Pharmacognostic studies on the flowers of Acacia nilotica Linn

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ABSTRACT

Acacia nilotica (Linn) family Mimosaceae commonly known as Karuvalem is used in Traditional System of Medicine for healing various diseases. It is used in the treatment of throat troubles, stomatitis, chronic dysentery, diarrhoea and in conjunctivitis. In the present investigation an attempt has been made for the pharmacognostical standardization of Acacia nilotica flowers. The pharmacognostical evaluation comprises of morpho-anatomy, histochemistry and physical constants such as ash, loss on drying and extractive values of A. nilotica flowers. The flowers extracts were subjected to preliminary phytochemical screening. The data obtained in present study will serve as valuable tool for identification, authentication and detection of adulterants, standardization and quality control of the drug. The developed technique will also be useful for the standardization of formulation containing A. nilotica.

Keywords: Acacia nilotica; Pharmacognostical evaluation; Physico-chemical data.

INTRODUCTION

Plants are the essential and integral part in complementary and alternative medicine. The ability for the formation of secondary metabolite like flavonoids, alkaloids, steroids and phenolic substances makes the plants to be used to restore health and heal many diseases. Natural products of plant and animal origin offer vast resources of newer medicinal agent with potential in clinical use.

Acacia, a leguminous genus belonging to the family of Mimosaceae, comprises approximately, 1200 species that are dispersed widely in tropical and subtropical regions of Australia, south America, Asia and Africa[1-2]. Many of these species are important for fuelwood, timbers, shelter belts and soil improvement[3]. It has been used extensively for the treatment of various diseases eg colds, bronchitis, diarrhoea, dysentery, biliousness, bleeding piles and leucoderma[4]. It also serves as a source of various products, including polyphenols[5-6]. The role of these natural products to the plant itself is not well understood, but for the human kind they can be of prime importance. Therefore, the bioprospection of naturally occurring polyphenolic compounds having ability to provide protection against certain types of mutagens and carcinogens is of great importance[7]. A. nilotica contains gallic acid, m-digallic acid, (+)-catechin, chlorogenic acid, catechin-5-galloyl ester[8]. A. nilotica has anticancer and antimutagenic, anti-inflammatory, antiplasmodial[9], antidiarrhoeal[10], antihypertensive, antiplatelet aggregatory, molluscicidal, antifungal, antimicrobial activity, inhibitory activity against Hepatitis C and HIV-I[11]. Eventhough the plant is rich in bio-active constituents and potential therapeutic activities there is a lacuna in the pharmacognostical standardization on the flowers of A. nilotica Linn. So the present investigation was aimed at evaluating the pharmacognostical features and phytochemical analysis for authentication and identification of the plant and also to evaluate the exact extract responsible for the biological activity.

Materials and Methods

The fresh flowers of A. nilotica Linn., were collected from Thanjavur Tamilnadu, India, (August 2010), which was identified by Taxonomist, and authenticated by Prof. P Jayaraman, Botanist, Director, Plant anatomy Research Centre, Tambaram. A voucher specimen no were deposited in the Department of CSMDRIA, Arumbakkam, Chennai for the future reference. The fresh flowers were collected and fixed immediately using FAA (Formalin: Acetic acid: Ethyl alcohol) as fixative agent for anatomical studies. The materials were cut into small pieces before fixing.

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Pharmacognostical studies

Macroscopic
Morphological studies were done by using simple microscope to determine the shape, nature, texture, colour, odour and taste of the flower.

Microscopic
For anatomical studies customary technique of microtomy was followed\[^{12}\]. Paraffin section of 10μm thick were stained with Toluidine blue. Photomicrography were taken with Nikon lab photo-microscopic unit. The chemomicroscopy were carried out according to the methods outlined by\[^{13-15}\].

Physicochemical studies
The ash values, extractive values and loss on drying at 105°C were performed according to the official methods prescribed in Indian Pharmacopoeia\[^{16}\] and the WHO guidelines on quality control methods for medicinal plant materials\[^{17}\].

Preliminary phytochemical analysis
For preliminary phytochemical analysis, extracts was prepared by weighing 1kg of the dried powdered flowers subjecting it to hot successive continuous extraction with different solvents in the order of increasing polarity namely petroleum ether, chloroform, ethyl acetate, methanol and finally with aqueous. The extracts were filtered separately, concentrated and the solvent was removed by rotary evaporator. The extracts were dried over desiccators and the residues were weighed. The presence of absence of the primary and secondary phytoconstituents was detected by usual prescribed methods\[^{18,19}\].

Results and Discussion

Macroscopy
The flowers are yellow in colour. The sepals and petals are five each, stamens are numerous. The pods are moniliform, white and tomentose.

Microscopy
Microscopical studies are usefulness to establish the botanical identity for the valuable herbal drugs.

Longitudinal section view
In longitudinal section, the inflorence has a peduncle, which dilates into hemispherical thalamus bearing numerous dense flowers. At the base of the thalamus is a ring of thick densely staining nectariferous gland (Fig 1). The flowers are sessile and are borne in the axil of a bract (Fig 2). The flower has gamosepalus calyx and gamopetalous corolla. The sepals are thin at the base and thick at the apex. The petals are uniform in thickness from the base to the tip (Fig 3). All the parts of the flower receive vascular strands from the surface of the thalamus. The prominent nectariferous gland and thick with slightly concave top portion. Vascular strands enter the nectary before extending to the flowers. The cells of the nectar are darkly stained (Fig 1).

Transverse section view
In transverse sectioned view, the flowers appear elliptical or circular. Each flower has a short, thick bract, thin outer cylinder of calyx and inner cylinder of corolla (Fig 4). Within the corolla tube occur sections of staminal filaments and anthers (Fig 5). The calyx tube consists of five thickened portions alternating with thin portion. The thick portion is about 6 layered including the inner and outer epidermal layers (Fig 6) and is 70µm in thickness. The thin portion is just three layered. The corolla tube also varies in thickness along five portions of the circumference. It consists of an epidermal layer of oblong thick walled cells and inner three or four layers of compact parenchyma cells (Fig 7). Anthers are dithecous and four celled. The anther wall consists of an outer epidermis, inner endothecium with spiral thickenings and inner epidermis which is mostly disintegrated during microsporogenesis. The pollen grains are large with prominent reticulate markings of the exine (Fig 8).

Powder microscopic observations
The powder character of a flowers are mainly used for the identification of drug in the powder form. The powder was yellow in colour with strong and characteristic in taste. On microscopical examination the powder showed the following inclusion;

Epidermal trichomes (Fig 9)
Unicellular, unbranched trichomes are occasionally seen in the powder. They have very thick reticulate lignified walls and narrow lumen. The trichomes are 250µm long and 10µm thick.

Stamens (Fig 10)
Isolated stamens and anthers are common in powder. The filaments are thick and cork like; they are multicellular, multiseriate with vertically oblong cells. The filament is 400µm long. There are also anthers attached to the filament and detached anthers. The anthers are squarish in side view, dark and measure 120µm thick and 150µm wide.

Pollen grains (Fig 11)
Pollen grains are seen scattered in the powder. They are spheroidal in shape with ridged and furrowed surface. The exine consists of deeply marked reticulate and capping. The pollen is 40µm in diameter it is darkly coloured.

Physicochemical standardization of flowers
The air dried, powdered plant materials were subjected for determination of various physicochemical standardization parameters as per the method described in\[^{21}\].
Determination of ash values
The percentage of loss on drying, total ash values, water soluble ash and acid insoluble ash were determined. The results obtained were; loss on drying (6.23 ± 0.19 %), total ash (4.48 ± 0.38%), water soluble ash (1.69 ± 0.39%), and acid insoluble ash (0.56 ± 0.10%) respectively. The ash value of any organic material is composed of their non volatile inorganic components. Control incineration of crude drugs result in ash residue consisting of an inorganic material (metallic salt and silica). This value varies within fairly wide limits and it is an important parameter for the evaluation of crude drugs. In certain drug, the percentage
variation of ash from sample to sample is very small and any marked difference indicates the change in quality. Unwanted parts of drug, some time possess a character that will raise the ash value. Ashing involves an oxidation of the components of the product. A high value is indicative of contamination, substitution, adulterations or carelessness in preparing the crude drug for marketing. The total ash value, acid insoluble ash value, water-soluble ash values were determined as per WHO guide lines. The results and observation are presented in Table 1.

### Table 1: Data showing the Physico-chemical standard vaues of Acacia nilotica Linn

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total ash</td>
<td>4.48 ± 0.38</td>
</tr>
<tr>
<td>Acid insoluble ash</td>
<td>0.56 ± 0.10</td>
</tr>
<tr>
<td>Water soluble ash</td>
<td>1.69 ± 0.31</td>
</tr>
<tr>
<td>Loss on drying at 105°C</td>
<td>6.23 ± 0.19</td>
</tr>
<tr>
<td>Extractive value</td>
<td></td>
</tr>
<tr>
<td>(i) Petroleum ether</td>
<td>0.569 ± 0.23</td>
</tr>
<tr>
<td>(ii) Chloroform</td>
<td>0.46 ± 0.25</td>
</tr>
<tr>
<td>(iii) Ethyl acetate</td>
<td>1.72 ± 0.32</td>
</tr>
<tr>
<td>(iv) Methanol</td>
<td>13.39 ± 0.12</td>
</tr>
<tr>
<td>(v) Aqueous</td>
<td>12.40 ± 0.20</td>
</tr>
</tbody>
</table>

The results are the mean of five estimation ± standard error.
different phytoconstituents. The compositions of these phytoconstituents depend upon the nature of the drug and solvent use. The use of a single solvent can be the means of providing preliminary information on the quality of particular drug. Extractive value also give the information regarding the quality of the drug (whether drug is exhausted or not). The mean value obtained for each of these parameters was found to be consistent with minimum standard deviation.

**Preliminary phytochemical analysis**

The various extracts were subjected to preliminary phytoconstituents analysis for their presence or absence of the constituents. The results are shown in Table-2. The plants are considered as biosynthetic laboratory for a multitude of compounds that exert physiological effects. Secondary metabolites are the compounds which are responsible for imparting therapeutic effects. The phytochemical screening showed that the flowers were rich in saponin, flavonoids, tannins and terpenoids. Steroids were found to be present in the flowers. It has been found that this plant contained steroidal compounds. It should be noted that steroidal compounds are of importance and interest in pharmacy due to their relationship with such compounds as sex hormones\(^{(2)}\). Therapeutic activity may be due to these compounds.

**CONCLUSION**

In the present days of modernization, Ayurveda no longer can afford to remain confined to use of conventional norms of medication. It has to accept the new challenges and be prepared to answer the queries of the modern man about the quality and efficacy of the herbal drugs administered to him and also how they are collected, processed, preserved and used. The above studies provide information in respect of their identification, chemical constituents and physicochemical characters which may be useful in standardization of herbal drugs of folk medicinal practice of present era and enrichment of Ayurvedic pharmacopoeia.

**REFERENCES**


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**Table 2. Data showing Qualitative analysis of the phytochemicals of the Acacia nilotica Linn.**

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Sterols</th>
<th>Terpenoids</th>
<th>Carbohydrates</th>
<th>Flavonoids</th>
<th>Alkaloids</th>
<th>Glycosides</th>
<th>Saponins</th>
<th>Tannins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Chloroform extract</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Methanolic extract</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ Presence of constituent; − Absence of constituent