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PHARMACOGNOSTICAL AND PHYTOCHEMICAL EVALUATION OF *CAESALPINIA BONDUC*

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ABSTRACT

The present paper deals with the detailed pharmacognostic evaluation of the crude drug, *Caesalpinia bonduc*. Morphoanatomy of the leaves of *C. bonduc* was studied with the aim to aid pharmacognostic and taxonomic species identification using light and confocal microscopy, WHO recommended physico-chemical determinations and authentic phytochemical procedures. Different extracts were prepared using the soxhlation method and these were used for phytochemical analysis and to determine various pharmacognostic parameters. The various procedures were performed following the WHO guidelines. Various pharmacognostic parameters were reported following the WHO guidelines. The physico-chemical and morphological parameters presented in this paper may be proposed as parameters to establish the authenticity of *C. bonduc* and can possibly help to differentiate the drug from its other species.

Keywords: *Caesalpinia bonduc*, Caesalpiniaceae, pharmacognosy, phytochemistry, standardization.

INTRODUCTION

Caesalpinia bonduc (family Caesalpiniaceae) is a climbing shrub, stem stout and woody. The branches are armed with strong, sharp yellowish hooked prickles. Leaves are 23-38 cm long, main rachis prickly, pinnae 5-10 pairs. Leaflets subsessile, 8-12 pairs on each pinna, 1.3-2 cm by 4.7-8 mm, oblong, rounded at the apex, pale green, glabrous above, glaucous beneath (Kirtikar and Basu, 1991).

C. bonduc has several ethnomedicinal uses. The leaves are used as laxative, tonic, carminative, antipyretic and emmenagogue (Nadkarni, 1976). In Madagascar, the root is used as an emmenagogue and a concentrated infusion of the leaves is prescribed as emetic and cathartic (Warrier *et al*., 2003). In Chamba district, Himachal Pradesh (India), the bruised leaves are applied to burns. The plant is widely used in the treatment of diarrhea in Parinche valley in Pune district, Maharashtra (Tetali *et al*., 2009). In China, the root is used as purgative (Kirtikar and Basu, 1991).

A new cassane diterpenoid, caesaldecan, along with spathulenol, 4, 5-epoxy-8(14)-caryophyllene, squalene, lupeol, trans-resveratrol, quercetin, astragalin and stigmasterol have been isolated from *C. bonduc* (Van *et al*., 2005).

Previous investigations on *C. bonduc* have reported antidiabetic effect of seed extracts (Kannur, 2006). In addition, anti-implantation activity has been reported (Chaudhurry, 1993).

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MATERIALS AND METHODS

Collection of plant material and its identification – The leaves of *C. bonduc* were collected from M/S Sheikh International, Dindigul, Tamil Nadu, India, during the month of August, 2009. The botanical identity of the plant was confirmed by Dr. Shiddamallayya N, Regional Research Institute (Ay.) Bangalore, India. A voucher specimen (RRI/BNG/SMP/Drug Authentication/2009-10/552) has been deposited at the Museum of the Department of Pharmacognosy, Lovely School of Pharmaceutical Sciences, Phagwara, Punjab, India.

Chemicals and instruments – Compound microscope, glass slides, cover slips, watch glass and other common glass ware were the basic apparatus and instruments used for the study. Microphotographs were taken using a Leica DMLS microscope attached with Leitz MPS 32 camera. Solvents viz. petroleum ether, benzene, chloroform, acetone, ethanol (99.9%), n-butanol and reagents viz. phloroglucinol, glycerine, HCl, chloral hydrate and sodium hydroxide were procured from Ranbaxy Fine Chemicals Ltd., Mumbai, India.

Macroscopic and Microscopic analysis – The macroscopy and microscopy of the leaf was studied according to the standard method (Brain and Turner, 1975a). For the microscopical studies, cross sections were prepared and stained as per the standard procedure (Johansen, 1940). The micro-powder analysis was done according to the standard methods (Brain and Turner, 1975b; Kokate, 1986a).

Physico-chemical analysis – The percentage of ash values and extractive values were performed according to the

official methods prescribed (Anonymous, 1996) and the WHO guidelines on the quality control methods for medicinal plant materials (Arzneibuch, 1998).

Preliminary Organoleptic Examination

Size: A graduated ruler in millimeters was used for the measurement of the length and width of crude materials.

Colour: Untreated sample was examined under diffused daylight.

Surface characteristics, texture: The material was touched to determine if it is soft or hard; bended and ruptured to obtain information on brittleness.

Odour: The material was powdered and the strength of the odour was determined whether none, weak, distinct or strong and then the sensation of odour whether aromatic, fruity, musty, mouldy, rancid, etc was observed.

Taste: Small amount of plant material was tasted and the taste was observed (Arzneibuch, 1998).

Determination of foreign matter: The plant samples were examined for the foreign matter (Arzneibuch, 1998).

Determination of ash: The ash remaining after ignition of medicinal plant materials was determined by two different methods which measure total ash and acid insoluble ash.

Total ash: The ground air-dried material (2g) was accurately weighed and placed in a previously ignited and tared crucible. The material was spread in an even layer and was ignited by gradually increasing the heat to 500-600°C until it was white, indicating the absence of carbon. It was cooled in a desiccator for 30 minutes and weighed without delay. The content of total ash was calculated in mg per g of the air dried material. (Arzneibuch, 1998).

Loss on drying: Air dried material was accurately weighed (4g) in a previously dried and tared weighing bottle. The sample was dried in an oven at 105°C. It was dried until two consecutive weighings did not differ by 5 mg. The loss of weight was calculated in mg per g of air dried material (Arzneibuch, 1998).

Determination of moisture content

Karl Fischer titration: Methanol (20 ml) was added to the titration beaker. Main plug was connected to 230 volts AC. and mains switch was switched on. The burette stopcock on the main unit was turned on. The stirrer was turned on and the speed of stirring capsule with magnetic stirrer regulator was adjusted. Red neon light indicated the instrument to be switched on. The timer knob was set to 20 seconds on the dial. The stopcock was kept closed and then the button to start the titration was pressed momentarily. The yellow neon light went off immediately and there was a click sound of solenoid valve indicating that the solenoid valve was energized. Then the stopcock of the automatic Karl Fischer burette was opened slowly so that Karl Fischer reagent from the bent tube was added dropwise to the methanol in the beaker. With addition of every Karl Fischer reagent drop, the pointer swinged to right side and came back again to its zero position. The solenoid valve kept on switching off and on until complete moisture in the methanol was removed. At the end point, the meter pointer remained on the right side. The reading on the Karl Fischer burette was noted and Karl Fischer factor was calculated as

$$\text{Karl Fischer factor} = \frac{15.66}{\text{Volume in ml of Karl Fischer reagent used}}$$

A specified quantity (50 mg) was weighed and was added to the methanol in the beaker. The button to start titration was pressed and the sample was titrated. The moisture content was calculated by the formula

Moisture content = 100 x Karl Fischer reagent reading x Factor / Weight of sample in mg (Rao, 2006).

Determination of extractable matter

Hot extraction: Coarsely powdered air-dried material (4.0g) was accurately weighed and placed in glass-stoppered conical flask. 100ml of water was added and total weight including the flask was noted. It was shaken well and allowed to stand for 1 hour. Reflux condenser was attached to the flask and gently boiled for 1 hour cooled and weighed. Original total weight was readjusted with the solvent specified in the test procedure for the plant material concerned. It was then shaken well and filtered rapidly through a dry filter. Filtrate (25ml) was transferred to a tared flat-bottomed dish and evaporated to dryness on a water bath. Then it was dried at 105°C for 6 hours and then cooled in a desiccator for 30 minutes and then weighed without delay. The content of extractable matter in mg per g of air dried material was calculated.

Cold extraction: Coarsely powdered air-dried material (4.0g) was accurately weighed and placed in glass-stoppered conical flask. It was macerated with 100ml of the solvent specified for the plant material for 6 hours, shaken frequently and then allowed to stand for 18 hours. It was filtered rapidly taking care not to lose any solvent. Filtrate (25ml) was transferred to tared flat-bottomed dish and evaporated to dryness on a water bath. It was dried at 105°C for 6 hours, cooled in a desiccator for 30 minutes and weighed without delay. The content of extractable matter in mg per g of air dried material was calculated.

Foaming index

Plant material (1g) was reduced to a coarse powder, weighed accurately and transferred to a 500ml conical

flask containing 100ml of boiling water. It was maintained at moderate temperature for 30 minutes. It was cooled and filtered into a 100ml volumetric flask and added sufficient water through the filter to dilute it. The decoction was poured into 10 stoppered test-tubes in successive portions of 1ml, 2ml, 3ml, 4ml, 5ml, 6ml, 7ml, 8ml, 9ml and 10ml. The tubes were stoppered and shaken in lengthwise motion for 15 seconds, two shakes per second and allowed to stand for 15 minutes and the height of foam was measured. The results were assessed as follows:

i. If the height of the foam in every tube was less then 1cm, the foaming index would be 100.

ii. If the height of foam of 1cm was measured in any of the test tubes, the volume of plant material decoction in that tube would be used to determine the index. If that tube was the first or second tube in a series, an intermediate dilution would be prepared in the same manner to obtain a more precise result.

iii. If the height of foam was more than 1cm in every tube, then the foaming index would be over 1000. In this case, determination would be repeated using a new series of dilutions of the decoction in order to obtain a result.

Foaming index was calculated using the following formula

$$\frac{1000}{a}$$

Where a= the volume in ml of the decoction used for preparing the dilution in the tube where foaming to a height of 1 cm was observed.

Determination of swelling index

Powdered plant material (4g) was accurately weighed and put into a 100ml glass-stoppered measuring cylinder. Water (100ml) was added into it and shaken thoroughly after every 10 minutes for an hour. Then the suspension was allowed to stand for 3 hours at room temperature. The volume occupied by the swelled plant material was measured in ml. This was repeated for three times and mean value was calculated for 1g of plant material.

Preliminary phytochemical evaluation

Preliminary phytochemical screening was carried out by using standard procedures (Kokate, 1986b; Harborne, 1998) without spray reagent.

Successive solvent extraction was performed on small scale with coarsely powdered dried plant material (22g). The plant material was first soxhleted with petroleum ether (300ml) at 60°C for 3hrs and then the plant material was dried at room temperature. The dried plant material was soxhleted with DCM (300ml) at 40°C for 3hrs and then it was dried at room temperature. Again the same process was repeated for ethyl acetate at 77°C and methanol at 70°C. Different extracts of plant material obtained were subjected to various chemical tests to detect the chemical constituents present in them.

Chromatographic studies

TLC plates- Silica gel GF 254 grade was used to prepare TLC plates (8.5 X 20).
Solvent system- Ethyl acetate: glacial acetic acid: Formic acid: water (100: 11: 11: 26)
Visualization - Plates were observed under UV at 254nm. (Wagner and Bladt, 1996)

The sample was spotted on the plate and was allowed to dry it for few minutes. Then the solvent system was prepared and allowed to stabilize for 10 min. Then the plate was dipped in the solvent chamber and allowed it to run up to three forth of the plate. Then it was removed and was air dried. The plate was kept in the UV chamber to visualize the spots. The Rf was calculated.

RESULTS

Preliminary organoleptic examination: (Table 1)

Table 1. Preliminary organoleptic examination.

S. No.	Property	Characteristics of *C. bonduc* leaves
1.	Taste	Bitter
2.	Colour	Pale green
3.	Odour	Not characteristic
4.	Size	Leaflets subsessile, 8-12 pairs on each pinna, 1.3-2 cm by 4.7-8 mm, oblong, rounded at the apex
5.	Surface characteristics	Smooth, glabrous above, glaucous beneath

***Determination of foreign matter*:** Foreign matter like soil particles, hair, other plant materials, insects and feathers were separated manually.
Determination of total ash: Total ash = 60.5 mg/g
Determination of acid insoluble ash: Acid insoluble ash = 38.3 mg/g
Determination of water and volatile matter: Loss on drying = 86.75 mg/g
Determination of moisture content: *Karl Fischer Titration*
KF factor = 15.66 / KF ml on the burette
KF factor = 15.66 / 13 = 1.2046
Moisture content = 100 x KF reagent reading (ml) x Factor / Weight of sample in mg
Moisture content = 100 x 1.5 x 1.2046 / 50 = 3.614 %

Determination of extractable matter*:* (Table 2)

Table 2. Determination of extractable matter

Method	Extract	Weight of empty petridish (g)	Weight of petridish + dried extract (g)	Difference (g)	Extractable matter (mg/g)	Colour of extract
Hot	Pet. Ether	29.415	29.437	0.022	22/4 =5.5	Grass green
	DCM	40.066	40.104	0.038	38/4 =9.5	Dark green
	Ethyl acetate	53.383	53.423	0.040	40/4 =10	Dull green
	Methanol	49.352	49.576	0.224	224/4 =56	Bottle green
	Ethanol	44.543	44.713	0.170	170/4 =42.5	Bottle green
	Aqueous	41.344	41.644	0.300	300/4 =75	Dark green
Cold	Pet. Ether	44.545	44.557	0.012	12/4 =3	Grass green
	DCM	53.251	53.284	0.033	33/4 =8.25	Dark green
	Ethyl acetate	49.569	49.624	0.055	55/4 =13.75	Dull green
	Methanol	43.266	43.455	0.189	189/4 =47.25	Bottle green
	Ethanol	43.265	43.445	0.180	180/4 =45	Bottle green
	Aqueous	51.613	51.855	0.242	242/4 =60.5	Dark green

Weight of drug taken every time = 4 g

Table 3. Foaming index

Test tube	1	2	3	4	5	6	7	8	9	10
Ratio of water: extract	9:1	8:2	7:3	6:4	5:5	4:6	3:7	2:8	1:9	0:10
Height of foam (cm)	0	0	0.1	0.1	0.1	0.1	0.1	0	0	0

Height of froth was measured and it was noticed that no froth measured was more than 1cm therefore the foaming index is less than 100.

Swelling index: (Table 4)

Table 4. Swelling index

Time	Volume of plant material (ml)			Mean (ml)	Difference for 4g plant material D=(b-a) (ml)	Swelling Index for 1g (D/4) (ml)
Initially	40	40	40	40	5.33	1.33
After 3hours	45	46	45	45.33		

Qualitative chemical examination of extracts*:*
Phytochemical screening (Table 5)

Table 5. Phytochemical screening

S. No.	Chemical constituents	Tests	Extracts			
			Pet. Ether	DCM	Ethyl acetate	Methanol
1.	Alkaloids	Mayer's test	-	-	-	+
		Dragendorff's reagent test	-	-	-	-
		Wagner's test	-	-	-	-
		Hager's test	-	-	-	+
2.	Carbohydrates	Molisch's test	-	-	-	+
		Benedict's test	-	-	-	+
		Fehling's test	-	-	-	+
3.	Glycosides	Modified Borntrager's test	-	-	-	-
		Legal test	-	-	-	-

Continued…

Table 5 continued

S. No.	Chemical constituents	Tests	Extracts			
			Pet. Ether	DCM	Ethyl acetate	Methanol
4.	Phytosterols	Salkowski test	-	-	+	-
		Tshugajeu test	-	-	-	+
5.	Phenols	Ferric Chloride test	-	-	-	+
6.	Saponins	Foam test	-	-	-	+
7.	Tannins	Gelatin test	-	-	-	+
8.	Diterpenes	Copper Acetate test	-	-	-	+
9.	Resins	Acetone water test	-	-	-	+
10.	Flavonoids	Alkaline reagent test	-	-	-	-
		Zinc Hydrochloride reduction test	-	-	-	+
		Lead Acetate test	-	-	-	-
		Shinoda test	-	-	-	+
11.	Amino acids and proteins	Xanthoproteic test	-	-	+	-
		Ninhydrin test	-	-	-	+
		Biuret test	-	-	-	-

Thin Layer Chromatography: (Table 6)

Table 6. Thin Layer Chromatography.

S. No.	Extract	Distance travelled by solute	Solvent front	R_f value	Colour of spots	
					Visible	Ultra violet
1.	Petroleum ether	12.7	12.9	0.98	Dark green	Fluorescent pink
2.	DCM	9.3	12.9	0.72	Translucent	Light brown
		12	12.9	0.93	Dark green	Fluorescent pink
		12.7	12.9	0.98	Dark green	Fluorescent pink
3.	Ethyl acetate	8.5	12.9	0.66	Translucent	-
		9.6	12.9	0.74	Translucent	-
		12.6	12.9	0.98	Light green	Fluorescent pink
		12.8	12.9	0.99	Dark green	-
4.	Methanol	1.1	12.9	0.08	-	Fluorescent yellow
		1.8	12.9	0.14	-	Fluorescent yellow
		5.2	12.9	0.40	Translucent	Brown
		6.2	12.9	0.48	Translucent	Light brown
		7.5	12.9	0.58	Translucent	Light brown
		9.4	12.9	0.73	Translucent	Light brown
		10.4	12.9	0.80	Translucent	Light brown
		12.7	12.9	0.98	Dark green	Fluorescent pink

DISCUSSION

The physicochemical and morphological parameters presented in this paper may be proposed as parameters to establish the authenticity of *C. bonduc* and can possibly help to differentiate the drug from its other species. This study will play vital role in future studies for the isolation of its various constituents which may prove to be pharmacologically active.

CONCLUSION

The detailed study of *C. bonduc* leaves provides the pharmacognostic data in the form of various values which is helpful in its standardization.

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