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PHYTOCHEMICAL AND PHYSIOCHEMICAL STUDIES OF *SOYAMIDA FEBRIFUGA* LEAF (MELIACEAE)

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ABSTRACT: The present study mainly focuses on the ethnomedicinal importance of *Soymida febrifuga*. The selected plant was reported to have wide ethnomedicinal use. The literature revealed that there is a lack of scientific reports on its leaf. So it is important to provide scientific means systematically. The Phytochemical analysis of the plant has stated about the presence of Carbohydrates, cardiac glycosides, Saponin glycosides, flavonoids, steroids, triterpenoids, tannins, phenolics, and fixed oil, *etc*. The ethnomedicinal documentation confirms about the potent activity of the leaf part of *Soymida febrifuga*. The present study provides evidence that solvent extract of *Holoptelea integrifolia* and *Celestrus emarginata* contains medicinally important bioactive compounds and this justifies the use of plant species as a traditional medicine for treatment of various diseases.

INTRODUCTION: *Soymida febrifuga* is a tall tree belonging to family Meliaceae; commonly known as Indian redwood, bastrol cedar. Pharmacologically the plant is of great importance in the ethnomedicinal, use. It contains some essential constituents like in bark lupeol, sitosterol, methyl angolensate, leaves contains Quercetin, rutin and fruits abundantly contains tetraterpenoids. The ethnobotanical use in the treatment of diarrhea, dysentery, and fever, as a bitter tonic in general debility, treatment of rheumatic swelling, in gargles, vaginal infection, *etc*.

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Plant Material and Extraction:

Plant Material: Fresh leaves of *Soymida febrifuga* collected in August to September from Amravati District, Maharashtra. A voucher specimen was botanically authenticated by Mrs. P.Y. Bhogaonkar head Botany Department, Vidarbha Institute of Science and Humanities College Amravati & deposited in the herbarium.

The fresh leaves were dried in a hot air oven for 24 h at 55 ºC under shed & powder in a mixture grinder. The powder sieved (40 mesh) leaves packed in a paper bags & stored in airtight container until use.

Extraction: Extraction was carried out by solvent extraction. 50 gm of dry powder was extracted with 200 ml of the solvent by Soxhlet for 20 cycles for Pet. ether, chloroform, methanol, and water. And also the total aqueous extract was obtained.

Physicochemical Evaluation:

Ash Values: Ash values are indicative to some extent of care taken in collection and preparation of a drug for market and of foreign matter content of natural drug. The object of ashing is to remove all traces of organic material interfering in an analysis of inorganic elements. The residue remaining after incineration is the ash content of the drug, adhering to it, or deliberately added to it as a form of adulteration. Many a time, the crude drugs are admixed with various mineral substances like sand, soil, calcium oxalate, chalk powder or other drugs with different inorganic contents. Ash value is a criterion to judge the identity or purity of the drug part of *Soymida febrifuga* A. Juss. was obtained by reported methods.

Total Ash: This method is designed to measure the total amount of material remaining after ignition. It includes both physiological ash and non-physiological ash. The physiological ash is derived from plant tissue itself, and non-physiological ash is a residue of extraneous matter (*e.g*., Sand and soil) adhering to plant surface. Total ash usually consists of carbonate, phosphate, silicates, and silica.

Procedure: 2gm of accurately weighed air-dried powder drug was taken in a tarred platinum crucible. Spread the drug material in fine even layer at the bottom of the platinum crucible. This platinum crucible with drug material was kept in a muffle furnace for ignition at high temperature. The temperature of the furnace increased gradually up to 450 ºC. The material was kept at this temperature for 6 h till complete ignition of drug occurred, that is till complete white colored ash was obtained, intermittent weighing was also done, and heating continued till constant weight of crucible.

Crucible was then taken out from the furnace, cooled and weighed. The total ash was calculated by subtracting the weight of crucible with the ash of drug after ignition from the weight of crucible with drug powder before ignition. Percentage of total ash was calculated concerning the air-dried drug.

Acid-Insoluble Ash: Acid-insoluble ash, which is a part of total ash insoluble in dilute hydrochloric acid, is also recommended for certain drugs. Adhering dirt, sand, as well as variation caused by calcium oxalate, may be determined by acid-insoluble ash content. From microscopical studies, it was evident that calcium oxalate crystals were present, although its percentage was less, the acid insoluble ash value has been undertaken to remove variation caused by calcium oxalate.

Procedure: The ash obtained in the total ash method was taken and boiled with 25 ml of 2N hydrochloric acid for 5 min. Insoluble matter was collected on ashless filter paper and washed with hot water. The material was further ignited and weighed. Percentage of insoluble acid ash was calculated concerning air-dried material.

Water Soluble Ash: The aqueous extract of crude drug *Soymida febrifuga* A. Juss shown to have various biological activities. Therefore, the exhausted powder may be used as an adulterant for this drug. Total ash value also varies from a wide range; therefore, water-soluble ash value, a quite reliable parameter was investigated to judge such type of adulteration.

Procedure: The ash obtained from total ash was taken, boiled with 25 ml water for 5 min. All insoluble matter was collected on ashless filter paper washed with hot water and ignited for 15 min at the temperature not exceeding 450 ºC. The percentage of water-soluble ash was calculated by subtracting the weight of insoluble matter from the weight of total ash. Percentage of water-soluble ash was calculated concerning air-dried drug.

Qualitative Tests for Determination of Inorganic Elem: Total ash was prepared, as per method mentioned above and added with 50 % v/v HCl and kept for 1hour, filtered. The filtrate was taken to perform qualitative tests listed in **Table 1.**

Extractive Values: The extractives obtained by exhausting crude drugs are indicative of approximate measures of their chemical constituents. Taking into consideration the diversity in chemical nature and properties of contents of drugs, various solvents are used for the determination of extractives. The solvent used for extraction is in a position to dissolve appreciable quantities of substances desirable.

It is employed for material to which yet no suitable chemical or biological assays exist. Extracts were prepared with various solvents by standard methods. Percentage of the dry extract was calculated in terms of air-dried powder drug part. Various extractive values are indicated in **Table 6.**

TABLE 1: PHYTOCHEMICAL TESTS

S. no.	Test	Observation	Inference
1	For Aluminum		
	a) Test solution + dilute ammonium solution.	No Gelatinous precipitate, soluble in hydrochloric acid, acetic acid and sodium hydroxide Solution but nearly Insoluble in dilute ammonium solution.	Aluminum absent
	b) Test solution + a solution of ammonium sulphides	Gelatinous precipitate, soluble in hydrochloric acid, acetic acid, and sodium hydroxide Solution but nearly Insoluble in dilute ammonium solution.	Aluminum absent
2	For Chlorides		
	a)Test solution+ Magnesium dioxide + sulphuric acid.	No odors of chlorine.	Chlorides absent
	b) Test solution+ A solution of potassium iodide.	No blue color.	Chlorides absent
	c) Test solution+ A solution of silver nitrate.	A white, curdy precipitate soluble in dilute ammonia solution but insoluble in nitric acid	
3	For Copper:		
	a) Test solution + hydrogen sulphide.	No brownish-black precipitate.	Copper absent
	b) Test solution + solution of sodium hydroxide.	No light blue precipitate. No black precipitate.	Copper absent
	c) Test solution +solution of ammonium thiocyanate.		
4	For Calcium:		
	a) Test solution + solution of ammonium carbonate.	No white precipitate which after boiling and cooling is insoluble in a solution of ammonium sulphide.	Calcium absent
	b) Test solution + solution of ammonium oxalate.	No white precipitate soluble in hydrochloric acid but insoluble in acetic acid.	Calcium absent
	c) Test solution + potassium chromate.	No yellow, crystalline precipitate.	Calcium absent
	d) Test solution +solution of potassium ferrocynide.	No immediate precipitate, but on the addition of an excess of reagent in the presence of an excess	
5	For Carbonates and Bicarbonates:		
	a) Test solution + dilute hydrochloric acid.		
	b)Test solution + A solution of mercuric chloride.	Effervescence due to liberation of carbon dioxide gas.	Carbonate present
	c)Test solution + A solution of silver nitrate.	Brownish-red precipitate.	Carbonate present
6	For Iron:		
	a) Test solution + dil. HCL and solution of potassium permanganate.	Faint pink color.	Iron present
	b) Test solution + dil. HCL and solution of ammonium thiocyanate.	Blood red color.	Iron present
	c) Test solution + solution of potassium ferrocyanide.	White precipitate.	Iron present
	d)Test solution +solution of NAOH.	Dull green precipitate.	
7	For Magnesium:		
	a) Test solution +solution of ammonium carbonate, boil.	White precipitate	Magnesium present
	b) Test solution +dilute ammonia solution and solution of sodium phosphate.	White crystalline precipitate	Magnesium present
	c) Test solution +solution of sodium hydroxide.	White precipitate	Magnesium present
8	For Nitrate		
	a) Test solution + sulfuric acid and copper, warm.	No liberation of red fumes.	Nitrogen absent
	b) Test solution + solution of ferrous	No brown precipitate.	Nitrogen absent

	sulphate.	No liberation of ammonia detected by its odor	Nitrogen absent
	c) Test solution + solution of sodium hydride and zinc powder, boil.	and its action on moistened litmus paper	
9	For Phosphate		
	a) Test solution + solution of silver ammonium nitrate.	No light yellow color precipitate, readily soluble in dilute ammonia solution and cold nitric acid	Phosphates absent
	b) Test solution + magnesium ammonium sulphate.	No white crystalline precipitate	Phosphates absent
	c) Test solution + solution of ammonium molybdate and nitric acid.	No yellow precipitate	Phosphate absent
10	For Potassium		
	a) Test solution + perchloric acid.	No white crystalline precipitate	Potassium absent
	b) Test solution + solution of sodium cobalt nitrite and acetic acid.	No yellow precipitate	Potassium absent
	c) Sample moistened with hydrochloric acid and introduced on the platinum wire into the flame of Bunsen burner.	No violet color to the flame	Potassium
11	For Sodium		
	a) Test solution + solution of uranyl zinc acetate.	No yellow crystalline precipitate.	Sodium absent
	b) Sample moistened with hydrochloric acid and introduced on the platinum wire into the flame of Bunsen burner.	No yellow color to the flame.	Sodium absent
12	For sulphates		
	a) Test solution + solution of barium chloride.	A white precipitate insoluble in hydrochloric acid.	Sulphate present
	b) Test solution + solution of lead acetate.	A white precipitate soluble in a solution of	Sulphate
13	For zinc		
	a) Test solution + solution of amm. Sulphide and solution of sodium hydroxide.	No white precipitate soluble in hydrochloric acid.	Zinc absent
		No white precipitate soluble in hydrochloric acid.	Zinc absent

Water-Soluble Extractive Values: This method is applied to drugs, which contain water-soluble active constituents of crude drugs, such as tannins, sugars, plant acids, mucilage, and glycosides.

Procedure: Accurately weighed 5 gm of the powdered drug in the glass-stoppered conical flask. Macerated with 25 ml of distilled water 6 h with frequent shaking, and then allowed to stand for 18 h. After completion of 18 h filtered the contents of the flask and transferred the filtrate in a tarred flat bottom porcelain dish. The filtrate was evaporated to dryness on a water bath and dried at 105 ºC for 6 h cooled in desiccators for 30 min and weighed. Calculated content of extractable matter in milligrams per gram of air-dried material.

Alcohol-Soluble Extractive Values: Alcohol is an ideal solvent for extraction of various chemicals like tannins, resins, *etc*. Therefore.

Procedure: Accurately weighed 5 gm of powdered drug placed in the glass Stoppard conical flask and macerated with the 25 ml of ethanol (95%) for 6 h with frequent shaking, mixture allowed to stand for 18 h. After completion of 18 h, filtered rapidly taking care not to lose any solvent. Transferred the filter in the tarred flat bottom porcelain dish. The filtrate was evaporated to dryness on the water bath, dried at 105 ºC for 6 h cooled in a desiccator for 30 min and weighted. Calculate content of extractable matter in milligram per gram of air dried material.

Ether Soluble Extractive Values: Same procedure was followed as per water soluble extractive, but instead of water, pet ether was used as a solvent.

Benzene Soluble Extractive Values: Same procedure was followed as per water soluble extractive but instead of water, benzene was used as a solvent.

Chloroform-Soluble Extractive Values: Same procedure was followed as per water-soluble extractive, but instead of water, chloroform was used as a solvent.

Ethyl Acétate Soluble Extractive Values: Same procedure was followed as per water soluble extractive, but instead of water, ethyl acetate was used as a solvent.

Ethanol Soluble Extractive Values: Same procedure was followed as per water soluble extractive but instead of water, ethanol was used as the solvent.

Methanol Soluble Extractive Values: Same procedure was followed as per water soluble extractive, but instead of water, methanol was used as a solvent.

Loss on Drying: Loss on drying is the loss in weight in percent w/w resulting from loss of water and volatile matter of any kind that can be driven off under specific conditions. 2 gram of air-dried drug reduced to powder was placed in a crucible of silica. Originally the crucible was cleaned and dried, and the weight of empty dried crucible was taken. The powder was spread in a thin uniform layer. The crucible was spread in a thin uniform layer. The crucible was then placed in the oven at 105 ºC. The powder was dried for 4 h and cooled in desiccators to room temperature, and weight of the cooled crucible + powder was noted.

TABLE 2: PHYTOCHEMICAL INVESTIGATION

S. no.	Tests Performed	Observation	Inference
1	CARBOHYDRATES:		
	a) Molisch test: To the test tube add with few drops of Molisch's reagent (Alcoholic α-napthol) 2ml of conc. Sulphuric acid is added slowly from the side of the test tube.	Violet ring is formed at the junction of two liquids. Red ppt. is obtained.	Carbohydrate present. Monosaccharide present
	b) Bar ford's test: Test solution heated with Barford's reagent on a water bath.	Rose color is formed.	Hexose sugar present.
	c) Selvanoff's test: To the test, solution add crystals of resorcinol and equal volumes of concentrated HCl acid and heat on a water bath.	The red color is formed.	Pentose sugar present.
	d) Test for pentoses: To the test, solution add equal volumes of HCL acid containing a small amount of phloroglucinol and heat.	Yellow crystals forme (observe under a microscope)	Carbohydrate present.
	e) Osazone formation test: Heat the test solution with the solution of phenylhydrazine hydrochloride, sodium acetate, and acetic acid.		
2	PROTEINS: a) Heat test: Heat test solution in a boiling water bath.		
	b)Biuret test: Test solution treated with Biuret reagent (40%sodium hydroxide and dilute copper sulphate solution)	Coagulation occurs. Violet or pink color obtained.	Proteins absent. Proteins absent.
	c) Xanthoproteic test: To the test, solution add weak aqueous iodine solution. Blue color indicates the presence of starch which disappears on heating and reappears on cooling.	Ppt. Turns orange.	Proteins absent.
3	AMINO ACIDS: a) Millon's test: Treat test solution with Millon's reagent and heat on a water bath. b) Ninhydrin test: Boil test solution with Ninhydrin reagent	No white ppt. forms and on warming it gets turns to red	Amino acids absent.
	GLYCOSIDES: a) Test A: Extract 200 mg of the drug with 5 ml of dilute sulphuric acid by warming on a water bath, filter it, and neutralize the acid extract with	No purple or blue color appears	Amino acids absent.

5% solution of sodium hydroxide. Add 0.1 ml of Fehling's solution A and B until it becomes alkaline (test pH-paper) and heat on a water bath for 2 min.	Red ppt. formed compared with ppt. of test A	If ppt. in test A is greater than in test B, then glycoside may be present.
b) Test B: Repeat test A procedure by using 5 ml of water instead of dilute sulphuric acid. Note the quantity of red precipitate formed.		
c) Test for Anthraquinone glycosides: Boil the test material with 1ml of sulphuric acid for 5 minutes. Filter while hot, cool the filtrate; Shake with an equal volume of dichloromethane or chloroform. Separate the lower layer of dichloromethane or chloroform; shake it with half of its volume of dilute ammonia.		Anthraquinone glycoside is absent.
d)Modified Borntrager's test: Boil 200mg of test material with 2ml of sulfuric acid. Treat with 2 ml of 5% aqueous ferric chloride solution (freshly prepared) for 5 min. shake well with an equal volume of chloroform and continue the test as above.	No Ammonical layer shows pink color No Ammonical layer shows pink to red color	Anthraquinone glycoside is absent. Anthraquinone glycoside is absent.
e) Test for hydroxyl anthraquinones: Treat the test solution with potassium hydroxyl solution.		
f)Test for cardiac glycosides:		
i) Keddie's test: Extract the drug with chloroform, evaporate to dryness. Add one drop of 90 % alcohole and 2 drops of 2% sodium hydroxide solution.	No Red color produced	Hydroxyanthraquinones absent.
ii) Killer killani's test: Extract the drug with chloroform and evaporate it to dryness. Add 0.4 ml of Glacial acetic acid containing ferric chloride; add carefully 0.5 ml of conc. Sulphuric acid by the side tube.	The purple color is produced.	Cardiac glycosides present. Cardiac glycosides present.
iii) Baljet's test: Treat test solution with sodium picrate or picric acid.	Upper layer shows bluish green color	
iv) Legal's test: Treat test solution with pyridine made alkaline with sodium nitroprusside.	At the junction of two layers reddish brown color	Cardiac glycosides present.
g) Test for coumarins glycosides: Place a small amount of sample in the test tube and covered it with filter paper moistened with dilute sodium hydroxide solution. Place the covered test tube on a water bath for several minutes. Remove the paper and expose it to ultraviolet light.	yellow to orange color Pink to red color	Cardiac glycosides present. coumarin glycosides absent.
h)Test for cyanogenetic glycosides: Place 200mg of drug in conical flask and moistened with few drops of water (flask should be completely dry because hydrogen cyanide produced will be dissolved in the water rather than come off as gas react with paper) moisten a piece of picric acid paper with 5% aqueous sodium carbonate solution and suspended in neck of flask. Warm gently at about 37^0C.Observe the change in color.	No Yellowish green fluorescence	cyanogenetic glycosides absent.
i) Test for saponin glycosides: Froth test: Place 2	No Reddish purple color	

	ml of a solution of drug in water in the test tube, shake well.	produced.	
	FLAVONOIDS:		
	a)Shinoda test: Treat test solution with fragments of magnesium ribbon and conc. HCL acid		
	b)Alkaline reagent test: Treat test solution with sodium hydroxide solution		
	b) Alkaline reagent test Treat test solution with zinc dust and few drops of HCL.		
	ALKALOIDS:		Saponin glycosides present.
	a) Dragendorff's test: Treat test solution with Dragendorff's reagent (potassium bismuth iodide)	Persistent foam forms.	Flavonoids present.
4	b)Mayer's test: Treat test solution with Mayer's reagent (mercuric potassium iodide)	The pink color produced.	Flavonoids present
	c) Wagner's test: Treat test solution with Wagner's reagent (Iodine in potassium iodide)	Yellow coloration	Flavonoids present.
	d) Hager's test: Treat test solution with Hager's reagent (saturated picric acid solution)		
5	e) Tannic acid test: Treat test solution with Tannic acid.	Magenta red color	Alkaloids absent.
	STEROIDS AND TRITERPENOIDS:		
	a) Libermann Burchard test: Treat extract with few drops of acetic anhydride, boil, cool, add conc. Sulphuric acid from the sides of test tubes.	Orange brown ppt	Alkaloids absent
	b)Salkowski test: Treat extract with few drops of conc. Sulphuric acid	Cream colored ppt occurs.	Alkaloids absent
	c) Sulfur powder test: Add a small amount of sulfur powder to test solution.	Reddish brown ppt	Alkaloids absent.
	TANNINS AND PHENOLICS:		Alkaloids absent.
	a) Ferric chloride test: Treat test solution with a few drops of 5% Ferric chloride solution.	Yellow ppt	
	b) Gelatin test: To test solution, add 1% gelatin solution containing 10% sodium chloride.	Buff colored ppt.	
6	c) Lead acetate test: Treat test solution with a few drops of 10% Lead acetate solution.		Steroids present.
		First red then blue and finally green color produced.	Triterpenoids present.
	MUCILAGE:		
	a) Ruthenium red test: Treat sample with Ruthenium red solution.	Chloroform layer appears red, and acid layers show greenish yellow fluorescence	Steroids present.
	GUMS:		Triterpenoids present.
	a) Treat sample with dil. HCl acid and then perform Fehling's or Benedict's test.	Sulfur sinks	Steroids present.
7	FIXED OILS:		
	Press sample on filter paper.		Hydrolyzable tannins present.
	VOLATILE OILS:	Deep blue color	
	Press sample on filter paper.	Green color.	
			Condensed tannins present.
		White ppt	
			Tannins present.

		White ppt No Red color produced.	Mucilage absent.
8		No Red color develops	Gums absent.
9		A permanent mark on filter paper.	Fixed oils present.
10		No Permanent mark on filter paper	No Volatile oils present.

TABLE 3: ASH VALUE

Types of ash values	% w/w
1) Total Ash	13.78%
2) Acid-insoluble ash	08.82%
3) Water-soluble Ash	18.16%
4) moisture content	06.98%

TABLE 4: INORGANIC CONSTITUENTS AND THEIR PRESENCE

S. no.	Test for Inorganic Elements	Inference
1	Calcium	-
2	Magnesium	+
3	Sodium	-
4	Potassium	-
5	Iron	+
6	Sulphate	+
7	Phosphate	-
8	Chloride	-
9	Carbonate	+
10	Nitrates	-

TABLE 5: EXTRACTIVE VALUE OF DIFFERENT SOLVENTS, PERCENTAGE, EXTRACTABILITY AND COLOR OF EXTRACT

Type of solvent	Extractive value	Colour of extract		consistency
		Day light	Under UV	
Pet. Ether	1.081%	Greenish	Greenish brown	Sticky
Benzene	0.828%	Greenish black	Black	Semisolid
Chloroform	5.172%	Brownish	Black	Semisolid
Ethyl acetate	1.224%	Brownish	Black	Sticky
Ethanol	3.121%	Brownish	Black	Semisolid
Methanol	4.641%	Brownish	Black	Semisolid
Water	3.256%	Brownish	Black	Dry

TABLE 6: PHYTOCHEMICALS INVESTIGATION

S. no.	Chemical tests performed	Pet ether	chloroform	methanol	Water	Total aqueous
1	Carbohydrates	-	-	-	+	+
2	Proteins	-	-	-	-	-
3	Amino Acids	-	-	-	-	-
4	Glycosides					
	I)Anthraquinone	-	-	-	-	-
	II)Cardiac	-	+	+	-	-
	III)Coumarins	-	-	-	-	-
	IV)Cyanogenetic	-	-	-	-	-
5	Saponin Glycosides	-	-	+	+	+
6	Alkaloids:	-	-	-	-	-
7	Flavonoids:	-	-	+	+	+
8	Steroids And Triterpenoids:	+	+	-	-	-
9	Tannins And Phenolics	+	+	+	+	+
10	Mucilage:	-	-	-	-	-

11	Gums:	-	-	-	-	-
12	Fixed Oils	+	+	-	-	-
13	Volatile Oils	-	-	-	-	-

+ indicates present and – indicates absent

CONCLUSION: In the present study it element detection results show the presence of magnesium, iron, sulphates, and carbonates. The phytochemical evaluation result of *Soymida febrifuga* leaves revealed the presence of Carbohydrates, cardiac glycosides, Saponin glycosides, flavonoids, steroids, triterpenoids, tannins, phenolics, and fixed oil.

The plant is blessed with immense potent activities in combining different types of diseases the requirement is to explore it the most for its active constituents and furthermore regarding its mode of action and structural analysis so that a better and more advanced formulation can be prepared for the mainstream administration of the drug.

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CONFLICT OF INTEREST: Nil

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