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Pharmacognostic, Phytochemical and study of different parts (Leaves, flowers, and bark) of the plant *Alstonia scholaris* Linn R. Br.

Saurabh Sharma [1] and Swapnil Goyal [2]

1. Ph.D Research Scholar, Faculty of Pharmacy, Mandsaur University, Mandsaur (M.P.) India, Email address: medilabujn@gmail.com, Mobile No. 8319097294.

2. Faculty of Pharmacy, Mandsaur University, Mandsaur (M.P.) India, Email address: swapnilmip@gmail.com, Mobile No. 9907860790.

Abstract:

Natural sources especially herbal drugs are a reliable source of treatment for various ailments in ancient to present times. *Alstonia scholaris* Linn R. Br. belongs to the family Apocynaceae also known as the devil's tree it is used in the treatment of many human diseases. Traditional knowledge related to *Alstonia scholaris* is useful in the treatment of malaria, abdominal disorders, dyspepsia, skin diseases, leprosy, chronic and foul ulcers, tumors, asthma, bronchitis, agalactia, helminthiasis, and various infirmities. The present research work is based on the Pharmacognostic and Phytochemical evaluation of different parts (Leaves, flowers, and bark) of the plant *Alstonia scholaris* Linn. R. Br. Important diagnostic and microscopic characteristics including phloem fibers, stone cells, and calcium oxalate crystals are found. Performed the successive extract of the different parts of the plant eg. (Leaves, flowers, and bark) and preliminary phytochemicals screening. Phytochemical screening results confirm the presence of different primary and secondary metabolites like alkaloids, glycosides, tannins etc. The study will provide referential initial information for the correct identification of the plant.

Keywords: *Alstonia scholaris*, Microscopic characters, Successive extraction, Phytochemicals screening.

1. Introduction

Medicinal Plants are a reliable source of drug discovery and development, in the past decade, scientists are more focused on this field. Phytoconstituents-based formulations showed advantages over synthetic medicaments like eco-friendly, non-hazardous, and fewer side effects. The acceptance of these formulations is increasing day by day.

Alstonia scholaris Linn. R. Br. belongs to the family Apocynaceae and has been used in traditional systems of medicine for treating various diseases. Different parts of the plants were used in the treatment of several diseases in ancient times. [(1)] Leaves of *Alstonia scholaris* contain very high amounts of alkaloids which can be extracted as a source of natural fungicide and bactericidal action. The ripe fruits of the plant are used in the treatment of syphilis and epilepsy. It is also used as a tonic, antiperiodic, and anthelmintic.[(2)] The milky juice of *Alstonia scholaris* has been applied to treat ulcers.[(3)] The bark is the most intensively used part of the plant and is used in many compound herbal formulas.[(4)] It is a bitter tonic, alternative, and febrifuge and is reported to be useful in the treatment of malaria, diarrhoea, and dysentery. Recently, the leaf extract has also been found to own antimicrobial properties.[(5)] *Alstonia scholaris* has also been reported to inhibit liver injuries induced by carbon tetrachloride, beta-d-galactosamine, acetaminophen, and ethanol as remarked by the reduced elevation of levels of serum transaminases and histopathologic changes such as cell necrosis and inflammatory cell infiltration.[(6)] Screening of natural products from plants provides the chance to discover new molecules of unique structure with high activity and selectivity**.** [(7)]

The aim of the present research is to perform the systematic study of the medicinal plant *Alstonia scholaris*, on the basis of their ethnobotanical data. First authenticate the plant parts (Leaves, flower, and bark) performed the powder microscopy of all three parts, successive extraction on these plant parts in respect of the different polarity solvents eg. (Petroleum ether, chloroform, methanol, and distilled water). After the extraction performed the phytochemical analysis of different parts of plant extracts.

2. Material and Method

2.1 Collection of plant parts Leaves, Flowers, and Barks.

The leaves of *Alstonia scholaris* were collected when flowers are just beginning to expand or the flowering is just arriving at its height after the rainy season. The collection should be done in dry weather since leaves collected in wet weather deteriorate in quality and may become discolored during drying.

Flowers of *Alstonia scholaris* are collected just before they are fully expanded in dry weather in the month of October and November because petals that are damp when gathered become badly discolored during drying.

Barks of *Alstonia scholaris* were collected in spring or early summer when the cambium is active as it is easy to separate them from wood in this season by the coppicing method. [(8)]

2.1.1 Reagents and chemicals

All the reagents and chemicals used in the present work were procured from M/s Merck India, Ltd. Bombay.

2.2 Authentication of selected plant material

The plant parts bark leaves and flowers of *Alstonia scholaris* were collected from the botanical garden of Ujjain (M.P.). The sample of the drug was also identified & authenticated by the botanist Department of Botany, Vikram University Ujjain (M.P.).

2.3 Pharmacognostic Studies

2.3.1 Physicochemical Parameters

The following physicochemical parameters were obtained using established techniques outlined in the Ayurvedic Pharmacopoeia of India and WHO guidelines standards.

2.3.2 Macroscopic Studies

In macroscopic studies, we focused on the organoleptic evaluation properties of crude drugs, without considering the activities (or events) happening at the molecular level in the crude drugs. In this approach, we determine the properties (e.g. shape, size, color, odour, taste, base, texture, margin, apex, venation, arrangement, of leaves and stem) of plants that were observed.[9]

2.3.3 Microscopic Studies

Macroscopic and microscopic characteristics of crude drugs are used as diagnostic features of medicinal plants. These studies are important tools for the quality control of medicinal plants. In this study photographs from different magnifications were taken. Microscopic studies were carried out by preparing thin sections of plant parts. The thin sections were further washed with water and decolorizing agents, stained with safranin, fast green, and mounted in glycerine for observation and to confirm its magnification of different capacities lenses of Microscopes (10x, 40x). The powder microscopic studies of crude drugs are also carried out to know the specific diagnostic characteristics of crude drugs.[10]

2.3.4 Qualitative Phytochemical Investigation

Medicinal plants contain different types of phytoconstituents and they are used for the treatment of different diseases and ailments. The medicinal properties of the plants are based on the presence of secondary metabolites [11]. Some of the important phytochemicals include Carbohydrates, amino acids, alkaloids, flavonoids, phenolics, tannins, saponins, steroids, glycosides, terpenes, etc. which are present in different parts of the plants[12]. Medicinal plants are a unique source of structures of high phytochemical diversity representing phenolics (45%), terpenoids and steroids (27%), and alkaloids (18%) as major groups of phytochemicals [13]. The present research work reveals the investigation and identification of the maximum possible phytoconstituents present in the different plant extracts.

3. Results and Discussion

3.1 Physicochemical Parameters

The following physicochemical characteristics were determined: total ash, acid-insoluble ash, 90% alcohol-soluble extractive, and water-soluble extractive. These were determined by following standard protocols specified in the official Ayurvedic Pharmacopoeia of India and WHO guidelines. [14,15].

3.2 Macroscopic studies

The macroscopic characters of different parts of the plant are summarized as follows.

Leaves in a bunch of 5-7, spreading, very shortly stalked 4-8 inch long oblong-lanceolate or obviate bluntly acuminate, dark green color, bitter taste.

Flowers are greenish white small shortly stalked and fragment, in small clusters, combined into compact long peduncles and rounded paniculate cymes, 2.5-5 cm of which are arranged in large stalked umbels.

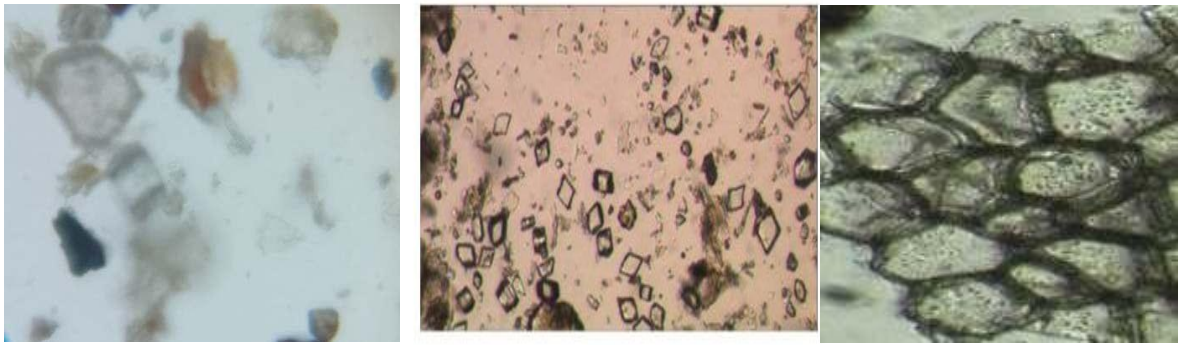
Bark is irregular more or less twisted pieces, with a spongy texture, and varying usually in thickness from about 1/8 to ½ an inch, it breaks easily with a short coarse fracture. The external surface is very rough and uneven, and grey or brownish. The inner surface is bright buff color, with a bitter taste, being neither aromatic nor acrid.[16,17].

3.3 Powder microscopy of selected plant part

A). Bark

Powder microscopic description: wood color powder showing abundant groups of stone cells of various sizes, shapes, and thicknesses with distinct radiating pits and striations. Plenty of sclereids with highly thickened and striated walls of various shapes and sizes, abundant prismatic crystals along with hexagonal to pentagonal beaded cork cells.[18]

Fig.1 Powder Microscopy of Bark of *Alstonia scholaris*



B). Leaf

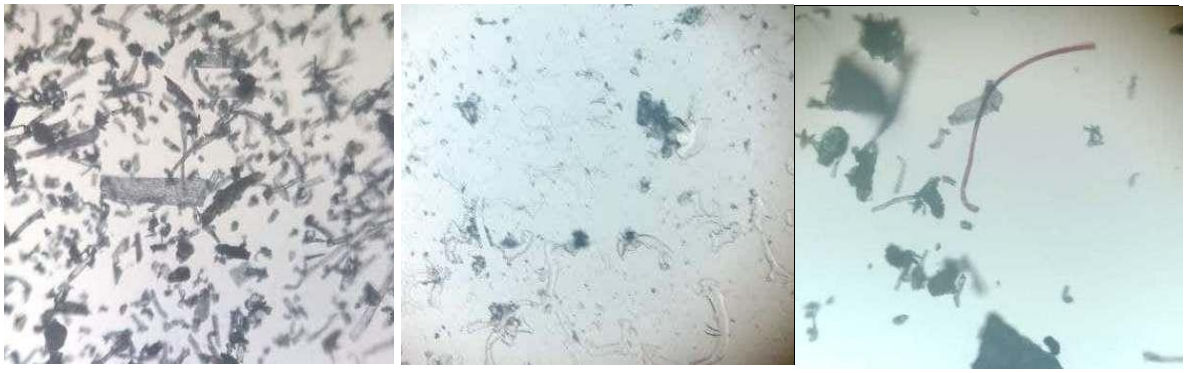
Powder microscopic description: The cluster crystals of calcium oxalate, which are found scattered and cells of spongy mesophyll. The lignified fibers of the pericycle of veins. The palisade cells are seen under the clusters and the calcium oxalate crystals are present in the cluster.[19]

Fig. 2 Powder Microscopy of Leaf of *Alstonia scholaris*

C). Flower

Powder microscopic description: The very abundant fragments of the corollas. The covering and glandular trichomes and pollen grains are present. The abundant fragments of the filaments and anthers of the stamens are also shown. [20,21]

Fig.3 Powder Microscopy of Flower of *Alstonia scholaris*



3.4 Successive extraction of selected plant part

First, collect the plant parts washed thrice with water remove the foreign substances, and shade dry. After the drying crude drug makes coarse powdered with the help of a grinder. The coarse powder of the flower *Alstonia scholaris* was extracted with different polarity solvents like petroleum ether, ethyl acetate, chloroform, methanol, ethanol, and distilled water.[22]

Table No.1 Physicochemical evaluation of Alstonia scholaris

		Physicochemical evaluation of *Alstonia scholaris* Leaf	**Physicochemical evaluation of *Alstonia scholaris* Bark**	**Physicochemical evaluation of *Alstonia scholaris* Flower**
Sr. No.	**Parameters**	**Result (in w/w %)**	**Result (in w/w %)**	**Result (in w/w %)**
1	Loss on Drying	3.20	4.70	3.58
2	Total Ash	4.20	5.02	4.10
3	Acid insoluble ash	0.64	0.72	0.43
4	90% Alcohol soluble extractive	8.22	9.32	10.52
5	Water soluble extractive	18.28	12.74	16.38

Table No.2: Estimation of % yield of various extracts of different parts of the plant

Sr. No.	**Plant Part**	**Parameters**			
		Solvent	**Nature**	**Color**	**% Yield in gm**
1	**Leaf**	Petroleum ether	Semi-solid	Greenish	1.8
		Chloroform	Crystals	Green to light green	1.25
		Methanol	Semi-solid	Brownish	1.20
		Distill water	solid	Brownish to green	6.29
2	**Bark**	Petroleum ether	Semi-solid	Brownish	1.1
		Chloroform	Crystals	Light Brownish	0.98
		Methanol	Semi-solid	Brownish	1.12
		Distill water	solid	Brownish	4.80
3	**Flower**	Petroleum ether	Semi-solid	Greenish to light green	1.2
		Chloroform	Solid crystals	Green to light green	0.84
		Methanol	Semi-solid	Brownish	0.91
		Distill water	solid	Brownish	4.02

3.5 Qualitative Phytochemicals Screening

The extracts obtained as above are then subjected to qualitative tests for the identification of different Phytoconstituents present in it mentioned in Tables 3, 4, 5.

Table No. 3: Phytochemicals analysis of bark extracts of *Alstonia scholaris.*

Sr. No.	Analytical Parameters	Petroleum Ether	Chloroform Extract	Methanol Extract	Aqueous Extract
1	Flavonoids	-	+	+	+
2	Alkaloids	-	-	++	+++
3	Tannin	-	+	-	+++
4	Protein	-	+	-	+++
5	Saponin	-	+	-	+
6	Glycosides	-	+	++	+
7	Phenols	-	+	++	+++
8	Thiols	-	-	++	-
9	Steroids	++	-	++	++
10	Carbohydrate	-	+	++	+++
11	Volatile oil	+	+	-	_-

Table No. 4: Phytochemicals analysis of leaf extracts of *Alstonia scholaris.*

Sr. No.	Analytical Parameters	Petroleum Ether	Chloroform Extract	Methanol Extract	Aqueous Extract
1	Flavonoids	-	+	+	+
2	Alkaloids	-	-	++	+++
3	Tannin	-	+	++	+++
4	Protein	-	+	++	++
5	Saponin	-	+	-	+
6	Glycosides	-	+	+	+
7	Phenols	-	+	+	++
8	Thiols	-	-	-	-
9	Steroids	++	-	++	++
10	Carbohydrate	-	+	+	++
11	Volatile oil	++	+	-	-

Table No. 5: Phytochemicals analysis of flower extracts of *Alstonia scholaris.*

Sr. No.	Analytical Parameters	Petroleum Ether	Chloroform Extract	Methanol Extract	Aqueous Extract
1	Flavonoids	-	+	++	+
2	Alkaloids	-	-	++	+++
3	Tannin	-	+	++	++
4	Protein	-	+	++	++
5	Saponin	-	+	+	+
6	Glycosides	-	+	+	+
7	Phenols	-	+	++	++
8	Thiols	-	-	-	-
9	Steroids	++	-	++	++
10	Carbohydrate	-	+	+	++
11	Volatile oil	+++	++	-	-

Results of the tests indicate very intense (+++), intense (++), Weak (+), or negative (-) reactions against, the particular crude drug sample in the respected solvent.

The value of all the different physiochemical determinations is summarized in Table 1. The results of the high value of water-soluble extractive value show the yielding of a higher quantity of phytoconstituents present in it rather than alcohol soluble extractive. The Ash value of a crude drug shows an idea of the inorganic matter composition and other impurities present along with the crude drug. The total ash value is more than acid-insoluble ash. Loss on drying of the powdered different parts of the plant revealed the presence of 4.70 % higher in the bark other than a flower 3.58 and leaves 3.20. The total estimation of the percentage yield of various extracts in different solvents is also mentioned in Table. 2.

Qualitative studies like a phytochemical screening of the bark, leaf, and flower parts of *Alstonia scholaris* reveal the presence of different phytoconstituents like (alkaloids, glycosides tannin, etc) in different solvent extracts of petroleum ether, chloroform, methanol, and aqueous extract are shown in Table 3,4, and 5.

Conclusion:

With the advancement in the field of healthcare, there is ample opportunity in the field of the Herbal drug industry. The ethnomedicinal uses of *Alstonia scholaris* prove that the well-known plant used in various traditional and folklore medicine and the wide distribution and richness of the Phytoconstituents are present in it, *Alstonia scholaris* can be of immense help in the discovery of new prototypic drugs and nanotechnology based new formulations for their better pharmacological actions. Pharmacological studies conducted on *Alstonia scholaris* indicate the immense potential of this plant with antimicrobial, immunomodulatory, adaptogenic, antistress, antimutagenic, antitumor, and anticarcinogenic properties.

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