

Original Research Article

Pharmacognostic Evaluation of the Bark of *Acacia suma* Roxb (Fabaceae)

Gouri Kumar Dash^{1*}, Mohd Syafiq Abdullah¹ and Suman Acharyya²

¹Faculty of Pharmacy and Health Sciences, Universiti Kuala Lumpur Royal College of Medicine Perak, 30450 Ipoh, Perak, Malaysia, ²Netaji Subhas Chandra Bose Institute of pharmacy, Tatla, Chakdaha, Nadia-741222, West Bengal, India

*For correspondence: Email: gk_dash@rediffmail.com, gkdash2@gmail.com; Tel: 0060105491614

Received: 9 September 2013

Revised accepted: 14 April 2014

Abstract

Purpose: To undertake the pharmacognostic evaluation of *Acacia suma* Roxb bark for the purpose of identification and differentiation from related species.

Methods: The macroscopic and microscopic features of the bark were studied, including the use of powder microscopy with the aid of suitable tools and reagents. Physicochemical parameters such as ash values, extractive values and loss on drying were also determined. The bark powder was successively extracted with different solvents followed by preliminary phytochemical screening of the extracts.

Results: Macro- and microscopic studies revealed an outer hard and woody exfoliating old bark consisting of dead elements of secondary bast alternating with tangential strips of compressed cork tissue. The outer layer consists of cork cell with lenticels, followed by phellum, phellogen and pheloderm layers. Concentric rings of secondary phloem tissue alternating with regularly arranged polygonal stone cells and radially traversed medullary rays were present. Preliminary phytochemical screening showed the presence of alkaloids, carbohydrates, proteins, tannins and phenolic compounds, gums and mucilages, steroids and triterpenoids, saponins and flavonoids in the bark.

Conclusion: The findings of this study will facilitate pharmacognostic standardization of the plant material and aid in the preparation of a herbal monograph for the species.

Keywords: *Acacia suma* var. *Acacia polyacantha*, Bark, Pharmacognostic evaluation, Standardization, Phytochemical, Pharmacopoeia

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

The family Fabaceae (alternatively known as the Leguminosae) is one of the largest families of flowering plants, consisting of 730 genera and over 19,400 species [1]. The genus *Acacia* comprises about 1200 species, indigenous to tropical and subtropical regions, but found throughout the world [2]. *Acacia suma* Roxb var. *Acacia polyacantha* Willd is a medium sized deciduous tree between 3.5 -20 meters in height, widely distributed in India in deciduous woodland

and groundwater forests in altitudes up to 1800 m [3].

Ethnopharmacological data revealed that preparations from stem barks of *A. suma* find its application in several traditional and folklore systems of medicine around the globe. The ground dried barks are applied externally over local sores for quick healing. The decoction of the bark is used orally to treat gonorrhoea, pneumonia, leprosy, malaria, diabetes and believed to be aphrodisiac [4,5]. Gessler *et al* [5]

reported the antimalarial activity of the stem bark. The hypoglycemic activities of different extracts of bark are reported by the authors [6]. Presence of gallocatechins in the barks and an indole alkaloid N,N-dimethyl tryptamine has been reported in the leaves [7].

In the light of the above and considering importance of this medicinal plant, the present investigation was carried out to study of some pharmacognostic features of the bark as a whole including its intact and powdered form that is not found in the literature. The studies were carried out in accordance with WHO General Guidelines for Herbal Drug Standardization methodologies [8]. The findings from this study would be useful as standards for the species as well as a source of reference for further scientific investigation of the species.

EXPERIMENTAL

Collection, authentication and preparation of plant material

Intact bark pieces were collected during June 2012 carefully from experimental plants inhabiting in forests of Ganjam district of Odisha, India and authenticated by Dr MS Mondal, Taxonomist, Botanical Survey of India, Howrah. A voucher specimen (no. BUB 0176) was deposited in the herbarium museum of Berhampur university, Berhampur for future reference. After authentication, fresh barks were collected in bulk, washed with potable water to remove adhering dirt followed by rinsing with distilled water, and were then dried under a shade and powdered.

Macroscopy

The following macroscopical characters for the fresh and dried barks were noted: size and shape, surfaces, fracture, texture, colour, odour and taste.

Microscopy

Transverse section of the bark

Fresh bark pieces were embedded into paraffin blocks and sectioned with the help of rotary microtome. Thin sections with thickness varying from 10 - 15 μ m were collected. Dewaxing of the sections was done by customary procedure [9]. The sections were stained with phloroglucinol and hydrochloric acid in the ratio 1:1 and mounted with glycerin. Microphotographs were taken using a Leica DMLS microscope attached

with Leitz MPS-32 camera. Photo micrographs of different magnifications were taken to study the anatomical features.

Powder microscopy

The shade dried powdered bark screened through sieve no. 40 was used for the powdered drug analysis. The specimens were separately treated with glycerin, N/20 iodine solution (for detection of starch grains), 10 % w/v alcoholic ferric chloride (for detection of phenolic compounds), phloroglucinol-hydrochloric acid (1:1) for detecting lignin and ruthenium red solution (for detection of mucilage). After staining, the samples were observed under a compound microscope [10].

Preliminary phytochemical studies

The dried and powdered bark (50 g) was successively extracted with petroleum ether (60 – 80 °C), chloroform, methanol and water by reflux for 2 h. Following extraction, the liquid extracts were concentrated under reduced pressure to yield dry residues. The extracts were subjected to preliminary phytochemical screening using standard procedures to determine the nature of phytoconstituents content [11-13].

Physicochemical analysis

The physicochemical parameters including ash values (total ash, acid insoluble ash, water soluble ash and sulphated ash), extractive values (ethanol, ether and water soluble) and loss on drying were performed according to the standard methods [14].

RESULTS

Macroscopic characters of the bark

The bark is usually in curved pieces with average size ranging from 2.6 cm – 7.2 cm long and 1.5 cm – 2.8 cm width. The upper surface of the matured bark is smooth and whitish grey in color, often with irregular longitudinal ridges and sometimes transverse cracks. Knobby persistent prickles in pairs are found below each node, straw colored to brown or black, with a black tip, 4 to 12 mm long. Inner surface longitudinally striated, fracture irregular when broken and coarsely fibrous. The cut surface is smooth and odourless with mucilaginous astringent taste. The bark has an average total thickness of 3.2 ± 0.2 mm.

Microscopic characters (transverse section) of the bark

The outer hard and woody exfoliating old bark consists almost entirely of the dead elements of secondary bast (Fig 1) alternating with tangential strips of compressed cork tissue. The outer layer is brown coloured cork cell where the lenticels are present. Below the cork cell, phellum layer is present (Fig 2). It takes red color by phloroglucinol and hydrochloric acid stain. Below

the phellum, phellogen and pheloderm layers are present. Following this, cortex layer is present where the phloem elements are found. Concentric rings of secondary phloem tissue alternating with regularly arranged, stone cells and radially traversed medullary rays are present in the former part (Fig 3). Medullary rays cells mostly biseriate and the stone cells are polygonal.

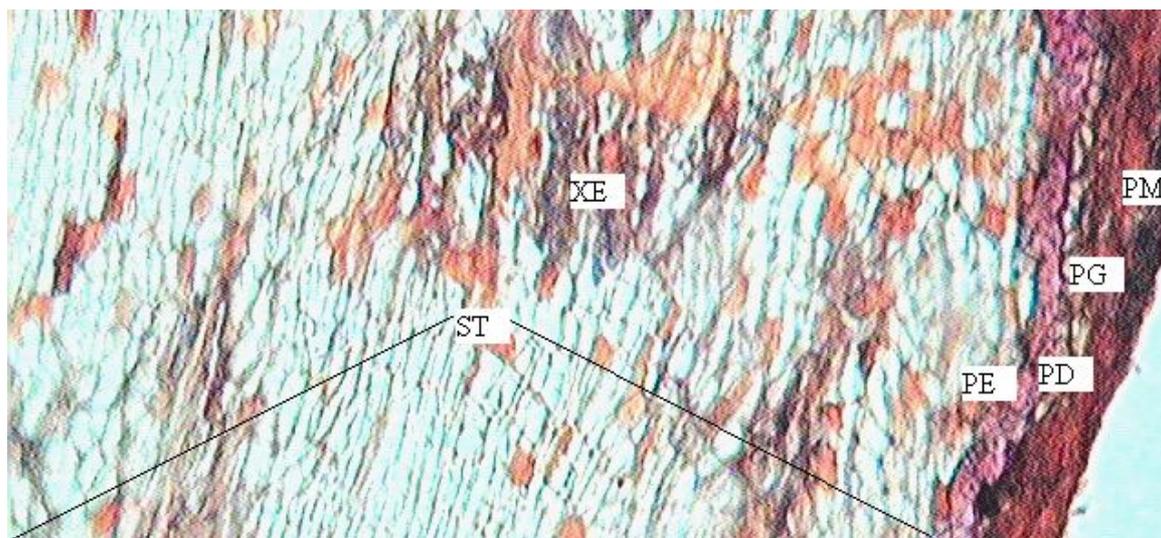


Fig 1: Anatomy of the bark – a sector of the transverse section of bark (PM = phellum; PG = phellogen; PD = pheloderm; PE = phloem elements ST = secondary tissue; XE = xylem elements)

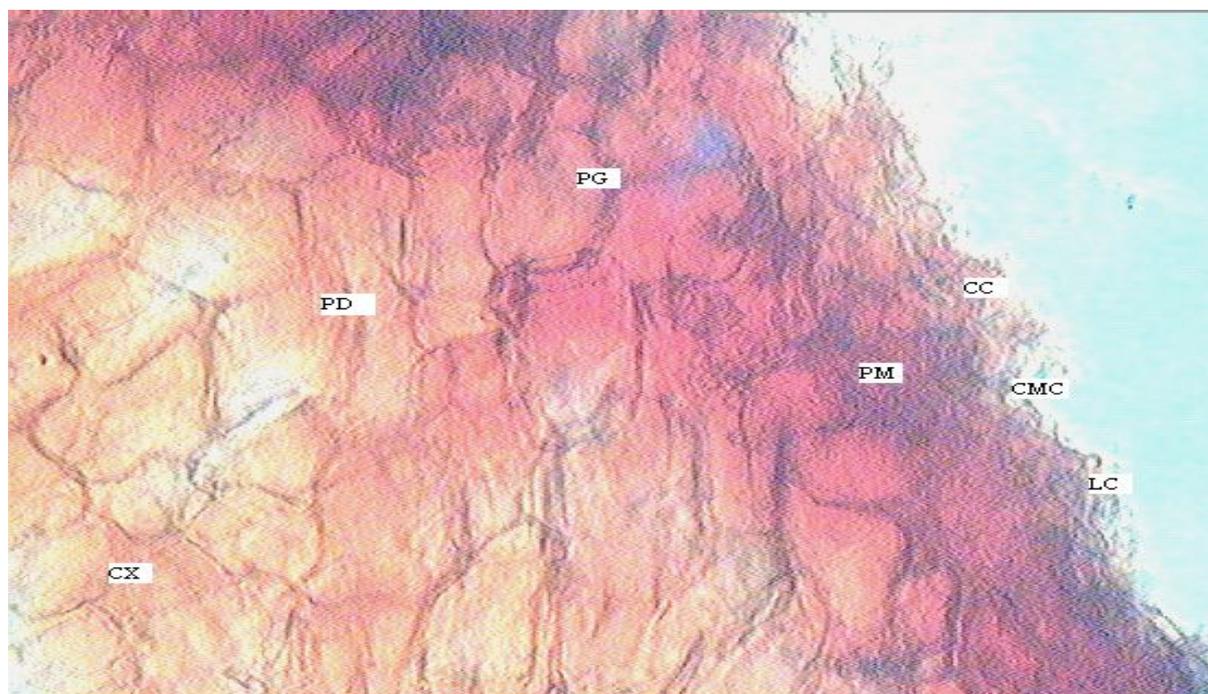


Fig 2: Anatomy of the bark – a sector of the transverse section of bark epidermis: (CC = closing cell; CMC = complementary cell; LC = lenticels; PM = phellum; PG = phellogen; PD = pheloderm; CX = parenchymatous cortex)

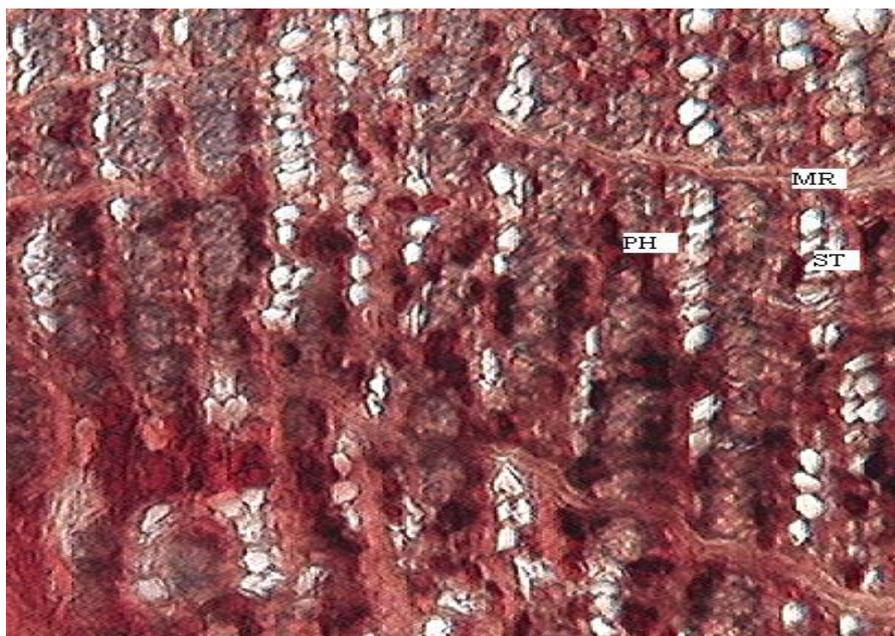


Fig 3: Anatomy of the bark – a sector of the transverse section of phloem elements of bark (ST = stone cell; MR = medullary ray; PH = phloem tissue)

Table 1: Preliminary phytochemical profiles of various extracts of *A. suma*

| Test | Pet. Ether | Chloroform | Methanol | Aqueous |
|--------------------------------|------------|------------|----------|---------|
| Steroids and sterols | + | + | - | - |
| Triterpenoids | + | + | - | - |
| Alkaloids | - | + | + | - |
| Saponins | - | - | + | + |
| Flavonoids | - | - | + | + |
| Carbohydrates | - | - | - | + |
| Gums and mucilages | - | - | - | + |
| Proteins and amino acids | - | - | - | + |
| Tannins and phenolic compounds | - | - | + | + |

+ = present; - = absent

Powder microscopy

The bark powder is grey in color with mucilaginous astringent taste. The powder consists of cork cells, vessel elements, fibers, parenchyma and few stone cells. The vessels elements are pitted long and cylindrical. They have simple, horizontal perforations. The lateral pits are circular to elliptic and dense. In the powder, the fibers are narrow and long with thick lignified walls. Parenchyma cells are pitted and lignified. Stone cells are round in shape and the cork cells are abundant in the powder.

Preliminary phytochemical studies

The result of the preliminary phytochemical screening of different extracts (Table 1) showed presence of alkaloids (in chloroform and methanol extracts), carbohydrates, proteins, tannin, gum (in aqueous extract), steroids and triterpenoids (in petroleum ether and chloroform

extracts), saponin and flavonoids (in methanol and aqueous extracts).

Physicochemical characteristics

The values obtained for physicochemical parameters are reported in Table 2.

Table 2: Physicochemical values for *A. suma* bark powder

| Parameter | Value (% w/w) |
|----------------------------|---------------|
| Total ash | 7.1 ± 0.698 |
| Acid insoluble ash | 2.1 ± 0.258 |
| Water soluble ash | 3.418 ± 0.354 |
| Sulphated ash | 8.625 ± 0.655 |
| Ether soluble extractive | 3.025 ± 0.275 |
| Ethanol soluble extractive | 6.075 ± 0.275 |
| Water soluble extractive | 11.0 ± 0.73 |
| Loss on drying | 6.675 ± 0.726 |

Values are expressed as mean ± SD (n = 4)

DISCUSSION

A plant may be considered as a biosynthetic laboratory, not only for the chemical compounds such as carbohydrates, proteins and lipids that are utilized as food by man, but also for a multitude of compounds like glycosides, alkaloids, volatile oils, saponins, steroids and sterols, flavonoids and phenolic compounds that exert a physiological effect. The compounds that are responsible for therapeutic effects are usually the secondary metabolites. A systematic study of a crude drug embraces through consideration of both primary and secondary metabolites derived as a result of plant metabolism in addition to its macro and microscopic studies.

Acacia has had a complex nomenclatural history. *A. suma* is often confused with other species due to their relative similarities. The species has been taxonomically described as distinct species by earlier workers [15]. The bark finds its application in several other traditional and folklore systems of medicine around the globe that have been previously described and surprisingly no pharmacopoeial standards are available for them in the literature. Owing to its importance in applications, the present study was designed and conducted. From the present study, it can be concluded that the macroscopic and microscopic findings together will help future investigators in proper identification of the plant. Further, the powder microscopy, preliminary phytochemical screening and physicochemical parameters would aid in standardization of the plant material. The wide spectrum of biological activity of this plant is due to presence of several phytoconstituents that needs to be studied further.

CONCLUSION

The standardization of a crude drug is an integral part of establishing its correct identity and is of paramount importance in justifying their acceptability in modern systems of medicine. The most established information with regard to the use of herbal preparations currently available in the public domain is in the form of pharmacopoeial monographs. These documents publish standardized parameters for their identification. Published monographs in a pharmacopoeia are the most practical approach for quality control of an herbal drug. The major advantage of an official monograph published in a pharmacopoeia is that standards are defined and available. But when pharmacopoeia monographs are unavailable, development of

suitable standards for the herbal drugs have to be done by the researchers. The best strategy is to follow closely the pharmacopoeia definitions of identity and set standardized parameters for the establishment of standard quality of raw material. The preparations made from the stem barks of *A. suma* are currently being used in several traditional and folklore systems of medicine for the treatment of various diseases without standardization. These findings would help as a tool for identification of *A. suma* with its pharmacognostic characteristics, discriminating it from its other species diversity and aid in the preparation of an herbal monograph for the species.

REFERENCES

1. Saha S, Shilpi JA, Mondal H, Hossain F, Anisuzzman M, Hasan MM, Cordell GA. *Ethnomedicinal, phytochemical, and pharmacological profile of the genus Dalbergia L. (Fabaceae)*. *Phytopharmacol* 2013; 4(2): 291-346.
2. Lorenzo P, Gonz'alez L, Reigosa MJ. *The genus Acacia as invader: the characteristic case of Acacia dealbata Link in Europe*. *Ann For Sci* 2010; 67: 101-111.
3. Orwa C, Mutua A, Kindt R, Jamnadass R, Simons A. *Agroforestry Database: a tree reference and selection guide version 4.0 [cited 2009 July 8]* Available from: <http://www.worldagroforestry.org/af/treedb/>
4. Headbarg I, Headberg O, Madati PJ, Mshigeni NK, Mshiu EN, Samuelsson G. *Inventory of plants used in traditional medicine in Tanzania, II. Plants of the families dilleniaceae-opiliaceae*. *J Ethnopharmacol* 1983; 9(1): 105-127.
5. Gessler MC, Nkunyak MHH, Mwasumbi LB, Heinrich M, Tanner M. *Screening Tanzanian medicinal plants for antimalarial activity*. *Acta Tropica* 1994; 56(1): 65-77.
6. Acharyya S, Dash GK, Mondal S, Acharyya A, Dash SK. *Studies on the hypoglycaemic activity of Acacia suma (Roxb.) barks*. *Int J Chem Analytical Sci* 2010; 1(1): 10-13.
7. Wadhwa SK, Elkheir YM. *Dimethyl tryptamine from the leaves of certain Acacia species of Northern Sudan*. *Lloydia* 1975; 38(2): 176-177.
8. Shrikumar S, Maheswari MU, Suganthi A and Ravi TK: *WHO Guidelines For Herbal Drug Standardization, Pharmainfo.net.*, [cited 2004 September 19]. Available from: www.pharmainfo.net/reviews/who-guidelines-herbal-drug-standardization
9. Johanson DA, *Plant Micro Technique*, McGraw Hill Book Co, New York, 1940, pp 183-203, 523.
10. Prabhu K, Karar PK, Ponnudurai K, Hemalatha S. *Pharmacognostic Investigation of the leaves and stems of Viburnum erubescens Wall.ex DC Trop J Pharm Res* 2009; 8 (6): 557-566.

11. Harborne JB. *Phytochemical methods. A guide to modern technique of plant analysis.* Chapman and Hill, London. 1992. 279.
12. Abere TA, Onwukaeme DN and Eboka CJ. Pharmacognostic evaluation of the leaves of *Mitracarpus scaber* Zucc (Rubiaceae). *Trop J Pharm Res* 2007; 6 (4): 849-853.
13. Abere TA, Onwukaeme DN. Pharmacognostic Evaluation of the Leaves of *Secamone afzelii* (Schult) K Schum (Asclepiadaceae). *Trop J Pharm Res* 2012; 11 (1): 125-131.
14. Evans WC. *Trease and Evans Pharmacognosy.* Saunders, London; 2002; pp 95-99.
15. Kshirsagar SR. Observations and Taxonomic assessment of *Acacia catechu* Willd. complex (Mimosaceae) in India. *Life Sci Leaflets* 2012; 6: 68-77.