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## Pharmacognostical studies on stem bark of *Acacia ferruginea* DC.

Jhuma Deb\*<sup>1</sup> and Gouri Kumar Dash<sup>2</sup>

<sup>1</sup>Department of Pharmaceutical Sciences, NIMS University, Shobha Nagar, Jaipur, India

<sup>2</sup>Faculty of Pharmacy and Health Sciences, Universiti Kuala Lumpur Royal College of Medicine Perak, Ipoh, Malaysia

### ABSTRACT

*Acacia ferruginea* DC. (Family: Mimosaceae) is an important Ayurvedic drug. Traditionally, the stem bark of this plant are used for treating various human ailments like leucoderma, ulcers, piles, inflammation, helminthiasis, dysentery, diabetes etc. In spite of having several medicinal properties, no standardization parameters have been laid down for the stem bark in the literature. Therefore, in the present paper, we report the macroscopical and microscopical characters, physicochemical parameters such as moisture content, ash value, extractive values, fluorescence analysis and preliminary phytochemical analysis of the stem bark. Transverse section of the bark shows presence of cork, phellogen, phelloderm, sclerenchymatous tissue and secondary phloem region. The powder microscopy revealed presence of pieces of cork, parenchyma, xylem vessels, phloem fibres, stone cells, calcium oxalate crystals and starch grains. The preliminary phytochemical screening of different extracts revealed presence of alkaloids, steroids, triterpenoids, saponins, flavonoids, tannins and phenolic compounds, carbohydrates, gums and mucilages, proteins and amino acids respectively in the bark. These findings will be useful towards establishing pharmacognostic standards on identification, purity and quality of the plant.

**Keywords:** *Acacia ferruginea* DC., Macroscopy, Microscopy, Physicochemical parameters, Preliminary phytochemical analysis

### INTRODUCTION

Plants have been the basis for medical treatments through much of human history. Traditional medicaments, chiefly obtained from plants have played a pivotal role in sustaining disease free human existence on this planet. It is rather difficult to date back the origin of these medicaments as a means of therapy. In spite of overwhelming influence of modern medicine and tremendous advances made on the production of synthetic drugs, traditional medicaments designated now a days as herbal drugs in different places in literature, have retained their place in therapy. Their effectiveness, low cost and comparative freedom from serious toxic effects make these medicaments not only popular but also an acceptable mode of treating diseases even in modern times. Ironically, not many herbal preparations are available in standardized form. Adulteration in market samples has been realized to be the greatest draw-backs in ensuring quality herbal products. Several countries including India have intensified their efforts in laying down standardization parameters for various herbal drugs otherwise not available in the literature. The World Health Organization has set up several guidelines for the standardization of herbal products starting from the raw materials to finished products. The objective of WHO guidelines is to define basic criteria for the evaluation of quality, safety and efficacy of herbal medicines. Protocols on standardization and documentation of herbal medicines have also been published by IUPAC Technical report [1].

*Acacia ferruginea* DC. (Family: Mimosaceae) is an important Ayurvedic drug used to treat 'Vata' and 'Kapha' disorders as cited in available literatures [2]. The drug also finds its application in other systems of medicine for curing various ailments. Traditionally, the decoction of the stem and root bark is used externally for treating itching,

leucoderma and ulcers. The aqueous extract is recommended in the inflammation of the mucous lining of the mouth, including the cheeks, gums, tongue, lips and throat. A decoction of the bark together with *Tamarindus indica* is recommended as a gargle in sore-mouth [2-4]. The decoction of the bark is used orally for treating helminthiasis. The bark decoction in conjunction with ginger is frequently used as an astringent for the teeth [5]. The natives of Tamil Nadu use the paste of the stem bark along with water as a remedy for dysentery, piles and diabetes. The fruits are consumed to treat dysentery, worm infestations and in cough [6]. The decoction of the leaves is believed to be good astringent. The juice or infusion of the leaves enriches blood and taken in the morning in liver complaints, diseases of the eye, diarrhoea and dysentery [4]. The leaf paste is applied externally in burns and scalds. The decoction from the pods is both used as an astringent and as a demulcent [2]. In spite of having several medicinal properties, no standardization parameters have been laid down for the stem bark in the literature. Therefore, in the present paper, we report few standardization parameters for the plant drug.

## MATERIALS AND METHODS

### Plant material

The fresh stem bark was collected from the well grown and matured trees from Tirumala, Andhra Pradesh during January 2011 and authenticated by the Botanist Dr. K. Madhava Chetty, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India. The fresh stem bark pieces were used for histological analysis. The remaining pieces of the bark were shade dried, milled in to coarse powder and used for other studies.

### Chemicals and reagents

The chemicals, solvents and reagents used in the study were of standard analytical grade obtained from S.D Fine Chem Ltd., Mumbai and Loba Cheme, Mumbai.

### Macroscopy

The bark was studied for the morphological characters including colour, odour, taste, texture etc.

### Microscopy

Thinnest possible transverse sections of the bark were taken out [7-12] and treated in chloral hydrate solution with gentle warming, stained with phloroglucinol and concentrated hydrochloric acid (1:1). They were then mounted with glycerin and studied under a binocular research microscope (Model BD 10, BD Instrumentation, India) fitted with Canon Photoshot (A3200IS) camera. Photomicrographs were obtained during observation of the sections in appropriate field.

### Powder microscopy

A small quantity of the bark powder was taken on a glass slide and treated with few drops of chloral hydrate solution and heated for 1-2 min [13-16]. Lignified tissues were confirmed after staining with a few drops of mixture of phloroglucinol and concentrated hydrochloric acid (1:1). In order to observe starch grains, a small amount of the powder was mounted separately in N/20 iodine solution. The starch grains appeared light blue in colour. Detection of calcium oxalate crystals were carried out by treating the powdered sample with water followed by observation under the microscope.

### Physico-chemical analysis

The dried powdered plant material was used for the quantitative determination of moisture content, ash and extractive values according to standard procedure of Indian Pharmacopoeia [17, 18]. The fluorescence analysis of powdered drug was carried out under ultra violet light after treatment with various chemical reagents [19, 20].

### Preliminary phytochemical studies

A known quantity of dried powder (50 g) was extracted successively with petroleum ether, chloroform, methanol and water. Following extraction, the liquid extracts were concentrated under vacuum. The extractive value for each extract was calculated with respect to the dried plant material. The extracts were further subjected to preliminary phytochemical studies to find out the nature of phytoconstituents they contain [9, 12, 21].

## RESULTS AND DISCUSSION

### Macroscopy:

The macroscopic characteristics of the bark of *A. ferruginea* were observed. The colour of the bark was rusty brown to dark reddish brown with characteristic odour. Taste is bitter and slightly astringent. Pieces of the bark were about 3-7 cm long and about 3-5 mm in thickness. The surface of the bark is rough.

**Microscopy:**

Transverse section of the bark shows presence of cork, phellogen, phellogen, phellogen, sclerenchymatous tissue and secondary phloem (Fig. 1).

**Cork:**

It consists of several layers of cells of which the outer ones are with thin walls and the inner walls are thick walled, flat, polygonal and lignified cells with reddish brown content.

**Phellogen:**

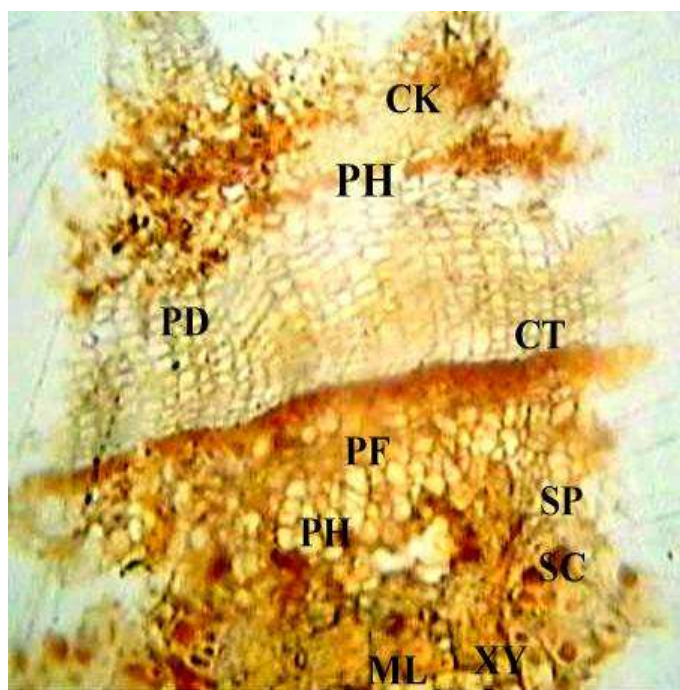
It contains 2-3 layers of thin walled cells.

**Phellogen:**

Phellogen comprises of 8-10 layers of thick walled rectangular parenchymatous cells without any cellular content which comprises the cortex. A belt of sclereids occur in between the primary cortex and secondary phloem which are highly lignified sclerenchymatous cells present in a row and sometimes scattered. The inner and radial walls of the sclereids are thicker than the outer walls. The surface of the sclereids is pitted. On the outer side of the sclerenchymatous band are found a few groups of small pericyclic fibres.

**Secondary phloem:**

This region comprises of phloem parenchyma, phloem fibres and medullary rays. Phloem fibres occur mostly singly and isolated, rarely in groups of two to three, embedded in phloem parenchyma. The fibres are mostly lignified and circular in shape. Parenchyma consists of polygonal cells with brownish matter throughout the medullary ray. The medullary ray consists of unlignified cells that are 3-4 cells wide.



**Fig. 1: Transverse section of the bark of *A. ferruginea***

[CK=Cork, PH=Phellogen, PD= Phellogen, CT= Cortex, PF= Pericyclic fibres, PH=Phloem, SP=Secondary phloem, XY=Xylem, ML=Medullary ray]

**Powder microscopy:**

The powder microscopy revealed presence of pieces of cork, parenchyma, xylem vessels, phloem fibres, stone cells, calcium oxalate crystals and starch grains (Fig. 2).



Fig.2: Powder characters of the bark of *A. ferruginea*

**Physicochemical analysis:**

The percentage of total ash, acid-insoluble ash, water soluble ash, water soluble extractive, ethanol soluble extractive and moisture content are presented in Table 1. The results of the fluorescence analysis of the drug powder is shown in Table 2.

Table 1: Physicochemical analysis of bark of *Acacia ferruginea*

Parameter	Obtained values (%w/w)
Total ash	5.36 ± 0.19
Acid insoluble ash	1.13 ± 0.10
Water soluble ash	2.59 ± 0.13
Water soluble extractive	11.73 ± 0.20
Ethanol soluble extractive	16.57 ± 0.34
Moister Content	10.2 ± 0.53

Results expressed as Mean ± SEM from three observations

Table 2: Fluorescence analysis of the bark powder of *A. ferruginea*

Sl. No.	Reagents	Daylight	Observed colour under UV light	
			Short wavelength (254 nm)	Long wavelength (365 nm)
1	Powder as such	Reddish brown	Reddish brown	Dark brown
2	Nitric acid	Grayish brown	Dark brown	Brownish black
3	1N Hydrochloric acid	Blackish brown	Dark brown	Brown
4	1N Sulphuric acid	Dark brown	Brownish black	Yellowish black
5	Glacial acetic acid	Reddish brown	Light brown	Brownish yellow
6	5% Iodine solution	Bluish black	Black	Black
7	5% Ferric chloride	Yellowish brown	Greenish yellow	Brownish yellow

**Preliminary phytochemical studies:**

The results of the preliminary phytochemical tests are presented in Table 3. The different extracts of the bark were found to contain alkaloids, steroids, triterpenoids, saponins, flavonoids, tannins and phenolic compounds, carbohydrates, gums and mucilages, proteins and amino acids respectively.

Table 3: Preliminary phytochemical studies of different extracts of *A. ferruginea*

Test for	Petroleum ether extract	Chloroform extract	Methanol extract	Aqueous extract
Alkaloids	-	+	+	-
Carbohydrates	-	-	-	+
Gums & Mucilages	-	-	-	+
Proteins and amino acids	-	-	-	+
Tannins and phenolic compounds	-	-	+	+
Steroids	+	+	-	-
Triterpenoids	-	+	-	-
Saponins	-	-	+	+
Flavonoids	-	-	+	+

+ = present, '-' = absent

The results of this investigation could serve as a basis for proper identification of the plant. The macroscopical and microscopical evaluation will help investigators to distinguish this plant from other members of the genera. These ash values are important pharmacognostic tools to standardize the crude drugs. The extractive values are indicative of approximate measures of their chemical constituents extracted with specific solvents [22]. Fluorescence is the phenomenon exhibited by various chemical constituents present in the plant material. Some constituents show fluorescence in the visible range in daylight. The ultra violet light produces fluorescence in many natural products (e.g. alkaloids like berberine), which do not visibly fluoresce in daylight. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives or decomposition products by applying different reagents. Hence, some crude drugs are often assessed qualitatively in this way and it is an important parameter of pharmacognostical evaluation [23]. The preliminary phytochemical tests are usually carried out to identify presence of various phytoconstituents in different extracts of crude drugs. Thus, all these parameters will augment in standardization of the plant material.

### CONCLUSION

Medicinal plants have been known for millennia and are highly esteemed all over the world as a rich source of therapeutic agents for the prevention of diseases and ailments. According to the World Health Organization, the macroscopic and microscopic description of a medicinal plant is the first step towards establishing the identity and degree of purity and should be carried out before any other tests are undertaken. In this context the pharmacognostical evaluation of the stem bark of *A. ferruginea* could serve as a basis for proper identification of the plant. The preliminary phytochemical studies further aids in providing valuable information about the chemical composition of the plant material. The tree has been enlisted under the IUCN Red list of Threatened Species [24]. There is an urgent need for conservation and research on this plant before it disappears from the earth. The present article will provide valuable information to the future investigators for proper identification of the plant.

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