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PRELIMINARY PHYSICO-PHYTOCHEMICAL STUDY OF STEM BARK OF *ALSTONIA SCHOLARIS* (L.) R. BR. – A MEDICINAL PLANT

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ABSTRACT

Alstonia scholaris (L.) R. Br. is a plant of family Apocynaceae and has a great medicinal importance. It is widely used by tribal people to treat various diseases and ailments. The present communication deals with the organoleptic and preliminary physico-phytochemical studies of the stem bark of the plant. The organoleptic study was done according to the W.H.O. guidelines for medicinal plants. The physicochemical parameters viz. loss on drying, ash values and extractive values were calculated. Soxhlet extraction of the powdered plant material was done with 80% ethanol and then partitioned with petroleum ether, ethyl acetate and n-butanol subsequently. The percentage yields of different solvent fractions were calculated and then they were subjected to preliminary phytochemical screening which indicated the presence of alkaloids, carbohydrates, tannins, terpenoids, saponins, flavonoids, steroids and fixed oils and fats.

INTRODUCTION: Medicinal plants have been used for years in daily life to treat diseases all over the world. Interest in medicinal plants reflects the recognition of the validity of many traditional claims regarding the value of natural products in healthcare [1]. Numerous useful drugs have been discovered from higher plants by following up ethnomedical practices [2]. *Alstonia scholaris* (L.) R. Br. is an important medicinal plant in folklore medicine. The plant belongs to family Apocynaceae and is native to India. It grows throughout India, in deciduous and ever-green forests and in plains. Juice of leaves and tincture of the bark acts as a powerful galactogogue and also used in cases of snake bite. Milky juice of the plant is applied on wounds and ulcers. The bark is bitter, acrid, astringent, digestive, laxative, thermogenic, antipyretic, galactogogue, cardiotonic and tonic. It is useful in abdominal disorders, fevers, leprosy, skin diseases, chronic and foul ulcers, asthma, bronchitis and helminthiasis [3]. The bark extract induces the cellular immune response at low doses and inhibited the delayed type of hypersensitivity reaction at high doses [4]. The methanolic extract of this plant exhibits pronounced antiplasmodial activity [5]. The alkaloid fraction of *A. scholaris* was found to have potential anticancer agent [6]. It's bark extract showed chemopreventive potential against skin tumorigenesis in swiss albino mice [7]. Ethanolic extracts of *A. scholaris* possess powerful *in-vitro* antioxidant activity [8].

The plant possesses valuable medicinal properties but most of the advantages are still confined to tribal areas because of raw knowledge and absence of proper scientific standardization. For the useful application of the plant parts in modern medicine, physico-chemical

and phytochemical standardization is very important so that the medical benefits of the plant may be used properly and scientifically and reach to the larger populations of the world.

MATERIALS AND METHODS: The stem bark of the plant *Alstonia scholaris* (L.) R. Br. was collected by scrapping the trunk from the local area of Vidisha District of Madhya Pradesh, India. The plant was identified by Taxonomist, S. S. L. Jain P. G. College, Vidisha (M. P.). After proper identification, voucher specimens were prepared and deposited in the herbarium of Pest Control and Ayurvedic Drug Research Laboratory of S. S. L. Jain P. G. College, Vidisha, (M. P.).

Organoleptic Evaluation: The freshly (just after collection) peeled stem bark of the plant was spread on a clean dry plastic sheet and investigated different organoleptic features by repeated observations using magnifying glass and ruler (where required) and recorded according to the guidelines of W.H.O. for medicinal plants.

Physico-chemical Properties of Plant Material: Physicochemical properties of the plant material were studied according to the guidelines of WHO for medicinal plants [9].

1. **Loss on Drying:** Fresh plant material was granulated by using pestle and mortar so that the thickness of plant part was less than 3 mm. 25 g of air dried plant material was placed in an oven at 100^{o} C. The material was dried until two consecutive weighing did not differ by more than 5 mg. After the procedure, the plant material was removed from the oven and weighed accurately by using electronic balance. The percentage of loss on drying was calculated.

2. **Ash Values**

   A. **Total Ash:** 3 gm of accurately weighed ground plant material was incinerated in a tarred silica dish at a temperature not exceeding 450^{o}C until it became free from carbon then it was cooled and weighed. The percentage of total ash was calculated with reference to the air-dried plant material.

   B. **Water-Soluble Ash:** 25 ml of water was added to the crucible containing the total ash and boiled for 5 minutes. The insoluble matter was collected on an ashless filter paper, washed with hot water and ignited in a crucible for 15 minutes at a temperature not exceeding 450^{o}C. The weight of this residue was subtracted from the weight of total ash. The percentage of water soluble ash was calculated with reference to the air-dried plant material.

   C. **Acid-Insoluble Ash:** 25 ml of hydrochloric acid was added to the crucible containing total ash. The insoluble matter was collected on an ashless filter paper and washed with hot water until the filtrate became neutral. The filter paper containing the insoluble matter was transferred to the original crucible, dried on a hot plate and ignited to constant weight. The residue was cooled in a desiccator for 30 minutes and weighed without delay. The percentage of acid-insoluble ash was calculated with reference to the air-dried plant material.

   D. **Sulphated Ash:** The silica crucible was heated to redness for 10 minutes. Allowed to cool in a desiccators and weighed. Put 2 gm of accurately weighed substance into the crucible, ignited gently at first, until the substance was thoroughly charred. It was cooled and moistened with 1 ml. of sulphuric acid, heated gently until white fumes were no longer evolved. Then ignited it at 800^{o}C until all black particles disappeared. Allowed the crucible to cool, added a few drops of sulphuric acid and heated. Ignited as before, allowed to cool and weighed. Repeated the operation until two successive weighing did not differ by more than 0.5 mg.

3. **Alcohol-Soluble Extractive and Water-Soluble Extractive:** 5 gm of the air dried and coarsely powdered plant material was macerated with 100 ml of ethanol in a closed flask for 24 hours, shaked frequently during 6 hours and allowed to stand for 18 hours. The mixture was filtered rapidly. 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish and dried at 105^{o}C, to constant weight and weighed. The percentage of alcohol-soluble extractive was calculated with

reference to the air-dried plant material. For the determination of water-soluble extractive, the same procedure had been followed using chloroform-water instead of ethanol.

Extraction by Soxhlet Method: The stem bark of *A. scholaris* was collected, washed with running tap water, shade-dried at room temperature and grounded in a manual mill to get a coarse powder of 60 mesh. Powdered plant materials of *A. scholaris* was extracted with 80% ethanol in a soxhlet apparatus at 40^{o}C. Extraction was done with solvent until the supernatant in the soxhlet apparatus became transparent (for 48 hours). The extracts were filtered through a Buchner funnel with Whatman filter paper no. 1. The filtrate was evaporated to dryness under reduced pressure using rotary evaporator at 40^{o}C. The crude extract was stored at 4^{o}C in airtight bottles until further use [10].

The ethanol crude extract of the plant was dissolved in water and partitioned subsequently with equal volumes of petroleum ether (60-80^{o}C), ethyl acetate and n-butanol. All solvents used were of analytical grade (Merck, Germany). All the extracts were filtered through Whatman filter paper no. 1, evaporated to dryness and percentage yields were calculated.

Preliminary Phytochemical Screening: The petroleum ether, ethyl acetate and n-butanol fractions were subjected to phytochemical screening for the presence of alkaloids, carbohydrates, tannins, terpenoids, saponins, flavonoids, steroids and fixed oils and fats according to the standard procedure [11, 12].

Tests for Alkaloids:

A. Dragendorff Test: 0.5 g of the extract was stirred with 5 ml of 1% aqueous hydrochloric acid on a water bath and filtered. 3 ml of the filtrate was divided into 3 parts. To the first 1 ml, few drops of freshly prepared Dragendorff reagent was added. An orange to brown precipitate indicates the presence of alkaloids.

B. **Mayer's Test:** To the second part, 1 drop of Mayer reagent was added. White to yellow or cream colour precipitate indicates the presence of alkaloids.

C. **Wagner's Test:** To the third part, Wagner reagent was added. Formation of brown or red or reddish-brown precipitate indicates the presence of alkaloids.

Tests for Carbohydrates:

A. **Molisch's Test:** The extract was treated with few drops of alcoholic alpha napthol Solution. Then 0.2 ml of concentrated sulphuric acid was added slowly through the sides of the test tube. A purple to violet colour ring appears at the junction indicates the presence of carbohydrates.

B. **Benedict's Test:** The extract was treated with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and upon boiling on water bath. Presence of reddish brown precipitate indicates the presence of reducing sugars.

C. **Fehling's Test:** Equal volume of Fehling's A (Copper sulphate in distilled water) and Fehling's B (Potassium tartarate and Sodium hydroxide in distilled water) reagents are mixed and few drops are added to the extract and boiled. A brick red precipitate of cuprous oxide forms, if reducing sugars are present.

Test for Tannins:

A. Ferric chloride Test: 0.5 g of extract was stirred with 10 ml of distilled water and then filtered. Few drops of 1% ferric chloride solution were added to 2 ml of the filtrate. Occurrence of a blue-black, green or blue-green precipitate indicates the presence of tannins.

Test for Terpenoids:

A. **Salkowski Test:** Five ml of extract was mixed in 2 ml of chloroform. Concentrated sulphuric acid (3ml) was carefully added to form a layer. A reddish brown coloration of the interface indicates presence of terpenoids.

Test for Saponins:

A. **Frothing Test:** 0.5 gm of the extract was shaken with distilled water in a test tube. Frothing which persists for 15 minutes indicates the presence of saponins.

Tests for Flavonoids:

A. **Shinoda Test:** Three pieces of magnesium chips were added to an alcoholic solution of the extract. Then few drops of concentrated hydrochloric acid was added. Appearance of an orange, pink or red to purple colour indicates the presence of flavonoids.

B. **Ferric Chloride Test:** The extract was boiled with water and filtered. Two drops of freshly prepared 10% ferric chloride solution was added to 2 ml of filtrate. Green, blue or violet colour indicates the presence of phenolic hydroxyl group.

C. **Sodium Hydroxide Test:** 2 ml of alcoholic solution of extract was dissolved in 10% aqueous sodium hydroxide solution and filtered to give yellow colour. Change in colour from yellow to colourless on addition of dilute hydrochloric acid indicates the presence of flavonoids.

D. **Lead Acetate Test:** 10% lead acetate solution was added to the aqueous solution of the extract. Yellow precipitate indicates the presence of flavonoids.

Test for Steroids:

A. **Liebermann Burchard Test:** 2 ml of diluted sulphuric acid was added to 0.5 g of crude extract dissolved in 2 ml of acetic anhydride. Formation of a blue or green solution indicates the presence of steroids.

Test for Fixed Oils and Fats:

A. **Spot Test:** A small quantity of extract was pressed between two filter papers. Oil stains on the paper indicates the presence of fixed oils and fats.

RESULTS AND DISCUSSION: The organoleptic studies were done in fresh and moist stem bark strips of the plant *A. scholaris* as mentioned in **Table 1**. The outer surface of the bark was grayish brown in colour while the inner surface was yellowish brown. No specific odour was present in the bark. Surface was rough, spongy, unevenly fissured and exude milky sap when cut. Fracture was short and smooth. These characteristics are in agreement of some previous literature about the plant.

TABLE 1: ORGANOLEPTIC EVALUATION OF PLANT MATERIAL

Features	Observation
Condition	Moist
Shape of Pieces	Flat Strips
Dimensions	8-14 cm long, 1-3 cm wide, 0.2-0.5 cm thick
Colour	Outer surface Grayish Brown, inner surface yellowish brown
Odour	No odour
Surface Texture	Rough, spongy, uneven fissured and exuding milky sap when cut
Fracture	Short and Smooth

The physicochemical parameters are given in **Table 2**. The percentage of loss on drying, total ash, water-soluble ash, acid- insoluble ash and sulfated ash are 5.5%, 6.8%, 2.98%, 1.12% and 6.78% respectively. The values of water soluble extractive and alcohol soluble extractive are 15.74% and 8.4% respectively.

TABLE 2: PHYSICOCHEMICAL PROPERTIES OF *A. SCHOLARIS* STEM BARK

Physicochemical Parameter	Value % (w/w)
Loss on Drying	5.5
Total Ash	6.8
Water-soluble Ash	2.98
Acid-insoluble Ash	1.12
Sulfated Ash	6.78
Water Soluble Extractive	15.74
Alcohol Soluble Extractive	8.4

The percentage yields in petroleum ether, ethyl acetate and n-butanol fractions are 1.87, 4.62 and 8.21 respectively as mentioned in **Table 3**. The yield is maximum in n-butanol fraction followed by ethyl acetate fraction and least in petroleum ether fraction which shows that polar phytoconstituents are present in much larger quantities in the stem bark of *A. scholaris*.

TABLE 3: PERCENTAGE YIELD OF *A. SCHOLARIS* STEM BARK EXTRACTS

Solvent	Colour	Consistency	Percentage Yield (w/w)
Petroleum Ether (40-60^{0}C)	Light Yellow	Sticky	1.87
Ethyl acetate	Brown	Nonsticky	4.62
n-Butanol	Brown	Nonsticky	8.21

The results of qualitative phytochemical screening of petroleum ether, ethyl acetate and n-butanol fractions of stem bark of *Alstonia scholaris* revealed the presence of alkaloids, carbohydrates, tannins, terpenoids, saponins, flavonoids, steroids and fixed oils and fats in the stem bark as mentioned in **Table 4**.

Alkaloids showed strong presence in the stem bark extracts. Khyade and Vaikos, 2009 reported the presence of alkaloids, flavonoids, reducing sugars, simple phenolics, steroids, saponins and tannins in the leaves of the same plant [13].

TABLE 4: PRELIMINARY PHYTOCHEMICAL SCREENING OF *A. SCHOLARIS* STEM BARK

Group	Phytochemical Tests	Petroleum Ether Extract	Ethyl Acetate Extract	Methanol Extract
Alkaloids:	Dragendorff Test	-	+++	+++
	Mayer's Test	-	+++	+++
	Wagner's Test	-	+++	++
Carbohydrates:	Molisch's Test	-	++	+++
	Benedict's Test	-	++	++
	Fehling's Test	-	++	++
Tannins:	Ferric chloride Test	-	+	++
Terpenoids:	Salkowski Test	+++	+	-
Saponins:	Frothing Test	-	+	+++
Flavonoids:	Shinoda Test	-	++	++
	Ferric chloride Test	-	++	++
	Sodium hydroxide Test	-	++	+
	Lead acetate Test	-	++	++
Steroids:	Liebermann Burchard Test	++	-	-
Fixed Oils and Fats:	Spot Test	++	+	-

(+++) - Strong Presence, (++)- Moderate presence, (+) -Weak presence, (-) - Absence of phytoconstituent by the phytochemical test.

Standardization of herbal drugs is a topic of great concern because of the great variability derived from heterogeneous sources. *Alstonia scholaris* (L.) R. Br. is a plant with large medicinal advantages and it's stem bark plays a major role in these medicinal properties. Thus the present study will provide a referential information for correct identification and standardization of this plant material.

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