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Preliminary Phytochemical Screening and Proximate Analysis of the trunk bark of *Alstonia scholaris* (L.) R.Br.

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Alstonia scholaris (L.) R.Br. (Apocynaceae), called satiyana in Assamese, is an evergreen tropical tree native to the Indian sub-continent and South East Asia. A. scholaris has a rough grayish bark and a milky sap rich in poisonous alkaloids. The tree is traditionally used by many ethnic groups of North-East India and elsewhere as a remedy for various ailments. Scientific standardization and pharmaco-botanical parameters for the trunk bark is limited; hence this study, which attempts to both analyze the phytochemical composition and establish a physicochemical standard. This information will be helpful in controlling quality parameters for the development of drugs

Keyword: *Alstonia Scholaris*, Trunk Bark, Phytochemical Analysis, Physicochemical Standard, Quality Control.

1. Introduction

Alstonia scholaris (L.) R.Br. belongs to the family Apocynaceae, is an evergreen tropical tree native to Indian sub-continent and South East Asia. It is called satiyana in Assamese having grayish rough bark and milky sap rich in poisonous alkaloid. [1,2] The bark also called dita bark is traditionally used by many ethnic group of North-East India and also other part of the world as a source of remedy against bacterial infection, malarial fever, toothache, rheumatism, snake bite, dysentery, bowl disorder asthma etc. And latex is used in treating coughs, through sores and fever.[3,4,5] In modern pharmaceutical, *Alstonia scholaris* is well studied for various activities viz., antimicrobial, antiamoebic, antidiarrhoeal, antiplasmodial, hepatoprotective, immunomodulatory, anticancer, antiasthmatic, free radical scavenging, antioxidant, analgesic, anti-inflammatory, antiulcer, antifertility and wound healing[6].

Several groups of Phytoconstituents have been isolated, characterized and formulated as drug from the bark, leaf and root. However, regarding scientific standardization or pharmaco-botanical parameter very few reports are available. The present work therefore, attempts to report phytochemical composition using different solvent and establishment of physicochemical standard of the trunk bark of *Alstonia scholaris*.

2. Materials and Methods

2.1 Plant Material

Healthy and disease free plant was selected in the Botanical garden of Dibrugarh University,

Dibrugarh, Assam (India). From the selected plant bark was collected by scrapping the trunk using neat and clean knife during the month of June 2008 and collected material was dried in shade and grinded in mechanical grinder to make course powder. The course powder was stored in plastic bags at room temperature and low humidity condition.

2.2 Preparation of Extracts[7]

The powdered plant material was successively extracted with Petroleum ether, Benzene, Chloroform, Methanol (Soxhlet extraction) and water (Cold maceration). Powdered plant material (100 gm.) was macerated overnight with 400 ml Petroleum Ether in Soxhlet apparatus and then continuously extracted at 45±5^0 C for 6 hours. The extract was shacked and filtered using Whatman No1 filter paper and Petroleum Ether was distilled off at 45±5^0 C and extract was made concentrated by heating on water bath for 1 hour and stored in sealed container at 4^0 C. The marc obtain after Petroleum ether extraction was successively extracted with Benzene, Chloroform, Methanol using Soxhlet apparatus and finally the marc was macerated with distilled water for 24 hrs. at room temperature with frequent shaking. This extract was also filtered and concentrated and stored. The extracts were named as Petroleum Ether extract (PEE), Benzene extract (BE), Chloroform extract (CE), Methanol extract (ME), and aqueous residual extract (ARE).

The powdered plant material was also cold macerated with Methanol and distilled water separately at room temperature with vigorous shaking for 2 days. After 2 days extract was filtered by using Whatman No 1 filter paper and dried by evaporating and stored at 4^0 C. The extracts were named as Cold Methanol extract (CME) and aqueous extract (CAE)

2.3 Phytochemical Screening[8,9]

The extracts were subjected to Phytochemical tests for the detection of organic constituents which include Carbohydrates, Proteins, Amino acids, Fats, Oils, Steroids, Volatile Oils, Glycosides, alkaloids, tannins and phenolic compounds and for the detection of inorganic constituents like calcium, magnesium, sodium, potassium, iron, phosphate, chloride etc. according to the procedure described by Kokate (1994) and Khandelwal (2005).

Chemical test for organic constituents were performed on extracts while for inorganic tests were performed on ash. Distilled water was taken as negative control.

2.4 Proximate analysis

The powdered plant material was subjected to proximate analysis to determine the moisture content, total ash value, water soluble ash value, acid insoluble ash value, alcohol and water soluble extractive value.

Determination of Moisture content: [10]

In order to determine moisture content 1.5 gm. of powdered plant material was dried at 105^0 C in an oven and after cooling weight was taken. Weight loss on drying was calculated as percentage (w/w) and expressed as Mean ±SD.

2.5 Preparation of Ash

Ash was prepared by incinerating the powdered plant material in Muffle furnace at 600^0 C for 6 hours followed by cooling. The ash was dissolved in 50 % HNO3 by keeping for 1 hour and the solution was filtered using Whatman No1 filter paper and used for detection of inorganic constituents

2.6 Determination of Total Ash value[10]

A clean silica crucible was ignited and 2 gm. of powdered plant material taken on it and weighted. The crucible along with plant powder was incinerated in a muffle furnace for 6 hrs. by gradually increasing temperature up to 600^0 C and allowed to cool. After cooling weight was taken and total ash content was calculated as percentage (w/w) and expressed as Mean ±SD.

Determination of Acid insoluble ash value: [10]

The ash above was washed into a clean beaker using 25 ml dil. HCl and boiled for 5 minutes. The boiled solution was filtered through an ash less filter paper previously weighted. The filter paper containing the insoluble ash was heated by

taking on silica crucible up to the removal of all carbon. And weighted and acid insoluble ash value was calculated percentage (w/w) and expressed as Mean ±SD.

2.7 Determination of water soluble ash value[10]

Water soluble ash value was determined by ashing 2 gm. of powdered material as mention in total ash determination. From that ash water soluble ash was determine in similar way to acid insoluble ash, using 25 ml of distilled water, in place of HCl. Finally water soluble ash value was calculated percentage (w/w) and expressed as Mean ±SD.

2.8 Alcohol soluble extractive value[11]

Alcohol extractive value was determined by macerating 5 gm. of powdered plant material with 100 ml of 90 % alcohol for 24 hrs. with frequent shaking at room temperature. The macerate was filtered and 25 ml filtrate was taken in a porcelain disc previously ignited and weighted. The filtrate was then dried by evaporating on water bath and finally in an oven at 105^0 C. after drying, weight of the disc along with extract was taken and alcohol soluble extractive value was calculated percentage (w/w) and expressed as Mean ±SD.

2.9 Water soluble extractive value[11]

Water soluble extractive value of powdered plant material was also determined similar way to alcohol soluble extractive value, 25 ml water instead of alcohol and water soluble extractive value was calculated percentage (w/w) and expressed as Mean ±SD.

3. Results

The qualitative Phytochemical tests on the trunk bark of *Alstonia scholaris* for organic constituents revel presence of carbohydrates, Gums and Mucilage, Proteins and amino acids, Fats and oils, Steroids, Glycosides and alkaloids in different solvent extracts. The results are shown in Table 1, 2 and 3.

Table 1: Results of qualitative phytochemical tests for organic constituents of the trunk bark of *Alstonia scholaris.*

Sl No.	Phytochemical constituents	PEE	BE	CE	ME	ARE	CAE	CME	NC
1.	Carbohydrates	-	-	-	+	+	-	+	-
2.	Gums and Mucilage	-	-	-	-	-	+	-	-
3.	Proteins and amino acids	-	-	-	-	-	-	+	-
4.	Fats and Oils	+	+	-	-	-	-	-	-
5.	Steroids	+	+	-	+	-	-	-	-
6.	Glycosides	-	-	-	+	+	+	-	-
7.	Alkaloids	-	-	+	+	-	+	+	-
8.	Tannins and Phenolic	-	-	-	-	-	-	-	-

Key: + =present, - =absent;
PEE = Petroleum Ether extract,
BE=Benzene extract,
CE=Chloroform extract,
ME=Methanol extract,
ARE= aqueous residual,
CME= Cold Methanol extract and
CAE= aqueous extract.

Table 2: Results of qualitative phytochemical tests for organic acids of the trunk bark of *Alstonia scholaris.*

S. No.	Organic acids	PEE	BE	CE	ME	ARE	CAE	CME	NC
1.	Oxalic acid	-	-	+	+	+	+	+	-
2.	Tartaric acid	-	-	-	-	-	-	-	-
3.	Citric acid	-	-	-	-	+	-	+	-

4.	Malic acid	-	-	-	-	-	-	-	-

Table 3: Results of qualitative phytochemical tests for inorganic constituents of the trunk bark of *Alstonia scholaris*.

S. No.	Constituents	Results
1.	Calcium	+
2.	Magnesium	-
3.	Sodium	-
4.	Potassium	-
5.	Iron	+
6.	Sulphate	-
7.	Phosphate	+
8.	Chloride	+
9.	Carbonate	-
10.	Nitrates	-

Table 4: Result of the proximate analysis of the trunk bark of *Alstonia scholaris*.

S. No.	Parameter		Value in percentage (Mean ±SD)
1.	Ash value	Total	24.73 ± 0.13 %
		Acid –insoluble.	13.17 ± 0.23 %
		Water-soluble	28.33 ± 0.47 %
2.	Extractive value	Alcohol-soluble	21.87 ± 0.87 %
		Water-soluble	34.13 ± 0.74 %
3.	Moisture content		10.12 ± 0.15 %

The values of all physicochemical determinations are summarized in Table 4. Water soluble ash was found to be quit greater than acid insoluble ash value. The result showed that water yielded higher extractive value. These all are important quantitative parameter for quality control of plant material.

4. Discussion and Conclusion

The quality of herbal products should be improved by starting with standard material. Thus in recent years there have been an emphasis in standardization of medicinal plants of therapeutic potential. According to World Health Organization (WHO) the macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken.[11]

In conclusion, the trunk bark of *Alstonia scholaris* has been shown to possess secondary metabolites and therefore may be important source for the development of plant based medicinal compounds. Further, author suggests before drug development plant material or its different compound should be purified and characterized using higher technique.

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