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VJ Deepthi
Postgraduate Scholar,
Department of Dravyaguna,
S.D.M College of Ayurveda,
Thanniruhalla, B.M Road,
Hassan, Karnataka, India

Pradeep
Associate Professor, Department
of Dravyaguna, S.D.M College of
Ayurveda, Thanniruhalla, B.M
Road, Hassan, Karnataka, India

Karanja Leaf –A pharmacognostical and phytochemical analysis

VJ Deepthi and Pradeep

Abstract

Background: *Karanja* is a very important drug which is botanically identified as *Pongamia pinnata* (Linn.) Pierre. and belongs to Papilionaceae family. It is commonly called as Indian beech in English, Ungu or Pongu in Malayalam and Honge mara in Kannada. *Karanja* is found almost throughout India, in tidal and beach forests and even cultivated as avenue trees. A medium sized semi evergreen glabrous tree with pale green and imparipinnate leaves. Mainly its seeds are used in case of skin ailments. Even its leaves are said to be having Anti-inflammatory activity, Anti-diarrhoeal Activity, Antioxidant and Anti-hyperammonemic Activity and even anti-bacterial activity. This work is an attempt to compare on the preliminary phytochemical and pharmacognostic parameters of leaves of *Karanja* with that of the established standardization in API.

Materials and Methods: Pharmacognostical, Analytical study, Thin Layer Chromatography and High Performance Thin layer chromatography were done.

Results: Phytochemical study showed the presence of Alkaloid, Steroid, Carbohydrate, Tannin, Flavonoids, Terpenoid, Coumarins, Phenol and Quinone. The pharmacognostical and analytical studies have confirmed the authenticity and purity of the drug *Karanja* (*Pongamia pinnata* (Linn) Pierre).

Keywords: *Karanja*, phytochemical study, pharmacognostical study

Introduction

Karanja (*Pongamia pinnata* (Linn) Pierre.) is an important drug which has been used since Vedic period. It is also known by the name Naktamala^[1-3] in Sanskrit. It is called as Pongu or Ungu in Malayalam and Honge mara in Kannada^[4-6]. It belongs to Papilionaceae family. It is a medium sized tree, upto 18m high. *Karanja* is a preferred species for controlling soil erosion and binding sand dunes because of its dense network of lateral roots. The literary review of the drug proved to be having *Vata kapha hara karma*. Root, bark, leaves, flower and seeds of this plant also have medicinal properties and traditionally used as medicinal plants. All parts of the plant have been used as crude drug for the treatment of tumors, piles, skin diseases, wounds and ulcers^[7]. It is also well known for its application as animal fodder, green manure, timber and fish poison^[8]. In traditional medicine, the fruits, seeds and even bark of *Karanja* are seen to be used. Seed oil is used in scabies, leprosy, piles, ulcers, chronic fever and lumbago^[9]. Powdered seed is used in bronchitis, chronic fever, whooping cough and chronic skin diseases and painful rheumatic joints. Seed extracts are having anti hypotensive activity and produce uterine contractions^[10].

The leaves are proved to be possessed with Anti-inflammatory activity, Anti-diarrhoeal Activity, Antioxidant and Anti-hyperammonemic Activity.

Anti-inflammatory activity: It has been reported that the 70% ethanolic extract of *Pongamia pinnata* leaves has potent anti-inflammatory activity against different phases (acute, subacute and chronic) of inflammation without side effect on gastric mucosa They also observed significant anti-pyretic action of the extract against Brewer's yeast-induced pyrexia^[11-13].

Anti-diarrhoeal Activity

In this study the crude decoction of dried leaves of *Pongamia pinnata* for its antimicrobial (antibacterial, anti giardial and antirotaviral) effect were evaluated; and its effect on production and action of enterotoxins (cholera toxin, CT; *Escherichia coli* labile toxin, LT; and *E. coli* stable toxin, ST); and adherence of enteropathogenic *E. coli* and invasion of enteroinvasive *E. coli* and *Shigella flexneri* to epithelial cells. The decoction had no antibacterial, anti giardial and antirotaviral activity, but it was found to reduce the production of CT and bacterial invasion to epithelial cells.

Correspondence

VJ Deepthi
Postgraduate Scholar,
Department of Dravyaguna,
S.D.M College of Ayurveda,
Thanniruhalla, B.M Road,
Hassan, Karnataka, India

These results indicated that the crude decoction of *P. pinnata* has selective anti-diarrhoeal action with efficacy against cholera and enteroinvasive bacterial strains causing bloody diarrhoeal episodes. They attributed the anti-diarrhoeal activity to antimotility, anti-secretory and antimicrobial actions of the compound [14].

Anti lice activity: In the study, various extracts of *Pongamia pinnata* leaves tested against the head louse *Pediculus Humanus Capitis*. A filter paper diffusion method was conducted for determining the potential pediculicidal and ovicidal activity of chloroform, petroleum ether, methanol and water extracts of *Pongamia pinnata* leaves. The findings revealed that petroleum ether extracts possess excellent anti-lice activity with values ranging between 50.3% and 100% where as chloroform and methanol extracts showed moderate pediculicidal effects. The chloroform and methanol extracts were also successful in inhibiting nymph emergence and the petroleum ether extracts was the most effective with a complete inhibition of emergence. Water extract was devoid of both pediculicidal and ovicidal activities. All the results were well comparable with the benzoyl benzoate (25% w/v) [15].

Anti-bacterial Activity: It is reported that the leaves of *Pongamia pinnata* show antibacterial effect. It is clear that the extracts have great potential as antibacterial compounds against enteric pathogens and that they can be used in the treatment of enteric infectious. This plant can be used to discover bioactive natural products that may serve as leads for the development of new pharmaceuticals that address hither to unmet therapeutic needs. It is hoped that this study would lead to the establishment of some compounds that used to formulate new and more potent antimicrobial drugs of natural origin [16-17]

Anti-oxidant and Anti-hyperammonemic: It has been observed that effect of *Pongamia pinnata* leaf extract on circulatory lipid peroxidation and antioxidant status was evaluated in ammonium chloride-induced hyperammonemia [16, 17] rats. It enhanced lipid peroxidation in the circulation of ammonium chloride-treated rats was accompanied by a significant decrease in the levels of Vitamin- A, Vitamin-C, Vitamin-E reduced glutathione, glutathione peroxidase, superoxide dismutase and catalase. It showed that PPET modulates by reversing the oxidant antioxidant imbalance during ammonium chloride induced hyperammonemia and this could be due to its anti-hyperammonemic effect by means of detoxifying excess ammonia, urea and creatinine and antioxidant property [18]

Materials and Methods

The leaves of the plant *Karanja* (*Pongamia pinnata* (Linn) Pierre.) was collected from the botanical garden of Shri *Dharmasthala Manjunatheshwara* Ayurveda College of *Ayurveda* and Hospital, Hassan. and was authenticated from the Department of *Dravyaguna*, *Shri Dharmasthala Manjunatheshwara* College of *Ayurveda* and Hospital, Hassan. Then the dried powdered samples were subjected for pharmacognostic, physico-chemical, phytochemical analysis and also undergone for TLC and HPTLC profile from the Department of pharmacognosy, *Shri Dharmasthala Manjunatheshwara* College of *Ayurveda*, Udupi.

Pharmacognostic study: Here macroscopy or organoleptic evaluation of the leaves along with its powder microscopy was done.

Analytical Study: It includes Physical evaluation of the leaves where Loss on drying, Total ash, Acid insoluble ash, Water soluble ash, Alcohol soluble extractive and Water soluble extractive were examined along with Preliminary Phytochemical tests for the presence of Alkaloids, Carbohydrate, Steroids, Saponin, Tannins, Flavonoids, Phenol, Coumarins, Triterpenoids, Carboxylic acid, Resin and Quinone were examined.

TLC and HPTLC: Thin Layer Chromatography and High Performance Thin Layer Chromatography of the leaves were determined under the retention factor (Rf) values and densitometric scanning at 254, 366 and 620 nm were done respectively.

Results and Discussion

Macroscopical / Organoleptic Evaluation

Karanja leaves are found to be oblong-ovate in shape, with smooth surface. It is dark green in colour and having bitter taste

Microscopy

Petiolute - circular in outline, covered with cuticle, epidermis single layered, consisting of tabular cells; cortex consisting of angular, isodiametric, parenchymatous cells without intercellular spaces, a few cells containing prismatic crystals of calcium oxalate; pericycle present in the form of sclerenchymatous sheath; vascular bundle single, arc-shaped, consisting of xylem and phloem; xylem vessels arranged radially, traversed by xylem rays; a few schizogenous cavities found scattered in cortex.

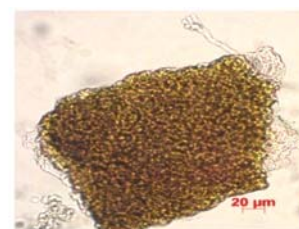
Mid rib - shows single layered epidermis, consisting of tabular cells, covered with thick cuticle, followed by 3-4 layered collenchymatous hypodermis; cortex consists of round to oval, thin-walled parenchymatous cells; pericycle present in the form of sclerenchymatous sheath; vascular bundle, collateral, conjoint and arranged in discontinuous ring; central portion occupied by oval to polygonal thin-walled parenchymatous pith; prismatic crystals of calcium oxalate present in cortex, phloem and pith.

Lamina -shows single layered epidermis covered with thick cuticle; palisade two layered; spongy parenchyma 3-5 layered, a few containing prismatic crystals similar to midrib, occasionally a few spongy parenchyma cells get elongated and look like palisade cells, palisade ratio 3.5-50; vein islet number 18-25 per mm square; stomata anisocytosis, present in lower surface; stomatal index 12.5-20.

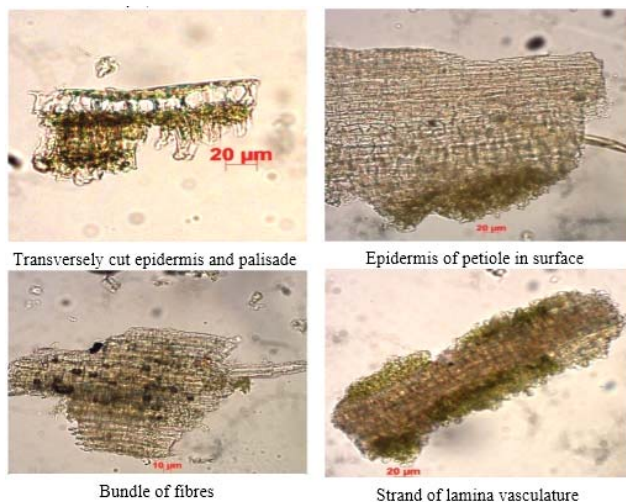
Powder Microscopy: The leaves of *Karanja* was found to be having Mesophyll tissue, Palisade with underlying epidermis, Transversely cut epidermis and palisade, Epidermis of petiole in surface, Bundle of fibres and Strand of lamina vasculature.



Mesophyll tissue



Palisade with underlying epidermis



Analytical study

(i) Physical evaluation

Table 1: Showing results of physical analysis of the drug

Parameter	Results n = 3 %w/w
	Karanja leaf powder
Loss on drying	12.12
Total Ash	7.98
Acid Insoluble Ash	0.69
Water soluble Ash	3.19
Alcohol soluble extractive value	4.38
Water soluble extractive value	14.60

Loss on drying – This is the method to determine the moisture content of a drug. It aids to prevent the decomposition of the drugs either due to chemical change or microbial contamination.

From the results obtained for loss on drying, *Karanja* (*Pongamia pinnata* (Linn.) Pierre) leaves have shown 12.12% of moisture content.

Determination of ash value

Ash value is the criterion to judge authenticity and purity of crude drugs. The residue remaining after incineration is the ash content of the drug. These could be inorganic salts such as carbonates, sulphates, phosphates, silicates etc. naturally occurring in the drug or adhered to it or deliberately added to it in order to adulterate the drug. Since the drugs were collected personally from their natural habitat there was no scope of any adulteration. Total ash is to measure the total amount of plant material remaining after ignition of the drug. Acid insoluble ash or water soluble ash content is the residue obtained after boiling the total ash either with dilute hydrochloric acid or water which measures the amount of sand and silica matter present in the drug.

The results of ash value of leaves of *Karanja* (*Pongamia pinnata* (Linn.) Pierre) showed total ash content of 7.98% w/w, acid insoluble ash was 0.69% w/w while water soluble ash was 3.19% w/w.

Determination of extractive value

Extractive value measures the nature of the chemical constituents present in a crude drug. It is essential for the estimation of specific chemical constituents soluble in that particular solvent used for extraction.

The results of alcohol soluble and water soluble extractive values of *Karanja* (*Pongamia pinnata* (Linn.) Pierre) leaves showed 4.38% w/w and 14.6% w/w respectively.

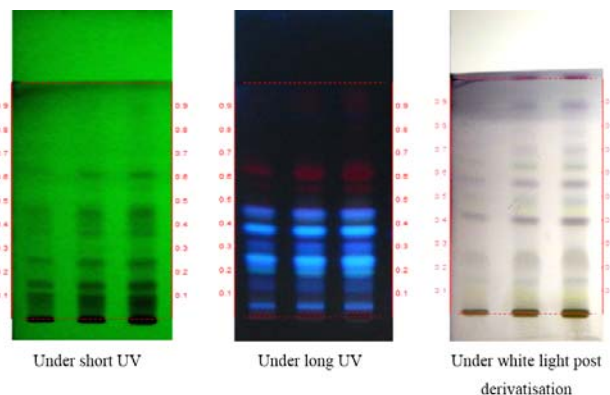
ii) Chemical evaluation

Table 2: Showing chemical constituents reported during preliminary phytochemical screening

Test	Inference
Alkaloid	+
Steroid	+
Carbohydrate	+
Tannin	+
Flavonoids	+
Saponins	-
Terpenoid	+
Coumarins	+
Phenol	+
Carboxylic acid	-
Amino acids	-
Resins	-
Quinone	+

Preliminary phytochemical screening is carried out to establish chemical profile of a crude drug and detect the various plant constituents. The chemical tests are used to identify crude drugs to determine purity. From the analysis of the results obtained from the preliminary phytochemical screening conducted in this study, Alkaloids, Steroids, Carbohydrate, Tannin, Flavonoids, Triterpenoids, Coumarins, Phenols and Quinone were the chemical constituents found in the drug.

(iii) TLC and HPTLC



Solvent system: Toluene: Ethyl acetate (7:1)

Track 1: *Karanja* (4µl)

Track 2: *Karanja* (8µl)

Track 3: *Karanja* (12µl)

This method helps in separation of chemical constituents between two phases: a mobile and a stationary phase. It is an important analytical tool for qualitative and quantitative analysis of the drugs. In this study, Thin layer chromatography (TLC) and High Performance Thin Layer Chromatography (HPTLC) methods were conducted. The plates developed were visualised in UV 254nm, 366nm and then post-derivatised.

From the results obtained, at 254 nm the drug has shown green spots, at 366 nm it showed blue and red spots and at post derivatised period it has shown yellow, blue, purple and green spots. The Rf values detected at 254 nm were 0.09, 0.15, 0.24, 0.29, 0.36, 0.47, 0.55 and 0.61 and at 366 nm were 0.05, 0.12, 0.19, 0.24, 0.29, 0.36, 0.44, 0.50, 0.55, 0.61 and 0.85. during the post derivatization period, *Karanja* (*Pongamia pinnata*(Linn.)Pierre) showed Rf values of 0.09, 0.15, 0.24, 0.41, 0.44, 0.47, 0.55, 0.63, 0.70, 0.78 and 0.88. From the results of HPTLC documentation, 12 chemical constituents at 254 nm, 11 chemical components at 366 nm and 9 chemical components at 620 nm were found in the leaves of *Karanja* (*Pongamia pinnata* (Linn.) Pierre).

Conclusion

The drug *Karanja* (*Pongamia pinnata* (Linn) Pierre) has been used since Vedic period for different diseases. This drug is easily available throughout India. Phytochemical study showed the presence of Alkaloid, Steroid, Carbohydrate, Tannin, Flavonoids, Terpenoid, Coumarins, Phenol and Quinone. The pharmacognostical and analytical studies have confirmed the authenticity and purity of the drug *Karanja* (*Pongamia pinnata* (Linn) Pierre).

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