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Isolation of 5, 7 dihydroxy 3, 4 Dimethoxy flavone from the stem bark of *Soymida febrifuga* Juss. (Meliaceae)

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Abstract
The plant *Soymida febrifuga* is called as Mamsaroyini in Sanskrit because of flesh coloured wood. It is an important medicinal plant belongs to family Meliaceae. This plant is used in Ayurveda, Unani and Sidda system of medicine and also in folk system. In North- eastern Karnataka it is commonly used for inflammation and also for joint pain, it is used for easy placental expulsion in cattle in the tribal areas. Keeping its medicinal significance and the literature available, the detailed phytochemical analysis was made. It shows the presence of phenols, flavonoids, saponin and alkaloids. A new flavone -5, 7 dihydroxy 3,4dimethoxy flavone is identified based on UV, IR and NMR data.

Keywords: Ethnobotany, Medicinal plant, Phytochemistry and Pharmacology

Introduction
Flavonoids are the largest group of plant nutrients with diverse therapeutic values. It is a complex group of polyphenol divided into 6 groups: Flavones, Anthocyanidin, Flavonones, Isoflavones, Flavonols, Flavanols. Each of them have their characteristic nutritive and medicinal values. Flavonoids in general has the following benefits when consume as food or medicine: Longevity, weight management, cardiovascular diseases, Diabetes, Cancer prevention and Neurodegenerative diseases. Therefore, the work on biology, pharmacology of flavonoids is increasing by leaps and bounds, Flavonoids are obtained not only by food plants but also by medicinal plants. Soymida is one of the important source of flavonoids used medicinally in Ayurveda, Sidda, Unani and folk system. It is subjected for a detailed phytochemical studies.
It is large tree growing to 20 m. height; Leaves greyish green and unipinnately compound, leaf rachis 25 cm long, leaf lets 8-10, ovate or obovate, obtuse at apex, coriaceous; Inflorescence is a panicle equalling the leaves; flower small whitish grey; calyx lobes ovate; sparsely pubescent; petals obovate, slightly fimbriate; stamens antipetalous; ovary glabrous; capsule obovoid; seeds oblong, flattened. Based on the population decline, habitat destruction and other factors, it is categorised under the threat status as Lower Risk (Seetharam *et al.*, 1998) [8] According to Ignace Kindo *et al.*, (2015) it is endangered in Madhya Pradesh because of over exploitation. However according to JCB herbarium, It is not evaluated.

Ayurveda
The use of plants for various ailments in Ayurveda is seen in Nighantus like Dhanavanthari Nighantu, Ratnakara Nigantu etc and also in Edinburgh Pharmacopoea 1803 and Dublin Pharmacopoea 1807. It is noted that the drug has Sheeta virya, Khasaya Rasa and Krimihara properties (Ashalatha and Tejaswani, 2015). The bark and leaves constitutes a drug in Ayurvedic system, the powdered bark is given to cure fever (perhaps the specific epithet is because of this property of curing fever), tridosha, diarrhoea, dysentery (Chandraraj Bhandari, 1997) [3]. Bhavamishra (1550) in his treatise mentioned the use of *Soymida* for tridosha, wound healing and for fractures.
The bark is acrid, anthelmintic, aphrodisiac, laxative, good for sore throat, used to cure fevers, cough, asthma, removes blood impurities, good for ulcers, leprosy and dysentery, used as antiperiodic, decoction of the bark is used in vaginal infection and also for application on rheumatic swellings (Kirtikar and Basu, 1980; Nadakarni, 1956; George Watt, 1972) [6, 5].

Ethnomedicobotany
In Puri district of Orissa, the bark juice of *Soymida febrifuga* is mixed with water and is taken orally for Kala ajar (Black water fever) and also used in general debility (Anonymous, 1989).

Jain (1980) in his ethnobotanical exploration in Madhya Pradesh has observed the usage of S. febrifuga leaves and bark as galactogogue. Pal (1981) [7] in his work on, tribals of eastern India mentioned the use of Soymida fibrifuga to cure diarrhoea in cattle (Pandey, 1989; Saiprasadgoud and Pullaiah, 1996) [4]. The Mundas use this plant for loose motion especially in goats. The Lodhas prescribe *Soymida febrifuga* when cattles stop mastication (Jain, 1981) [7]. Chopra *et al*., (1956) have reported the use of bark in dysentery and as febrifuge. In North-Eastern Karnataka,it is used for easy placental expulsion at the time of delivery of calf, it is also used to reset the fractured bone in veterinary system (Seetharam *et al*., 1998; 1999) [8]. Formerly the bark was used as an antimalarial and as a substitute for Cinchona bark. It was found to be of inferior quality. This is also a substitute for Oak bark and adopted for gargles (Anonymous, 1966) [2].

Phytochemistry

Many of the organoleptic properties of the bark may be ascribed to the chemical constituents. Occurrence of tannin in the bark may be responsible for the astringent properties of the bark. Anti-oxidant properties are due to flavanoids. Bitterness of the bark may be due to alkaloids. Similarly, triterpenes are the source of many drugs. Many therapeutic properties like anticancer is ascribed to occurence of triterpenes like Cucutbitacin, Ajiboye B.O *et al* (2013). Terpenoids are known to inhibit cancer cell proliferation and induce apoptosis through inhibition of oncogenic and antiapoptotic signelling pathway as well as suppression or nuclear translocation of various translocation factor including Nf kB (Kuttan, Preethesh kumar *et al* 2011). The bark extract was used earlier to cure malarial fever it may be due to alkaloids present in th bark extract. It was leftout as the quality of the drug was inferior to quinine of Cinchona. Work of Shreelatha Devi and Basha (2017) has shown the prevalence of many flavonoids (8 flavanoids are reported) Flavanoids in plants plays a very important role in disease control and prevention. They are known for their anti-oxidant properties. As result of the multifarious activities of flavonoids intensive study is being done all most all the medicinal plants. According to Sreelatha Devi and Basha 2017, their screening of 20 medicinal plants of Tirumala have shown flavanoids in many plants. However, Soymida plant extensively used in traditional system in Tirumala area has all the eight flavanoids tested indicating rich source of flavonoid and its useful ness as medicine. According to Krishna palei *et al* (2013}, their study on root bark of Soymida, it is used for leucorrhoes, menorrhagia, for phytochemicals have reported the occurence of alkaloids, tannins and glycosides. HPTLC has supported the occurence of more number of chemical moieties from the bark. Phytochemistry of Soymida crude fractions were tested for the detection of secondary metabolites such as flavonoids, phenols, quinones, terpenoids, and tannins in callus cultures by Kishore K. Several such reports on the chemistry of Soymida are seen.

The isolation, characterization and structure elucidation of compounds isolated from plants is not yet been carried out. In the present study we have made a phytochemical study using column chromatography, characterized and isolated a compound by spectral studies and elucidate the structure through IR, UV, and NMR spectroscopy.

Materials and Method

The plant material was collected from the Gulbarga University Campus, Identity was confirmed with the voucher specimen deposited in Herbarium of Gulbarga University, Gulbarga (HGUG- 574) A Specimen collected for the present study with other details are deposited in HGUG for future reference.

The bark of *Soymida fubrifuga* was shade dried, powdered and extracted using Soxhlet extractor with solvents like petroleum ether, chloroform, ethanol (95%) and distilled water. The extraction was carried out exhaustively to obtain crude extract and the solvents were recovered by distillation under reduced pressure using rotary vacuum evaporator.

Preliminary phytochemical tests

The qualitative phytochemical tests were carried out for phenols, flavonoids, steroids, triterenes, diterpenes, lactones, tannins, lignins, saponins, alkoloids following the methods of Gibbs (1974), Kleipool (1952), Peach and Tracey (1959), Rangaswami and Rao (1951).

Quantitative estimations of secondary metabolites

The quantitative estimations are carried out for total phenols by Folin-Danis method, (1939), flavones by Swain-hills method, (1959), Estimation of alkaloids Ikan method, (1969).

Extraction and separation of phenolic compounds

Extraction

20 g of powdered plant material was immersed in 40 ml of methanol, allowed to stay overnight. It was then filtered and condensed to 1/4th of the volume. The extract was directly used for chromatographic studies. Chromatographic plates were prepared as suggested by E. Stahl (1965).

Separation

The TLC plates were prepared as formulated by Ergon Stahl (1965).Solvents used for the development of chromatogram are benzene and acetone (6:4) and benzene, acetone and ethyl acetate (9:4:6). The colour of the spots and hRf values are recorded hRf values of all the spots were directly read with the aid of spotting guide and were recorded.

Extraction and separation of alkaloids

Extraction

The alkaloid is extracted from the plant material using 95% ethanol. To the ethanolic extract 10 ml of 10% KOH is added and centrifuged the residue obtained is discarded and the supernatant is mixed with $CHCl_3$ and $CHCl_3$ layer is separated. Thus obtained $CHCl_3$ layer is evaporated to dryness and to obtain crystals. These crystals were again dissolved in $CHCl_3$ and used for chromatographic studies.

Separation

The TLC plates were prepared as formulated by Ergon Stahl (1965).The solvent systems used for the separations are $CHCl_3$ and CH_3OH (1:9) and $CHCl_3$ CH_3COCH_3 and Diethyl amine (5:4:1). The solvent were mixed in the proportion mentioned above and were allowed in developing chamber for saturation at room temperature. The hRf values of all the spots were directly read with the aid of spotting guide and were recorded.

Extraction and separation of flavonoids

Extraction

10g of plant material was powdered with cold petroleum ether. The supernatant was evaporated to dryness, to this methylene chloride was added and left for evaporation. To

this residue few drops of $CHCl_3$ was added and directly used for chromatographic studies.

Separation

The solvent system used was Butanol, Acetic acid and Water (BAW 5:4:1) the compounds eluted by TLC are further Subjected to UV, NMR, IR spectrum to know the structure.

Observations and Discussion

Preliminary phytochemical tests

In the present study an attempt is made to see the occurrence of therapeutically important secondary metabolites such as phenols, flavonoids, steroids, triterpenes, diterpenes, lactones, tannins, saponins, lignans and alkaloids using ethanolic extract of the bark of *S. febrifuga*.

Table 1: Indicating the occurrence of secondary metabolites in studied taxa

Sl. No.	Tests	Observstion
1.	Test for phenols	
	a) Phenol test	+
2.	Test for flavonoids	
	a) Shinoda test	+
	b) Flavonoid test	+
3.	Test for steroids	
	a) Salkowski test	+
	b) Leiberman-Buchardt test	-
4.	Test for triterpenes	
	a) Salkowski test	+
	b) Leiberman-Buchardt test	+
5	Test for tannins	
	a) Tannin test	+
	b) Gelatin test	+
6	Test for saponins	
	a) Foam test	+
7	Test for alkaloids	
	a) Mayer's test	+
	b) Wagner's test	+
	c) Dragendroff's test	+

Table 2: Quantitative estimation of secondary metabolites from the bark.

Phenols	Flavonoids	Alkaloids
192mg/100g.	0.25mg/100g.	0.32mg/100g.

Separation of saponins

In the present study saponins are separated on TLC plates using Ethyl acetate: Hexane (1:9) as a mobile phase.

Table 3: Showing colour and hRf values after separation of Saponins

Sl.	Plant	I_2 saturated chamber		1% vanillin in methanol	
No.	Material	Colour	hRf value	Colour	hRf value
1.	S. febrifuga	Yellow	32.00	Grey	48.00
		Yellow	48.00	Grey	68.00
		Yellow	73.00		

The plates kept under saturated I_2 chamber indicated 3 yellow bands with hRf values 32, 48 and 73. When plates were sprayed with 1% vanillin methanol 2 spots were encountered with grey colour having hRf values 48 and 68 respectively.

Separation of phenols

Methanolic extract showed 3 spots on the TLC with following hRf values 18 (light green), 48.2(Greyish Blue), and 64.40 (Grey).

Table 4: Showing separation of phenols

Sl. No.	Plant material	Colour	hRf value
1.	S. febrifuga	Light green	18.00
		Greyish blue	48.20
		Grey	64.40

Separation of alkaloid

Ethanolic extract has indicated the occurrence of 4 spots with 4 different colour and hRf values in Visible and UV light as indicated in the Table-2.

Table 5: Showing colour and hRf values of alkaloid extract from bark of *S. febrifuga*

Sl.	Plant	Visible light		UV light	
No.	Material	Colour	hRf value	Colour	hRf value
1.	*S*. febrifuga	Pale yellow	02.33	Sky blue	33.33
		Yellow	38.01	Pink	42.10
		Light brown	83.04	Fluorescent	76.02
		Pale yellow	94.73	blue	

Separation of Flavonoid

Petroleum ether was used as a solvent to extract flavonoid. It showed 3 bands with hRf values 14 (Light green), 24.8 (Yellow) and 78.00 (Chacolate). Yellow appearence under visible light is the characteristic of Flavonoids.

Table 6: Showing separation of flavonoids

Sl. No.	Plant material	Colour	hRf value
1.	S. febrifuga	Light green	14.00
		Yellow	24.80
		Chocolate	78.00

Spectral studies of a flavone

The Methanolic extract was separated on column chromatography with silica. Elution from the column with n-hexane-ethyl acetate (60:40) has yielded a yellow solid crystals (35 mg) with m.p. 261-62^{0}C. This compound responded positively to flavonoid test with Magnesium and HCl. UV, IR and NMR studies was made to understand the structure.

UV Spectra

Ultra violet spectrum of eluted compound was studied to determine the nature of chromophoric groups. The UV spectrum was recorded in methanol indicated the presence of a chromophoric group with an extended conjugation. A bathochromic shift was observed when the spectrum was recorded in the presence of $AlCl_3$ in the same solvent. No change was observed when a drop of HCl was added to it. Studies on flavonoids by spectroscopy have revealed that most flavones and flavonols exhibit two major absorption bands: Band I at 328 represents the B ring absorption, while Band II 268 corresponds to the A ring absorption.

λ max (MeOH): 268, 328; ($AlCl_3$): 278, 302, 344, 382 (sh); ($AlCl_3$+HCl): 278, 300, 338, 388 (sh).

Infrared absorption spectrum

The IR spectrum of same compound (4) in KBr disc was studied. The IR spectrum (KBr) exhibited absorption bands at 1675 and 3000 cm-1which revealed the presence of carboxyl and hydroxyl functions of the molecule, respectively. The presence of -OH in the compound was indicated by the appearance of a peak at 3000. cm^{-1} and C=O group by the appearance of absorption peak at 1675.2 cm^{-1}. The IR spectrum also showed absorption at 2910 and 1580 cm-1due to C-H and C=C functions, respectively.

Nuclear magnetic resonance spectroscopy (NMR)

The H'NMR spectrum exhibited a sharp singlet of one proton at 12.25 δ which is due to the presence of -OH group and it is well supported by IR. Two peaks appeared at 8.06 δ and 7.82 δ have integrated for 2 protons each indicating the presence of 2 sets of equivalent protons in the molecule. The appearance of three singlets of one proton at 6.88 δ, 6.45 δ and 6.26 δ each accounted for a single proton, they may be the protons of aromatic CH. The appearance of a singlet at 3.86 δ accounted for three protons, the place where normally OCH_3 protons are appeared.

It may be inferred that the UV, IR, H' NMR and m.p. will well match with 5,7 dihydroxy 3,4 dimethoxy flavone.

Flavanoid mode of action is understood. According to literature, in flavanoid, the total number of hydroxyl groups substantially influence the several machanism of antioxidant activity such as radical scavenging and metal ion chelating ability. A ring with hydroxyl configuration is most significant in scavenging of free radicals and therby prevents damage to cell.

Conclusion

The results indicated that S. febrifuga has secondary metabolites such as phenols, flavonoids, alkaloids, steroids, tannins in them. But, diterpenes are absent in S. febrifuga The quantitative estimations have shown the occurrence of varying quantity of primary and secondary metabolites. Protein, phenol, flavonol and alkaloid content is more than amino acid in S. febrifuga. The IR spectral studies and preliminary phytochemical studies confirms that the isolated compound I and III are alkaloidal in nature, whereas, compound II is phenolic in nature. This needs further studies like, UV, NMR etc.

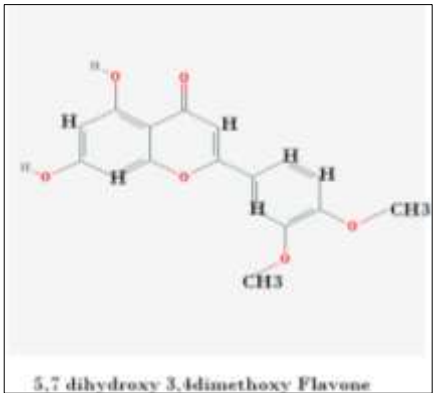

Fig 1: C17 H14 O6

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