

Pharmacognostical Evaluation of *Caesalpinia sappan* Heartwood

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Received : 12-04-2003

Accepted: 27-07-2003

ABSTRACT : *Caesalpinia span heartwood* is used in folklore in India. The present work attempts to summarize the pharmacognostical characters of the heartwood of this plant. Ash and extractive values, phytochemical tests, HPTLC and fluorescence analysis were carried out. The present work contributes for the standardization of this medicinal heat wood.

Keywords: *Caesalpinia sappan*, Pharmacognostical evaluation, Phytochemical tests and Fluorescence analysis.

INTRODUCTION

Caesalpinia sappan (Family-Caesalpinaceae) is found in India, Peru Malaya, etc., According to Ayurveda, the heartwood is used in vitiated conditions of *Pitta*, burning sensation, wounds, ulcers, leprosy, skin diseases, diarrhea, dysentery, epilepsy, menorrhagia, leucorrhoea, diabetes and other diseases (1-3). The plant is one of the ingredients of an indigenous drug "Lukol"TM that is administered orally for the treatment of non-specific leucorrhoea (post IUD) and gave encouraging results for bleeding following IUD insertion (1). It is also one of the ingredients in many Ayurvedic formulations. A decoction of the heart wood is commonly used in Kerala, India for its antithirst, blood purifying, anti-diabetic, improvement of complexion and for several other properties (Personal communication). Its anticonvulsant (4) anticomplementary (5), modulation of immune function (6),

hepatoprotective (7), anti inflammatory (8), cytotoxic (9), hypoglycemic (10) and several other (1) biological activities have been reported. Several triterpenoids, flavonoids, oxygen heterocycles, steroids have been isolated from the heartwood (1). Due to its medicinal properties, the present investigation was undertaken to standardize the heart wood *Caesalpinia sappan* by carrying out various pharmacognostical studies.

MATERIALS AND METHODS

Collection: *Caesalpinia sappan* heart wood collected during October 2001 from the campus of M.G.University, Kottayam, India. Dr.S.Rajan, Survey of Medicinal Plants and Collection Unit, Govt. Arts College Ootacamund, TN, India, authenticated the plant. A voucher specimen is preserved in our laboratory for further reference. The

macroscopical characters such as, odour, taste, shape, texture and surface of the heartwood were observed and show in Table-1.

Extraction: The shade dried heartwood powdered and extracted (135 g) successively with 700 ml each of petroleum ether (40-60⁰), chloroform, ethyl acetate and methanol in a Soxhlet extractor for 18-20 h. The powdered heart wood (60g) was also subjected to crude extraction with 50% methanol (350 ml) in a Soxhlet extractor for 18-20 h. All the extracts were preserved in a refrigerator till further use. These extractive values are shown in Table-2.

Ash and Extractive values: Ash values such as total ash, acid insoluble ash, water soluble ash and sulphated ash and water soluble and ethanol soluble extractive values were determined using the powdered heartwood according to India Pharmacopoeia (11). These values are shown in Table-3.

Phytochemical screening and other studies:

The Successive petroleum ether, chloroform, ethyl acetate, methanol and crude 50% menthanol and distilled water extracts were subjected to various chemical tests for the identification of Phytocostituents (12) and the results are show in Table-4. The HPTLC studies were also performed for all the extracts on precoated silica gel GF₂₅₄ plates and the suitable solvent system, R_f values and the percentage of the constituents in each extract were found out and the results are shown in Table-5. The behavior of the powdered heartwood with different chemical reagent (13) and fluorescence characters of the powered heartwood and the extracts were observed under UV (254 and 366nm) and visible light (14, 15). These results are shown in Table 6-8.

RESLUTS AND DISCUSSION

The extractive values for the powdered heartwood were found to be 1.40, 1.60, 3.80, 8.90 and 13.45 present, respectively for the petroleum ether, chloroform ethyl acetate, methanol, and 50% methanol extracts (Table-2). The total ash, acid insoluble ash, water soluble ash and sulphated ash values of the heartwood powder were found to be 1.22, 0.3, 0.38 and 1.14 respectively. The ethanol and water soluble extractive values were found to be 4.80 and 2.69, respectively (Table-3). The phytochemical tests indicated the presence of steroids and fixed oils in petroleum ether extract, steroids and flavonoids in chloroform extract, saponians, flavonoids, tannins and triterpenoids in ethyl acetate, carbohydrate, glycosides, saponins, flavonoids, tannins and triterpenoids in methanol and 50% methanol extracts. (Table - 4).

In high performance thin layer chromatography studies, petroleum ether: ethyl acetate (80:20) was found to be the better solvent system for the separation of constituents of the petroleum ether extracts. Similarly, the suitable solvent systems were found out for the other extracts (Table-5).The heartwood powder gave brown, yellow, orange and other colours with various chemicals reagents (Table-6&7).

In conclusion the present study on pharmacognostical characters of *Caesalpinia sappan* may be useful to supplement information in regard to identification.

ACKNOWLEDGEMENTS

The authors wish to place on record their heart felt thanks to Jagadguru Sri Sri Shivarathreeshwar Deshikendra Maha Swamigalavaru of Sittur Mutt, India for providing the facilities.

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Table – 1. Macroscopic Characters of *Caesalpinia sappan* heart wood

S. No.	Macroscopic Parameters	Observations
1	Colour	Orange-red
2	Taste	Bitter
3	Odour	Slight
4	Surface	Grained
5	Texture	Fine and even
6	Shape	Straight

Table – 2. Data Showing the Extractive Values *Caesalpinia sappan* heart wood

S. No.	Name of the extract	Percentage Yield
1	Petroleum ether	1.40
2	Chloroform	1.60
3	Ethyl acetate	3.80
4	Methanol	8.90
	Crude	
5	50% Methanol	15.90

Table – 3. Ash and Extractive values of *Caesalpinia sappan* heart wood

S. No.	Name of the extract	Percentage Yield
	Ash Values	
1	Total	1.22

2	Acid Insoluble	0.13
3	Water Soluble	0.38
4	Sulfated	1.14
Extractive Value		
5	Ethanol Soluble	4.80
6	Water Soluble	2.69

* Average of three determinations

Table – 4. Phytochemical Analysis of Successive and Crude Extracts of *Caesalpinia sappan* heart wood

S. No.	Phytoconstituents	Successive Extracts				Crude Extract
		Petroleum ether	Chloroform	Ethyl acetate	Methanol	50% Methanol
1	Alkaloids	-	-	-	-	-
2	Carbohydrates	-	-	-	+	+
3	Proteins & Amino Acids	-	-	-	-	-
4	Steroids	+	+	-	-	-
5	Glycosides	-	-	-	+	+
6	Saponins	-	-	+	+	+
7	Flavonoids	-	+	+	+	+
8	Tannins & Phenolics	-	-	+	+	+
9	Triterpenoids	-	-	+	+	+
10	Fixed Oils	+	+	-	-	-

+ = Positive; - = Negative

Table – 5. The HPTLC Profile of Various Extracts of *Caesalpinia sappan* heart wood

S. No.	Extract	Solvent System	Number of Peaks	R _f values	Percentage peak area
1	Petroleum ether	Pet. Ether ₈₀ : Ethyl acetate ₂₀	10	0.11, 0.13, 0.19, 0.24, 0.30, 0.57, 0.65, 0.67, 0.72, 0.80	0.11, 1.36, 1.75, 0.41, 4.02, 38.98, 0.38, 0.24, 1.46, 51.28
2	Chloroform	Chloroform ₄₀ :	5	0.20, 0.30, 0.56,	0.64, 8.13, 21.64,

3	Chloroform	Ethyl Acetate ₆₀ Chloroform ₉₀ : Methanol ₁₀	6	0.78, 0.94 0.10, 0.14, 0.20, 0.24, 0.31, 0.44	58.41, 11.18 4.90, 19.71, 2.35, 24.28, 40.58, 8.16
4	Methanol	Chloroform ₉₀ : Methanol ₁₀	8	0.08, 0.13, 0.18, 0.25, 0.32, 0.44, 0.76, 0.84	9.51, 4.24, 3.95, 28.95, 42.68, 9.01, 1.01, 0.65
5	50% Methanol	Chloroform ₉₀ : Methanol ₁₀	8	0.07, 0.14, 0.16, 0.23, 0.31, 0.42, 0.79, 0.84	29.33, 3.60, 3.14, 15.05, 40.29, 6.87, 1.01, 0.70

Table – 6. Data Showing the Fluorescence Analysis of Powdered Heartwood of *Caesalpinia sappan* with Various Chemicals

S. No.	Analysis	UV light		Visible light
		Short (254 nm)	Long (366 nm)	
1	Powder as such	Yellow	Brown	Orange Red
2	Powder + 1N NaOH	Reddish Brown	Violet	Red
3	Powder + Picric acid	Brown	Green Fluorescence	Light Orange
4	Powder + Acetic acid	Yellow	Dark Brown	Orange
5	Powder + 1N HCl	Yellowish Brown	Brown	Orange
6	Powder + 1N HNO ₃	Brown	Cherry Brown	Red
7	Powder + 5% Iodine	Black	Dark Brown	Brown
8	Powder + 5% FeCl ₃	Black	Brown	Brown & Black
9	Powder + HNO ₃ + NH ₃	Brown	Green & Brown	Yellow & Brown
10	Powder + 1N NaOH in methanol	Brown	Violet	Red
11	Powder + Methanol	Yellow	Brown	Orange & Yellow
12	Powder + 50% HNO ₃	Orange Brown	Yellowish Brown	Orange & Brown

Table – 7. Data Showing the Behavior of *Caesalpinia sappan* Heartwood Powder with Different Chemical Reagents

S.No.	Parameters	Percentage*(w/w)
1	Picric acid	Light Orange
2	Acetic acid	Orange
3	Conc. HCl	Pinkish Red
4	Conc. H ₂ SO ₄	Black
5	Conc.HNO ₃	Brown
6	Ferric chloride	Brownish Black
7	Iodine solution	Brown
8	Ammonia solution	Red
9	Aqueous 10% NaOH	Red
10	Powder as such	Orange Red

Table – 8. Data Showing the Fluorescence Analysis of Different Extracts of the Heartwood of *Caesalpinia sappan*

S. No.	Analysis	UV light		Visible light
		Short (254 nm)	Long (366 nm)	
	Successive			
1	Petroleum ether	Light Green	White	White
2	Chloroform	Yellowish Green	Brown	Brownish Violet
3	Ethyl acetate	Brownish Green	Brownish Green	Wine Red
4	Methanol	Yellow	Brown	Wine Red
	Crude			
5	50% Methanol	Yellow	Brown	Wine Red