Establishment of Quality Parameters for Flowers of Karanja [Pongamia pinnata (L.) Pierre] through Powder Microscopy and Phytochemical Studies

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ABSTRACT

Background: Karanja (Pongamia pinnata (L.) Pierre), generally known as “Indian beech,” is a plant of high medicinal importance, possessing several beneficial effects such as antimicrobial, wound healing, anti-inflammatory, anti-diabetic, gastroprotective, and neuroprotective, which is widely used in the Ayurvedic system of medicine.

Aim: The aim of this study is to establish the pharmacognostical and physicochemical standards for flowers of an ayurvedic plant, Karanja.

Materials and methods: Pharmacognostical analysis was done by morphological, macroscopical, and powder microscopy. Physicochemical standards were established by ash values, extractive values, phytochemical screening, and high performance liquid chromatography (HPLC) methods.

Results and conclusion: Flower powder microscopy shows diagnostic characters like unicellular trichomes of different sizes and triangular-shaped pollen grains. Loss on drying value of flower powder was 9.7% w/w. Total ash values of drug were found to be 6.15% and acid insoluble ash 0.3% w/w with respect to air-dried crude drug. Water soluble and alcohol-soluble extractives were found to be 25.5 and 6.37% w/w, respectively. Phytochemical characterization of alcoholic extracts revealed the presence of phenols, flavonoids, alkaloids, glycosides, and steroids. Aqueous extract revealed the presence of proteins, carbohydrates, and saponins. Various powder microscopic and phytochemical studies observed in this study can serve as a valuable tool for the authentication of Karanja flowers.

Keywords: Fluorescence analysis, Karanja flowers, Phytochemical analysis, Powder microscopy.


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Conflict of interest: None

INTRODUCTION

Karanja—Pongamia pinnata (L.) Pierre (Family—Leguminosae) Syn. Pongamia glabra Vent. is a medium-sized glabrous tree with a short bole and spreading crown and found almost throughout India up to an altitude of 1,200 m. It is a deciduous tree that grows to about 15–25 m in height with a large canopy that spreads equally wide. Its seeds, stem bark, root, leaves, flowers, fruits, and oil from the seeds are being used in Ayurveda for the treatment of piles, intestinal worms, infections, leprosy, gonorrhea, fever, malaria, skin diseases, bronchitis, diabetes, and in liver affections. Karanja flowers cure vata and kapha, biliousness, and diabetes. The different extracts of Karanja flowers have been scientifically reported for their pharmacological actions. Oral administration of ethanolic extract of Karanja flowers (300 mg/kg body weight) showed a significant antihyperglycemic and antilipid peroxidative effect and an enhancement in antioxidant activity in alloxan-induced diabetic rats. Due to antioxidant property, ethanolic extract of flowers shows nephroprotective activity when administered at a dose of 300 mg/kg and 600 mg/kg. Aqueous, ethanolic extracts, and fresh juice of flowers is reported to possess antibacterial activity. Acetone and petroleum ether extracts of flowers (1,600 µg) have shown good antibacterial activity against Escherichia coli, Bacillus cereus, Bacillus subtilis, Klebsiella pneumoniae, Enterobacter aerogenes, and Staphylococcus aureus. Methanolic and ethyl acetate extracts...
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of flowers show antimalarial activity with IC\textsubscript{50} values of 32 µg/mL and 58 µg/mL, respectively.\textsuperscript{8} Despite the great significance of Karanja flowers, much work has not been reported on pharmacognostical and physicochemical standardization. Hence, an attempt was made for the detailed study of macroscopy, organoleptic characters, powder microscopy, and physicochemical parameters including preliminary phytochemical screening, fluorescence studies, and HPLC analysis of Karanja flowers.

MATERIALS AND METHODS

Collection of Flowers and Identification

The flowers of \textit{P. pinnata} were collected from Suryapet, Telangana, India. The authentication and identification of the plant was done by Dr V Subhose, AD (Ayu), MD (Dravyaguna) at the Department of Pharmacognosy, Central Ayurveda Research Institute for Drug Development, Kolkata. Fresh flowers were used to study the macroscopical parameters, whereas shade-dried flower powder was used for the microscopical and physicochemical investigations.

Chemicals and Instruments

All the solvents and chemical reagents used for the study were of analytical grade. HPLC grade solvents were used for HPLC analysis. Simple microscope, compound microscope, watch glass, microscopic slide, and other common glassware’s were used in this experiment. Photomicrographs were taken with Olympus CX21i LED microscope attached with Magcam DC14 camera. Chloral hydrate, phloroglucinal, iodine, and picric acid were the major chemicals used for powder microscopy. Chemical reagents like Dragendorff’s, Millon’s, and Benedict’s were used for physicochemical studies.

Macroscopy

Macroscopy was done by observing the fresh flowers under a simple microscope and the color, size, texture, etc., were observed. Different macroscopic parameters like shape, odor, and taste were noted.\textsuperscript{9}

Powder Microscopy

The coarse powder of Karanja flower was studied under the compound microscope. The flower powder was macerated in a chloral hydrate reagent. A little quantity of the sample was taken onto a microscopic slide; 1–2 drops chloroglucinol reagent was added and a cover slip was placed above the sample. Then microscopic slides were sealed with the paraffin wax. The prepared slides were mounted and examined under a microscope. Tracing of characteristic structures and cell components was done.\textsuperscript{10}

Photomicrographs

Photomicrographs of the different cellular tissues in macerated sample were taken with various magnifications by Magcam DC14 camera attached to the trinocular microscopic unit. For normal observations, 10× magnification was used for the study of cellular structures, and for micro-observations, 40× magnification was employed.\textsuperscript{11}

Physicochemical Studies

Physicochemical parameters such as loss on drying, total ash, acid insoluble ash, alcohol and water soluble extractives as well as foreign organic matter were determined according to standard methods. Air-dried sample was used for the all these quantitative studies. Extractive values with ethanol and water were determined.\textsuperscript{12}

Phytochemical Screening

Phytochemical screening was performed by using standard procedures. The alcohol soluble extract of flowers was subjected to chemical tests for the detection of different phytoconstituents like alkaloids, glycosides, phenols, flavonoids, etc. and aqueous extract was used for detection carbohydrates, proteins, and saponins.\textsuperscript{13}

Fluorescence Analysis

The chemical nature of the active constituents can be determined by fluorescence analysis. By using standard methods, fluorescence analysis of flower powder was carried out.\textsuperscript{14} The shade-dried powder and after treatment with various chemical reagents like 1 N hydrochloric acid, 5% sodium hydroxide, 50% sulfuric acid, 5% acetic acid, 50% nitric acid, etc., and their fluorescence behaviors were observed in ordinary visible light and also under UV light.

HPLC Analysis

HPLC analysis was carried out on the Waters HPLC Autosampler model 717 plus operated with the Empower2 software. The separation was achieved by reversed-phase Phenomenex C18 column. The mobile phase was being methanol and water (70:30). Karanja flower powder was soaked in methanol for 24 hours. The soaked sample was filtrated with a Whatman filter paper (pore size 0.45 µm). The filtrated sample was taken for the dilution preparations with the mobile phase. The standard concentration sample, i.e., 10 ppm, was injected.\textsuperscript{15,16}
RESULTS AND DISCUSSION

Macroscopy

Flowers pedicellate, bisexual, zygomorphic, 13–16 mm long, purple white when fresh and becomes light brown after drying. Calyx cup-shaped, truncate, gamosepalous, 5-toothed, aestivation valvate; corolla papilionaceous and glabrous, petals 5, polypetalous, aestivation vexillary; stamens 10; monadelphous, the vexillary stamen free below and above; anthers uniform; ovary subsessile, inferior, 1-celled, ovules 2; style in curved, beardless; stigma capitates. Fresh flowers have characteristic odor and bitter taste. Figure 1 shows morphology of flowers.

Powder Microscopy

Flower powder is yellowish brown, with characteristic odor and bitter taste. It shows diagnostic microscopic characters like different sizes of unicellular trichomes, triangle-shaped pollen grains, reticulate vessel, parenchyma cells, epidermal cells with trichomes, and petal veins. Figure 2 represents powder microscopy of Karanja flowers.

Physicochemical Studies

The results of physicochemical parameters are shown in Table 1. Total ash and acid-insoluble ash values were found to be 6.15% and 0.3%, respectively. The extractive values for ethanol and water were found to be 6.37% and 25.5%, respectively. The loss on drying (at 105°C) was found to be 9.7%.

Phytochemical Screening

The results of phytochemical screening are shown in Table 2. Preliminary phytochemical screening revealed that ethanolic extract is a rich source of phenols, flavonoids, alkaloids, glycosides, and steroids. Aqueous extract reported carbohydrates, proteins, and saponins.

Fluorescence Analysis

Karanja flower powder in various reagents was treated with UV radiation of long (365 nm) and short wavelengths (254 nm) and ordinary visible light. They emitted various color radiations at short wavelength but
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Table 3: Fluorescence analysis of Karanja flower powder

<table>
<thead>
<tr>
<th>Chemical reagent</th>
<th>Visible light</th>
<th>Short UV light</th>
</tr>
</thead>
<tbody>
<tr>
<td>As powder</td>
<td>Yellowish brown</td>
<td>Green</td>
</tr>
<tr>
<td>1 N hydrochloric acid</td>
<td>Pale pink</td>
<td>Ash color</td>
</tr>
<tr>
<td>5% sodium hydroxide</td>
<td>Yellowish green</td>
<td>Green</td>
</tr>
<tr>
<td>50% sulfuric acid</td>
<td>Red</td>
<td>Dark red</td>
</tr>
<tr>
<td>5% acetic acid</td>
<td>Cream</td>
<td>Red</td>
</tr>
<tr>
<td>50% nitric acid</td>
<td>Pinkish red</td>
<td>Brown</td>
</tr>
<tr>
<td>Picric acid</td>
<td>Yellowish red</td>
<td>Reddish brown</td>
</tr>
<tr>
<td>Dilute iodine solution</td>
<td>Brown</td>
<td>Dark brown</td>
</tr>
<tr>
<td>5% ferric chloride</td>
<td>Black</td>
<td>Reddish black</td>
</tr>
<tr>
<td>Water</td>
<td>Cream</td>
<td>Ash color</td>
</tr>
<tr>
<td>Alcohol</td>
<td>Cream</td>
<td>Ash</td>
</tr>
</tbody>
</table>

failed to emit radiations at long wavelength. The color change for the flower powder and individual extracts were distinctive and reproducible revealing the solvent properties to the phytoconstituents and data are presented in Table 3.

HPLC Analysis

HPLC chromatogram of Karanja flower methanolic extract showed six fractions of components visualized in the form of peaks (Fig. 3). Six components appeared at different retention times between 0 and 8 minutes. The component number (6) has the highest sharp peak with 25.38% in height and 7.5 minutes of retention time. The results are summarized in Table 4. This HPLC chromatogram acts as a finger printing of herbal medicines and is utilized for the authenticity and quality control of herbal medicines.

CONCLUSION

The present investigation established the qualitative and quantitative diagnostic features for flowers of Karanja through anatomical, powder microscopical, physico chemical, phytochemical, fluorescence, and
HPLC analysis. Phytochemical analysis revealed that alcoholic extract is rich sources of alkaloids, phenols, and flavonoids. HPLC chromatogram of methanolic extract confirmed the presence various chemical components and established the fingerprint profile for identification Karanja flower. These results will help in minimizing adulteration, authentication of original crude drug, and in carrying out further research on Karanja flowers.

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REFERENCES

हिंदी सारांश

पाउडर माइक्रोकोपी एवं फाइटोकेमिकल अध्ययनों के माध्यम से करंज (पोगामिया फिन्नेटा (एल.) पिर्रे) के फूलों के लिए गुणवत्ता मापदंडों की स्थापना

पृष्ठभूमि: आम तौर पर "इंडियन बीच" के नाम से जाना जाने वाला करंज (पोगामिया फिन्नेटा (एल.) पिर्रे) एक उच्च औषधीय महत्व का पौधा है, जिसमें एंटी माइक्रोबियल, वूंड होलिंग, एंटी पायरेटिक, एनाल्जेसिक, एंटी-इन्फ़्लमेंटरी, एंटीडायबिटिक, गैस्ट्रोप्रोटेक्टिव एवं न्यूरॉ प्रोटेक्टिव जैसे कई लाभकारी प्रभाव हैं, जिसे आयुर्वेद चिकित्सा प्रणाली में व्यापक रूप से प्रयोग किया जाता है।

उद्देश्य: इस अध्ययन का उद्देश्य आयुर्वैदिक पौधे करंज के फूलों का फार्माकोग्नोस्टिकल, फिजिकोकेमिकल मानक स्थापित करना है।

सामग्री एवं विधि: मोर्फोलोजिकल, मैक्रोकोपिक एवं पाउडर माइक्रोकोपी द्वारा फार्माकोग्नोस्टिकल विश्लेषण किया गया। फिजिकोकेमिकल मानकों को एश वैल्यूएशन, एक्सट्रैक्टिव वैल्यू, फाइटोकेमिकल स्क्रीनिंग और हाई परस्मोर्स लिवरिड क्रोमेटोग्राफी (एचपीएलसी) विधियों द्वारा स्थापित किया गया।

परिणाम एवं निष्कर्ष: फूल पाउडर का माइक्रोकोपी अध्ययन विभिन्न आकार के यूनिसेंटलार ट्राइकोमस एवं ट्रिकोपीय आकार के पराग कणों जैसे डायग्नोस्टिक कोरेक्ट्र्स को प्रदर्शित करता है। फूल पाउडर को सुरक्षित रूप से इसी की वैल्यू हालिन 9.7% w/w थी। ड्रग की कुल एश वैल्यू 6.15% पायी गई और एयर- ड्राइड क्रूड ड्रग के संबंध में एसिड अघुलशील एश 0.3% w/w थी। पानी में ग्रुननशील और अल्कोहोल-पुलामंग रस्सी क्रमशः 25.5 और 6.37% w/w पाए गए। अल्कोहोलिक एस्ट्रैक्ट्स की फाइटोकेमिकल कैटकराइजेशन से फिनोल, फलेवोग्राफ, अल्कोहोलस, ग्राउकोसाइड और स्टेरॉइड की उपस्थिति का पता लगाया गया। जलीय सतत से प्रोटीन, कार्बोहाइड्रेट और सेपोनिन की उपस्थिति का पता लगाया। इस अध्ययन में पाया गया कि विभिन्न पाउडर माइक्रोकोपिक एवं फाइटोकेमिकल अध्ययन करंज फूलों के प्रमाणीकरण के लिए एक मूल्यवान उपकरण के रूप में कार्य कर सकते हैं।

शब्दकुंजी: प्रतिदिनित विश्लेषण, करंज फूल, फाइटोकेमिकल विश्लेषण, पाउडर माइक्रोकोपी