, the ancient medicine systems of India. The plant contributes to the biodiversity of the forests where it grows, helps in protecting the soil and protects the wildlife. Nowadays the pharmaceutical industry is focussed towards the design and development of new plant based drugs through investigations from traditional system of medicine since they are well tested for their efficacy and generally believed to be safe. The over exploitation of the roots of *C bonduc* may cause the extinction or threat in the survival of this genus. Hence here an attempt was made to trace out the presence of similar phytoconstituents of root from leaves and bark of the plant that enables the sustainable utilization of this medicinal plant.

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