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# Pharmacognostical Studies and Preliminary Phytochemical Investigations on Roots of *Sophora interrupta* Bedd., Fabaceae

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Summary

*Sophora interrupta* Bedd., is a woody perennial shrub which belongs to the family Fabaceae. Many species of this genus like *S. flavescens* Ait., and *S. japonica* L. are used in traditional Chinese medicine. Several phytochemical investigations on this genus revealed the presence of many bioactive constituents like matrine and oxymatrine alkaloids, flavonoids, glycosides and polysaccharides which has medicinal importance. In view of its allied species, their importance in the Chinese medicine and in the absence of its scientifically reported pharmacognostical parameters, the present study attempts to undertake the study of qualitative and quantitative microscopic evaluation of the root along with physicochemical parameters and fluorescence analysis of root powder which helps to establish diagnostic characters and quality parameters for the identification of powdered form of root. Phytochemical evaluation of roots revealed the presence of flavonoids, alkaloids, saponins, glycosides and carbohydrates. TLC and HPTLC profile of Benzene extract was performed for flavonoids.

Key Words: *Sophora interrupta* Bedd., *S. flavescens* Ait., *S. japonica* L., matrine, oxymatrine, flavonoids and polysaccharides

## Introduction

*Sophora* is a genus of about 40 species in the family Fabaceae of herbaceous (*Sophora flavescens*) to trees (*Sophora japonica*). More than 15 species in this genus have a long history of use in traditional Chinese medicine [1]. Several phytochemical researches, invivo and invitro experiments and clinical practices have demonstrated that *Sophora* contains many phyto-constituents like matrine, oxymatrine type of alkaloids [2,3], flavonoids [4,5], saponins and polysaccharides [6] which possess wide-reaching pharmacological actions, including anti oxidant [7], anti cancer [8], anti asthamatic, anti neoplastic, antimicrobial [9], anti viral, antidote, anti pyretic, cardiotonic, anti inflammatory, diuretic and in the treatment of skin diseases like eczema, colitis and psoriasis [10].

*Sophora interrupta* Bedd., is a woody perennial shrub which grows endemically in seshachalam hill ranges, sesha theertham and kumaradhara theertham in Tirumala, India. Leaves are odd-pinnate, leaflets sub opposite, broadly ovate, pubescent below emarginated mucronate. Flowers are golden yellow in axillaries and terminal recemes. Calyx tube widely companulate, oblique at mouth, teeth short and deltoid. Corolla much exerted petals 5 clawed, obtuse, stamens 10, and free anthers versatile. Ovary stipulate, many ovules, style incurved, stigma terminal and minute, pods 4 winged constricted between seeds. Seeds are 3-6, obovoid or globbose and strophiole [11] (Figure 1).

## Materials and Methods

### *Plant Material*

The whole plant (*Sophora interrupta* Bedd., Fabaceae) was collected from surroundings of Seshachalam hill ranges of Tirumala, Tirupathi, Andhra Pradesh, India in the month of November. The plant material was identified and authenticated by Mr. A. Laxma Reddy, Retired lecturer (Botany), Nagarjuna Govenment College, (Affiliated to Osmania University) Nalgonda. The plant specimen was prepared and submitted in the Department of Pharmacognosy under the voucher no: NCOP-NLG/Ph'cog/10-11/040.

### *Chemicals and Instruments*

All the chemicals and reagents like chloral hydrate, phloroglucinol, hydrochloric acid, nitric acid, potassium hydroxide, picric acid, lead acetate etc., used were of analytical grade.

Sisco muffle furnace (3003137), Stage micrometer and eye piece micrometer.

### *Macroscopic and Microscopic Analysis*

The macroscopy and microscopy of the plant was studied according to the methods of [12, 13], the cross sections were prepared and stained by using phloroglucinol and hydrochloric acid in the ratio 1:1 was used as a stain and mounted on a glass slide and focused under a microscope. The microscopic analysis of root powder was done according to the method of [14] and Kokate et al. (1986).

### *Physico-Chemical Analysis*

Air dried plant material was used for the quantitative determination of ash and extractive values [15]. Fluorescence analysis of the extract(s) was carried out by the method of [16, 17].

*Preliminary Phytochemical Screening*

Preliminary phytochemical screening was carried out by using standard procedure described by [18, 19]. 100 gm of powdered root material was subjected to successive solvent extraction using petroleum ether benzene, chloroform, ethylacetate, acetone, alcohol and water based on increasing polarity using soxhalet apparatus. The extracts were concentrated under vacuum using rotary vacuum evaporator, dried and weighed. Each extract was tested for the presence of phytoconstituents *viz*. flavonoids, alkaloids, glycosides, saponins and carbohydrates. The TLC pattern of benzene extract was carried using precoated silica gel plates (Merck). The plates were developed using Benzene-Acetone-Ethyl acetate (8: 1: 1) mobile phase. The developed plate was sprayed with respective spray reagent 1% ethanolic aluminium chloride for flavonoids and dried at 100°C in Hot air oven and the spots were observed in UV 366 nm, it is followed by HPTLC studies using CAMAG LINOMAT 5 instrument, photodocumentation chamber (CAMAG REPROSTAR 3), CAMAG TLC SCANNER 3. An alluminum plate (2x10 cm) precoated Silica gel $60F_{254}$ was used as adsorbent. The plates were developed using Benzene-Acetone-Ethyl acetate (8: 1: 1) mobile phase. The developed plate was sprayed with respective spray reagent 1% Ethanolic Aluminium chloride for flavonoids and dried at 100°C in Hot air oven. The plate was photo-documented at Day light and UV 366nm mode using Photo-documentation (CAMAG REPROSTAR 3) chamber. After derivatization, the plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was done at UV 365nm. The Peak table, Peak display and Peak densitogram were noted.

## Results and Discussion

*Macroscopic Characters*

The roots are woody, tuberous, and perennial, about 4 to 8 cm in diameter, light brownish yellow in colour with characteristic odour and highly bitter taste. Fractured surface is fibrous. Inner tuber is whitish cream coloured. The tubers show many rootlets (Figure 1).

*Microscopic Characters*

Cork cells are arranged in 12-14 layers with lignified, suberised rectangular shaped cells. Cortex composed of several layers of loosely arranged thin walled parenchymatous cells. In cortex the cells are arranged without any intercellular spaces. Calcium oxalte crystals are seen in this region. Vascular bundles are radially arranged, xylem and phloem constitutes xylem bundles, phloem bundles respectively and present alternatively on different radii. Uniseriate medullary rays are seen in between the vascular bundles, they are formed of radially arranged thin walled parenchymatous cells from centre to cortex (Figure 2)

*Powder Characters*

Powder of root material showed the presence of xylem vessels with annular and scalariform thickenings, cork cells, starch grains and calcium oxalate crystals. These powder characteristics can be used for diagnostic purposes of crude drug (Figure 3). Quantitative analysis of root powder was also done and the results were shown in the Table 1.

Table 1. Quantitative microscopy

| Parameter | Length | Width |
|---|---|---|
| Phloem fibres | 250-562.5-1250 µ | 12.5-18.75-40µ |
| Xylem vessels | ----- | 37.5-62.5-87.5 µ |
| Starch grains | ----- | 12.5-18.75-31.25 µ |

Table 2. Extractive values

| S.No. | Parameters | Value{% w/w±SD} |
|---|---|---|
| | Water soluble | 17.6±0.08 |
| | Alcohol soluble | 12.8 ±0.12 |
| | Benzene soluble | 16.9±0.09 |

Results are mean±S.D of 3 replicates.

Table 3. Ash values

| S.No. | Parameters | Value{ % w/w±SD} |
|---|---|---|
| | Total ash | 3.95±0.12 |
| | Acid insoluble | 0.6±0.04 |
| | Water soluble | 7.6±0.152 |
| | Sulphated ash | 3.56±0.13 |

Results are mean±S.D of 3 replicates.

Figure 1. *Sophora interrupta* Bedd.

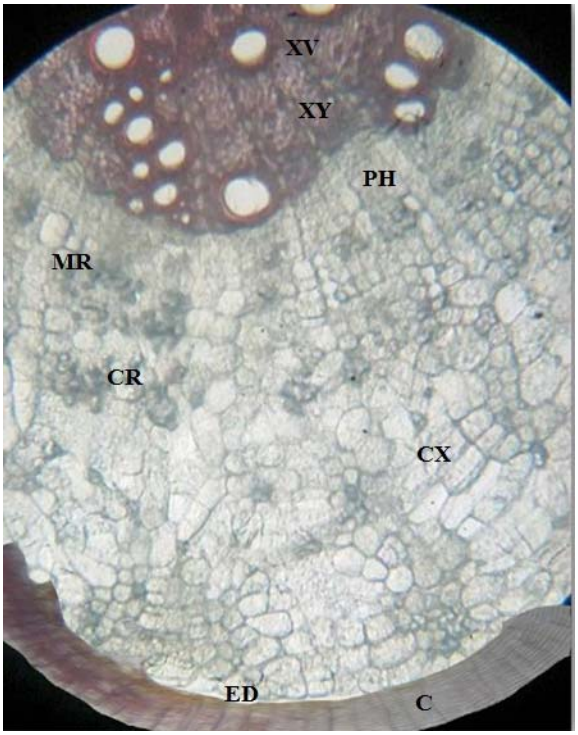


Figure 2. Transverse section of root: *S. interrupta* Bedd. (XV-Xylem vessel, XY-Xylem, PH-phloem, MR, Medullary rays, CR-Calcium oxalate crystals, CX-cortex, ED-Epidermies, and C-Cork)

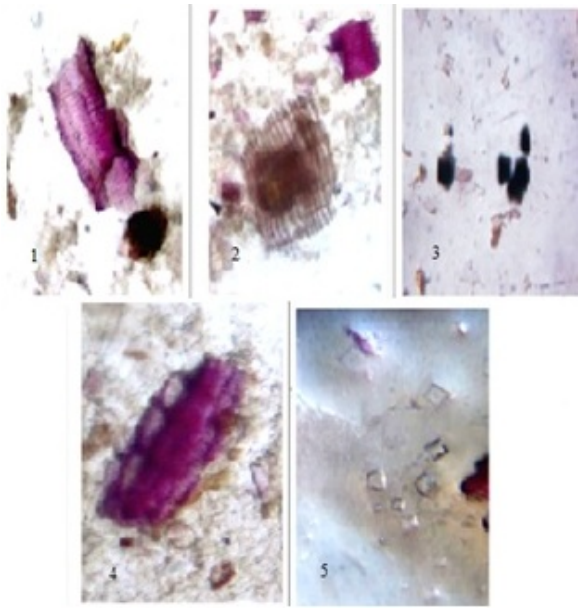


Figure 3. Powder characters of *S. interrupta* Bedd. root. (1. Scalariform xylem vessel, 2. Cork, 3. Starch grains, 4. Scalariform vessel with annular thickenings, 5.Calcium oxalate crystals.)

*Physicochemical Analysis*

Air dried root material was made into fine powder and used for quantitative determination of physiochemical values. Total, acid insoluble and water soluble ash (Table 2) was determined in triplicate and its mean+SD was calculated. Similarly, benzene, alcohol and water soluble extractives were determined in triplicate and its mean+SD was calculated (Table 3). Alcohol and water extractive values were determined as per WHO recommendations while benzene soluble extractive was determined due to the medicinal attributes of the extract. Water extractive was found to be very high when compared to other extractable matter in the powder. Fluorescence analysis of powder was carried out with different chemical reagents and observed under UV short, long and visible light (Table 4). The physical properties and nature of extracts prepared by successive extraction method are recorded (Table 5). Evaluation of physicochemical parameters is prerequisite for the preparation of drug monograph, thus ash values, extractive values, loss on drying, total fibre content and fluorescence characteristics can be used for standardizing of crude drugs and establishment of its quality parameters.

Table 4. Colour & Consistency of *S. interrupta* Bedd., root extracts

| S.No | Extracts | Colour | Consistency | Yield % w/w |
|---|---|---|---|---|
| | Petroleum ether | Yellow | Sticky | 7.4 |
| | Benzene | Orange | Flakes | 18.2 |
| | Chloroform | Brown | Flakes | 13.0 |
| | Ethyl acetate | Brown | Amorphous | 16.3 |
| | Acetone | Red | Amorphous | 11.6 |
| | Alcohol | Reddish brown | Amorphous | 12.5 |
| | Water | Reddish brown | Amorphous | 17.9 |

Table 5. Behaviour of root powder on treatment with different chemical reagents.

| S.No. | Reagent | Long (366 nm) | Short (265 nm) | Day light |
|---|---|---|---|---|
| | Powder+50% $H_2SO_4$ | Black | Black | yellow |
| | Powder+50%$HNO_3$ | Black | Pale green | Yellow |
| | Powder+5% NaOH | Fluorescent green | Green | Yellowish brown |
| | Powder+1N Me NaOH | Yellowish brown | Green | Pale green |
| | Powder+1N KOH | Dark green | Green | Yellowish brown |
| | Powder+5% KOH | Black | green | Yellowish brown |
| | Powder+$FeCl_3$ | Black | Green | Yellow |
| | Powder+Methanol | ------- | White | Yellow |
| | Powder+Conc HCl | ------- | Green | Yellow |
| | Powder+Conc $H_2SO_4$ | ------- | Black | Orange |
| | Powder+Ammonia | Pale green | Green | orange |
| | Powder+Conc $HNO_3$ | ------- | Green | Yellowish orange |

Table 6. Preliminary phytochemical screening.

| Test | Pet. ether | Benzene | Chloroform | Ethylacetate | Acetone | Alcohol | Water |
|---|---|---|---|---|---|---|---|
| Carbohydrates | - | - | - | - | - | + | + |
| Amino acid | - | - | - | - | - | - | - |
| Protiens | - | + | - | + | - | - | - |
| Alkaloids | - | + | + | + | + | - | - |
| Tannins | - | - | - | - | - | - | - |
| Steroids | + | + | - | - | - | - | - |
| Flavonoids | + | + | + | + | + | - | - |
| Saponins | - | - | - | + | + | + | + |
| Glycosides | - | - | + | + | + | - | - |

Table 7. Peak profile of benzene extract of root for flavonoids.

| Peak | Rf | Height | Area | Assigned substance |
|---|---|---|---|---|
| 1 | 0.02 | 199.6 | 3368.3 | Flavonoid 1 |
| 2 | 0.10 | 172.8 | 5433.7 | Flavonoid 2 |
| 3 | 0.16 | 163.7 | 9159.9 | Flavonoid 3 |
| 4 | 0.21 | 117.2 | 3508.8 | Flavonoid 4 |
| 5 | 0.26 | 84.2 | 2792.4 | Unknown |
| 6 | 0.34 | 110.3 | 6316.6 | Unknown |
| 7 | 0.40 | 106.2 | 4983.0 | Flavonoid 5 |
| 8 | 0.53 | 487.0 | 26733.1 | Flavonoid 6 |
| 9 | 0.58 | 132.8 | 4478.6 | Flavonoid 7 |
| 10 | 0.66 | 31.8 | 901.8 | Flavonoid 8 |
| 11 | 0.72 | 227.2 | 8946.3 | Flavonoid 9 |
| 12 | 0.81 | 34.9 | 1009.4 | Unknown |
| 13 | 0.87 | 29.0 | 1109.4 | Unknown |

*Preliminary Phytochemical Screening*

Preliminary phytochemical analysis revealed the presence of flavonoids, alkaloids, glycosides, saponins and carbohydrates (Table 6)

*Chromatographic Studies*

*TLC Profile*

Yellow coloured fluorescent zones at UV 366 nm in benzene extract of *S. interrupta* Bedd., was observed in the TLC chromatogram after derivatization with spraying reagent for flavonoids four spots were observed with Rf values 0.40, 0.56, 0.64 and 0.69.

*HPTLC Finger Print Profile*

Yellow coloured fluorescent zones at UV 366nm in benzene extract of *S. interrupta* Bedd., track observed in the chromatogram after derivatization (Figure 4) which may be the presence of flavonoids in the given sample. It revealed the presence of 13 phytoconstituents, out of these 9 compounds are assigned to be flavonoids with Rf values 0.02, 0.10, 0.16, 0.21, 0.40, 0.53, 0.58, 0.66 and 0.72 with a most pronounced spot of maximum area at Rf 0.53 (Figure 5; Table 7). HPTLC developed profiles used for the detection, isolation and standardization of phytoconstituents profile in the extracts and it is useful in quality control of herbs and its extracts.

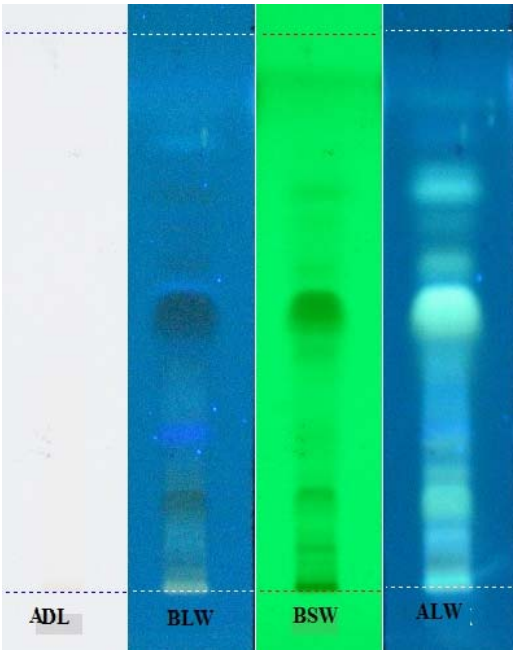


Figure 4. HPTLC chromatogram of benzene extract.
ADL-After derivatization day light, BLW-before derivatization long wave length (UV 366 nm), BSW-before derivatization short wavelength (UV 254 nm), ALW-After derivatization long wave length (UV 366 nm).

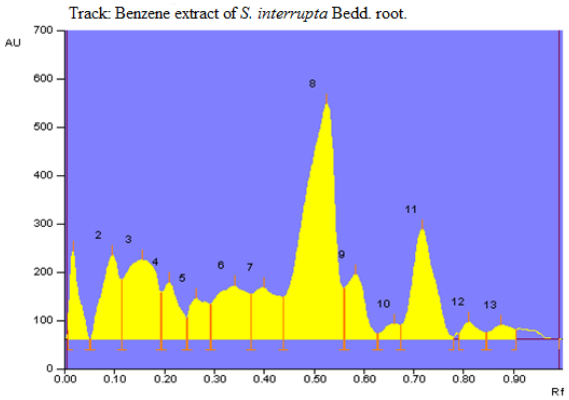


Figure 5. Benzene extract of *S. interrupta* Bedd., root Peak densitogram display (Scanned at 365nm)

## Conclusion

The macro and microscopical characters along with physicochemical and fluorescence characters of root powder of *Sophora interrupta* Bedd., is used to establish the pharmacognostical standards and qualitative parameters as per pharmacopoeia and WHO guide lines. Preliminary phytochemical screening of different plant extracts revealed the presence of alkaloids, flavonoids, saponins, glycosides and carbohydrates. TLC profile of benzene extract was confirmed the presence of flavonoids followed by HPTLC finger print profile has given further conformation for the presence of flavonoids, thus further research on *Sophora interrupta* Bedd., is necessary for isolation and characterization of important bioactive constituents which have wide medicinal values in view of its allied species like *S. flavescens, S. japonica* and *S. alopecuroides* which has importance in traditional Chinese medicine [1].

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