



Preliminary Phytochemical Pharmacognostic Studies on the Different Source Plants of *Kadamba*

Research article

Anand Kumar M P^{1*}, Srilakshmi M²

1. Reader & H.O.D, 2. PG Scholar,
P.G, Dept of Dravyaguna, Dr.B.R.K.R.Govt. Ayurvedic College, Hyderabad.

Abstract

Ayurvedic Acharyas had a great knowledge of the medicinal plants because they used to live near the forests which are a good source of the natural resources. But now days due to urbanization, deforestation and industrialization certain medicinal plants have become extinct and became difficult to make use of them in therapeutics. Hence demand is naturally increased as there was restriction of entry into forests imposed to protect the endangering plants.

By increasing demand of such drugs which are considerably less in availability, by adulteration/substitution of the raw materials has taken its place creating havoc in the Ayurvedic pharmaceutical industry. Hence the need is to standardize these herbs through botanical surveys, Pharmacognostical studies, assessment of the quality material available in particular area of market etc.

In the present study of *Anthocephalus cadamba* MIQ (*Kadamba*), *Adina Cordifolia BENTH & HOOK* (Dhara *Kadamba*), *Mytragyna parvifolia KORTH* (Dhuli *Kadamba*), for standardization collected from different places.

Keywords: Standardization, Biological assay, Nipa, Nighantu, H.P.T.L.C, NIN method

Introduction:

Kadamba is a plant drug that is widely used in many instances in the classical Ayurvedic texts for various ailments (1,2,3). Charaka mentioned *Kadamba* to be used as vegetable and fruit (4). Susrutha has described it in first group of sour fruits(5).

History of *Kadamba* can be traced back to Vedas, Puranas and Samhita. There is a reference of root and branches of *Kadamba* in different books of vedic period. It was used for dantadhavana. In

paraskara Guhyasutra 1/21, Atharva parishista 26/5/1-4, Yajna valkya shiksha 34, Manduki shiksha 4/1 etc., *Kadamba* has been mentioned. It is mentioned as Nipa in Panineeya Asthadhyayi, Pathanjali Mahabhashya, Gubhilagruhya sutra and Shulwa Prathishakhya.

Susrutha has mentioned *Kadamba* and *Nipa* as two different plants. But in most instances *Kadamba* and *Nipa* are used as synonyms(5).

In Ramayana while describing *Chitrakuta*, *Nipa* is mentioned one among the fruit bearing plants. In Mahabharata while describing *Dwaitavana*, *Nipa* and *Kadamba* are included among fruit bearing plants. There is mentioning of *Kadamba* tree in Srimad Bhagavata during the occasion of Kaliya mardana. It is also told that Lord Sri Krishna was very fond of this tree. Pathanjali in his Mahabhasya,

*Corresponding author:

Dr. Philip Anand Kumar

Reader & H.O.D,

P.G, Dept of Dravyaguna,

Dr.B.R.K.R.Govt. Ayurvedic college,

Hyderabad.

E-mail: philipmudumala@gmail.com



mentions the *Kadamba*, while describing fruit varieties. *Kadamba* was favourite tree of ancient India.

In spite of the above history about the plant, the identity of the plant still remains a controversy, because many plants are being used as the source plants of *Kadamba*. Susrutha mentioned *Kadamba* and *Nipa* as two different plants. But latter in the *nighantus*, they are mentioned as the synonyms of the same plant. And different *nighantus* have mentioned different varieties of *kadamba* like *Dhara Kadamba*, *Dhuli Kadamba*, *Bhoomi Kadamba* *Raja Kadamba* etc. The source plants available for these include *Anthocephalus cadamba* MIQ., *Adina Cordifolia* BENTH & HOOK., and *Mytragyna parvifolia* KORTH.

To evaluate the phytochemical similarities between these plants, a preliminary phytochemical study has been carried out on the above mentioned source plants of *Kadamba*.

Pharmacognostic Study:

The present Pharmacognostic study of *Anthocephalus cadamba* MIQ (*Kadamba*), *Adinacordifolia* BENTH & HOOK (*Dhara Kadamba*) and *Mytragyna parvifolia* KORTH (*Dhuli Kadamba*) includes the following.

1. Morphological Identification
2. Collection.
3. Powder Microscopic study.
4. Organoleptic properties.
5. Preliminary phytochemical analysis.
 - a. Determination of the moisture content
 - b. Total ash
 - c. Acid soluble ash
 - d. Water soluble ash
 - e. Alcohol soluble extract
 - f. Water soluble extract
 - g. Determination of PH

Identification:

The plants were identified properly by their morphological features and are

compared with the standard literature available. The morphological features of the plants are as follows:

Anthocephalus cadamba MIQ., is a medium sized tree and is identified by its dark brown bark with irregular woody scales, elliptic – oblong leaves, small orange yellow coloured globose heads and globose pseudocarpic fruit.

Adina cordifolia BENTH & HOOK., is a large deciduous tree with dark grey bark with exfoliating in irregular woody scales, orbicular shortly acuminate leaves, Yellow flowers in globose pedunculate heads and dehiscent capsule.

Mytragyna parifolia KORTH., is also a large tree with light grey smooth exfoliating bark with elliptic leaves, greenish yellow fragrant flowers and an oblong capsule.

Collection and processing:

Leaves of the plants were collected and are shade dried and powdered. The powdered plant materials are used for the study.

Powder microscopy studies:

Materials and methods:

The collected leaves of *Anthocephalus cadamba* MIQ., *Adina Cordifolia* BENTH & HOOK and *Mytragyna parvifolia* KORTH., were fixed in 3:1 Alcohol: Acetic acid solution and kept for two days. After two days, the powder material is mounted on the glass slides and is studied under compound microscope at different magnifications and the following were observed.

Powder microscopy of *Anthocephalus cadamba*:

Isolated fragments of uniseriate conical hairs either whole or broken are found. Few, whole unicellular conical hairs, pieces of epidermis of lower surface with wavy anti clinical walls and stomata, few pieces of isolated stomata and prismatic crystal of calcium oxalate are found in the microscopy.

**Powder microscopy of *Mitragyna parviflora*:**

Pieces of tracheary tissue with vessels and tracheary tissue with helical, calceiform and pitted walls were found in the microscopy. Pieces of translucent resinous masses, few, isolated sphaeroraphides of various sizes, pieces of epidermis with parasitic stomata are also found in the microscopic study.

Powder microscopy of *Adina Cordifolia*:

Pieces of broken tracheary tissue with calciform, reticulate and broadened pitted elements with attached fibres and parenchyma, some pieces of parenchyma with polygonal to rounded cells with intercellular spaces, few translucent resinous droplets appearing are found in the microscopic study of *Adina cordifolia*.

Organoleptic characters:

It involved the identification of the colour, touch, odour and taste. The results are as follows:

Samples	Colour	Touch	Odour	Taste
<i>Adina cordifolia</i>	Green	Slightly coarse.	Slightly pungent, causes choking.	Bitter
<i>Mitragyna parvifolia</i>	Moss green	Slightly coarse	Not characteristic.	Slightly Bitter, Astringent.
<i>Anthocephalus cadamba</i>		Slightly coarse	Pungent.	Acrid, causes tingling.

Physico-Chemical Analysis:**Determination of moisture content:****Method:**

2 gms of sample is taken in a previously weighted Petri plates. Petri plates with the samples were kept in the oven and maintained at 110°C for drying. After 3 hrs Petri plates were taken out weight was noted down. This procedure is repeated for 4-5 times until the constant weight is reached.

% of moisture = difference in weight / weight of the sample X 100

Result:

Anthocephalus cadamba 4.0% W/W
Adina cordifolia 3.0% W/W
Mytragyna parvifolia 1.25% W/W

Total Ash:**Method:**

2 gms of each powder is taken in 3 heated silica dishes to avoid any moisture content. The materials are ignited to 100° - 150°C in an electric ignition till the

charring of the drug material. Then it is kept in an incinerator at 50°C, temperature allowed to roll back to Zero; then it is removed from furnace and cooled in a desiccators to room temperature and weighed.

Total ash = weight of residue / weight of the sample X 100

Result:

Anthocephalus cadamba 5.0% W/W
Adina cordifolia 5.45% W/W
Mytragyna parvifolia 7.5% W/W

Acid Insoluble Ash Estimation:**Method:**

The total ash, which was obtained was boiled for 5 minutes with 25ml of diluted hydrochloric acid, collect the insoluble matter in a ash less filter paper, wash with hot water and ignited to constant heat.

% of acid insoluble ash = difference in weight / weight of sample X 100

Result:

Anthocephalus cadamba 1.50% W/W



<i>Adina cordifolia</i>	0.65%W/W
<i>Mytragna parvifolia</i>	1.5% W/W

Water Soluble Ash:**Method:**

The total ash, which was obtained, was boiled for 5 minutes with 25 ml of water. Collect insoluble matter in Gooch crucible or on an ash less filter paper, wash with hot water and ignite it for 15 minutes at a temperature not exceeding 450°C.

Water soluble ash=Weight of total ash-weight of insoluble matter

% of water soluble ash=Difference in weight /weight of sample x100

Result:

<i>Anthocephalus cadamba</i>	1.0%W/W
<i>Adina cordifolia</i>	2.18%W/W
<i>Mytragna parvifolia</i>	1.5%w/w

Alcohol Soluble Extract:**Method:**

5 gm powder was taken in a volumetric flask, 100 ml of alcohol was added to it and flask was kept for 24 hrs. The solution was filtered next day and 25 ml of this filtrate was evaporated in a previously weighed evaporating dish on a water bath. Later it was dried in the oven at 110 C to remove the traces of alcohol. Constant weight was noted down.

% alcohol soluble extractive = difference in weight / weight of the sample X 100

Result:

<i>Anthocephalus cadamba</i>	20.0%W/W
<i>Adina cordifolia</i>	12.0%W/W
<i>Mytragna parvifolia</i>	16.0%w/w

Water soluble extract:**Method:**

5 gm powder was taken in a volumetric flask few drops of chloroform and subsequently 100 ml of distilled water was added to it. It was kept for 24 hrs, shaking frequently for the first 6 hrs. next day the solution was filtered and 25 ml of this filtrate was evaporated in a previously

weighed evaporating dish on a water bath. Later it was dried in the oven at 110 C to remove the traces of water. Constant weights were noted.

% of water soluble extractive =Difference in weight /weight of sample x100

Result:

<i>Anthocephalus cadamba</i>	10.0%W/W
<i>Adina cordifolia</i>	10.0%W/W
<i>Mytragna parvifolia</i>	14.0%w/w

Determination of pH:

The determination of p^H was carried out at room temperature of 25°C. Calibration of the apparatus was done using buffer solution to pH7 water soluble and alcohol soluble solutions was kept ready, then the electrodes were immersed in both the solutions and readings were recorded. The results are as follows:

SAMPLES	Ph alcohol	Ph Water
<i>Anthocephalus cadamba</i>	5.46	4.34
<i>Adina cordifolia</i>	5.63	4.53
<i>Mytragna parvifolia</i>	4.87	4.26

HPTLC:

HPTLC was performed to develop phytochemical finger printing. It was performed using 10 x 10 cm silica gel 60 F254 precoated HPTLC plates [MERCK, Germany]. 10 µl Volume of each extract was applied on plates with the help of Camag linomat - 5 applicator [CAMAG, Switzerland] fitted with 100 µl Hamilton micro syringe. The chromatograms were developed at room temperature in a 10 x 10 cm twin trough chamber using solvent systems Toluene:Ethyl acetate in a ratio of 6:4 for the etanolic extract of *Anthocephalus cadamba* MIQ, *Adinacoordifolia* BENTH & HOOK, and *Mitragyna parvifolia* KORTH, respectively.



After the development chromatograms of saponin were derivatized with 20% Antimony trichloride in chloroform in a ratio of 20:100ml followed by heating at 110°C in preheated oven for 10 min. These chromatograms were scanned and evaluated under wave lengths of 254nm & 366nm using a camag TLC scanner [CAMAG Switzerland] to get graphical representation of finger prints.

From the HPTLC finger printing of the three drugs, presence of the saponins as the principle chemical compounds were identified.

U.V.Visible spectrophotometer:

Preparation of sample

5 gm of powder of each viz. *Anthocephalus cadamba* MIQ, *Adina cardifolia* BENTH and HOOK and *Mitragyna parvifolia* KORTH were extracted with 100ml Methanol. From the filtrate 3ml of extract is treated with charcoal and centrifuged.

Scanning of extract:

50 µl of *Anthocephalus cadamba* MIQ and *Adina cardifolia* BENTH AND HOOK diluted to 3ml with Methanol, while 150 µl *Mitragyna parvifolia* was diluted to 3ml with Methanol. The above extracts were scanned in the U.V.Visible region i.e 190-700nm using methanol as a plank solution by using Sphectronic Unicam Helios Spectrophotometer.

It is evident from the UV graph that the *Anthocephalus cadamba* MIQ has revealed 4 peaks at wave lengths of 230nm, 235nm, 282 nm and 324-329nm.

Mitragyna parvifolia KORTH exhibits 3 peaks 324 nm- 329nm and

Adina Cordifolia BENTH & HOOK exhibited 2 peaks between 230-235nm and 324-329nm.

This shows that the peaks obtained at 324nm to 329nm correspond to the same chemical content in all the three specimens.

DISCUSSION:

Kadamba has synonyms such as Kadanbarya, Nipa, Pavrishenya, Kalambaha, Pulaki, Priyaka etc. all these indicates that the tree bears fruits which are sour taste which produces horripilation. Flowers are beautiful which attract people by its fragrance, also blossoms during rainy season, are the specific characters of *Kadamba*. In other words resembling on the characters the homonym names were also given to Dhara *Kadamba*. Since *Kadamba* and Dhara *Kadamba* bears synonyms and also morphologically similar characters in some aspects, such as yellowish orange round flowers with sweet fragrance, fruits which are spear shaped, also flower blossoms during rainy season. All these indicate Dhara *Kadamba* is a variety of *Kadamba*.

Physic chemical study of the powder of the 3 drugs have been carried out and it was found that *Mytragna parvifolia* KORTH is having higher total ash, acid insoluble ash and water soluble extractive in comparison to other 2 varieties, while *Anthocephalus cadamba* MIQ showed higher moisture content and alcohol soluble extractive. *Adina Cordifolia* BENTH & HOOK has higher water soluble ash.

U.V.Visible spectrophotometer of extracts of 3 varieties of *Kadamba* exhibited that the peaks obtained at 324nm to 329nm correspond to the same chemical content in all the three specimens.

HPTLC studies revealed that the samples contained saponins as the main chemical contents.

Conclusion:

From the preliminary phyto chemical study it can be concluded that the three drugs being used as the source plants of the *Kadamba* are having some similarity in the phytochemical properties. As the study is only a preliminary work



further phytochemical and clinical analysis of the drugs have to be done to know the proper identity of the drug.

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