Pharmacognostical Evaluation of *Caesalpinia sappan* Heartwood

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**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- Caesalpiniaceae) 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, menstruation, 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* heartwood 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 (40-60\(^{0}\)), 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\% methanol and distilled water extracts were subjected to various chemical tests for the identification of Phytoconstituents (12) and the results are show in Table-4. The HPTLC studies were also performed for all the extracts on precoated silica gel GF\(_{254}\) 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 powdered heartwood and the extracts were observed under UV (254 and 366nm) and visible light (14, 15). These results are shown in Table 6-8.

**RESULTS 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 phyyochemical tests indicated the presence of steroids and fixed oils in petroleum ether extract, steroids and flavonoids in chloroform extract, sapnonians, flavonoids, tannins and triterpenoids in ethyl acetrate, 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.

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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      | Petroluem ether         | 1.40             |
| 2      | Chloroform              | 1.60             |
| 3      | Ethyl acetrate          | 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 |
|--------|---------------------|------------------|
| 1      | Ash Values          | 1.22             |
| 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<sub>80</sub>: Ethyl acetate<sub>20</sub> | 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<sub>40</sub>: | 5               | 0.20, 0.30, 0.56,           | 0.64, 8.13, 21.64,    |
| 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 HNO₃               | Brown               | Cherry Brown           | Red                    |
| 7      | Powder + 5% Iodine             | Black               | Dark Brown             | Brown                  |
| 8      | Powder + 5% FeCl₃              | Black               | Brown                  | Brown & Black          |
| 9      | Powder + HNO₃ + NH₃            | Brown               | Green & Brown          | Yellow & Brown         |
| 10     | Powder + 1N NaOH in methanol   | Brown               | Violet                 | Red                    |
| 11     | Powder + Methanol              | Yellow              | Brown                  | Orange & Yellow        |
| 12     | Powder + 50% HNO₃             | Orange Brown        | Yellowish Brown        | Orange & Brown         |

Table – 6. Date Showing the Fluorescence Analysis of Powdered Heartwood of *Caesalpinia sappan* with Various Chemicals
Table – 7. Date 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. Date 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        |