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Pharmacognostical Evaluation and Phytochemical Screening of *Neolamarckia Cadamba*

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Abstract

The Pharmacognostical studies of *Neolamarckia Cadamba* (Roxb.) Leaf the purpose of identification and differentiation from related species. The macroscopic and microscopic features of the Leafs were studied, including the use of powder microscopy with the aid of suitable tools and reagents. Physicochemical parameters such as ash value, extractive value and weight loss on drying were also determined. The Leafs powder was successively extracted with different solvents followed by preliminary phytochemical screening of the extracts. Preliminary phytochemical screening of different extracts revealed the presence of alkaloids, carbohydrate, protein, gum, steroid, tri-terpenoids, saponin, flavonoids and tannin in the Leafs. The scientific parameter is necessary to identify the exact plant material and to find its quality and purity. These studies indicated the possible information for correct identification and standardization of this plant material.

Keywords: *Neolamarckia Cadamba*; Leafs; Pharmacognostical studies; Macroscopic; Microscopic; Phytochemical

Abbreviations: WHO: World Health Organization; Pet. Ether: Petroleum Ether; *N. Cadamba*: *Neolamarckia Cadamba*; Phytochemical

Introdution

Neolamarckia Cadamba Miq; syn. *Anthocephalus Kadamba* (Family: Rubiaceae) commonly known as 'Kadamba' in Ayurveda1 is a large deciduous tree between 37.5-45meter height. The stem of younger trees appears greyish-green with smooth bark. As it gets older, the bark gets rough and grey with longitudinally fissured. Leafs glossy, dark green, opposite, simple pulvinus base sub sessile to petiolate, broadly ovate to elliptical-oblong, entire, apex marinate and venation pinnate. The flowers that appear from August to October are orange to yellow. Inflorescence in clusters, terminal globose heads, subsessile and fragrant. Fruitlets numerous with upper parts containing 4 hollow or solid structures. Seed trigonal or irregularly shaped. The trees found in the greater part of India in moist localities in West Bengal, Bihar, Andhra Pradesh, Karnataka, Kerala and peninsular India [1-2].

The plant finds its application in several traditional and folklore systems of medicine around the globe. The tribal of India use the Leafs paste orally against dyspepsia and locally applied in mouth ulcer in children. Leafs are nutritious, astringent and their decoction is reported to be used for gargling in aphthae or stomatitis [3]. Dried powdered Leafs used as anthelmintic and the tribal people of India used hot water extract of the Leafs as an astringent, stomatitis and for washing wounds in throat [4-8]. The flowers are used as vegetable and as gurgle to remove the foul smell from mouth. The fruit is cooling and said to destroy the phlegm and impurity of blood when eaten. The ripened fruit are aromatic, acidic with astringents property. Lod has taken ripened fruits as carminative/masticate. Fruit juice is given during fever and gastric disturbance. Similarly stem barks reported to possess astringent, febrifuge, antiseptic and acts as diuretics. Juice of the bark given orally against cough, fever and in inflammation of eyes. Dried stem bark also used as folk medicine (ethno medicines) in the treatment of various skin diseases, anemia, uterine complaints and for improvement of semen quality. Lod has apply stem bark paste on swelling of legs and juice to cure eye inflammation.

The fresh juice of the bark is applied to the heads of infant when the fontanels sunken. Mundas prescribe the bark paste duly suspended in water in reducing blood sugar in the patients with diabetes mellitus [4]. Several pharmacological and biological tests have been reported on this plant are evident from literatures. Alcoholic extracts of dried Leafs possess analgesic, anti-inflammatory antimicrobial, wound healing, antioxidant and anti- alarial antibacterial and antifungal, activities. However, only a few phytochemicals have been reported on this plant in the literature types of sapogenins such as cadambagenic

acid, quinovic acid and ß-sitosterol was isolated from the bark. Few alkaloids are also reported from the bark and Leafs like cadambine, 3α-dihydrocadambine and isohydrocadambine [4]. In the light of all the above and keeping the medicinal overview of *N. Cadamba*, the present investigation was being carried out to study some Pharmacognostical features of the Leafs as a whole including its intact and powdered form available in the literature [5]. The studies were carried out in accordance with WHO General Guidelines for Herbal Drug Standardization methodologies. 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 [6].

Materials and Methods

Collection, authentication and preparation of plant material

The Leafs of the *Neolamarckia Cadamba* was collected from Monad University Hapur, Uttar Pradesh. For authentification, I made a herbarium in which plant part are attached. Then it was authentified from the taxonomist of NISCAIR, New Delhi. After authentication, fresh Leafs were collected in bulk, washed with potable water to remove adhering dirt followed by rinsing with distilled water, and then shade dried and powdered [7].

Microscopy

The following macroscopic characters for the fresh and dried Leafs were noted: surfaces, size and shape, fracture, texture, color, odour and taste. Leafs are slightly aromatic with unpleasant taste. Anatomical characteristics of the Leafs Fresh Leafs pieces were subjected to dehydration procedure from aqueous and alcohol [8].

Powdered microscopic characteristics

The shade dried powdered Leafs screened through sieve no. 40 was used for the powdered drug analysis. The specimens were separately treated with glycerin, N/20 iodine solution, 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 through temporary micro slide preparation taking the mount ant glycerin and were observed under a compound microscope [9-10].

Preliminary phytochemical studies

The dried and powdered Leafs (50g) was successively extracted with petroleum ether (60-80°C), chloroform, ethanol and water by reflux for 24h by Soxhlet apparatus. Following extraction, the liquid extracts were concentrated under reduced pressure using rotary evaporator to yield dry residues. The extracts were subjected to preliminary phytochemical screening using standard procedures to determine the nature of phytoconstituents content [11]. The result of the preliminary phytochemical screening of different extracts (Table 1) showed presence of alkaloids (in chloroform and methanol extracts), carbohydrates, proteins, gum (in aqueous extract), steroids (in petroleum ether, chloroform and ethanol extracts), triterpenoid (in petroleum ether and chloroform extracts), saponin (in chloroform, ethanol and aqueous extracts) and flavonoids and tannin (in ethanol and aqueous extracts) [12].

Table 1: Preliminary phytochemical profiles of various extracts of *Neolamarckia cadamba* Test.

Test	Pet. Ether	Chloroform	Ethanol	Aqueous
Steroids and sterols	+	+	+	_
Triterpenoid	+	+	_	_
Alkaloids	_	+	+	_
Saponins	_	+	+	+
Flavonoids	+	_	+	+
Carbohydrates	_	_	+	+
Gums and mucilage	_	_	_	+
Proteins and amino	_	+	+	+
Tannins and phenolic compounds	+	_	+	+

+ = present; - = absent

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 treatises [13].

Results

Macroscopic characteristics of the Leafs

Leafs coriaceous, entire margin, elliptical-oblong or ovate, pulvinus base, with acute or shortly acuminate. It is often used in the form of powder (nygrodhadi kvatha churna) which is a herbal formulation.

Powder microscopy of *Neolamarckia 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.

Microscopic characteristics

The microscopic study of *Neolamarckia Cadamba* leaf showed the presence of simple elongated, unicellular trichomes, rubiaceous types of stomata on the lower side of the leaf, starch grains, crystals of calcium oxalate, wedge-shaped vascular bundles, and phloem in the form of ring and oil globules. The

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leaves of *Neolamarckia Cadamba* having methyl salicylates aroma when crushed by hands.

TLC

TLC was performed to develop phytochemical finger printing. It was performed using 2x10cm TLC plates coated by silica gel G. 10µl Volume of each extract was applied on plates with the help of capillary a thin layer (0.25mm). In addition, a binder like gypsum is mixed into the stationary phase to make it stick better to the slide. TLC plate on the side with the white surface draw a thin line with pencil. The thin end of the spotter is placed in the dilute solution; the solution will rise up in the capillary (capillary forces). Touch the plate briefly at the start line. Allow the solvent to evaporate and spot at the same place again. This way you will get a concentrated and small spot. A TLC plate can be developed in a beaker. Place a small amount of solvent (mobile phase) in the container. The solvent (eluent) travels up the matrix by capillarity, moving the components of the samples at various rates because of their different degrees of interaction with the matrix (stationary phase) and solubility in the developing solvent. Non-polar solvents will force non-polar compounds to the top of the plate, because the compounds dissolve well and do not interact with the polar stationary phase. Allow the solvent to travel up the plate until~1 cm from the top. Take the plate out and mark the solvent. The components, visible as separated spots, are identified by comparing the distances they have traveled with those of the known reference materials. Measure the distance of the start line to the solvent front. Then measure the distance of center of the spot to the start line. Divide the distance the solvent moved by the distance the individual spot moved. The resulting ratio is called Rf – value.

The Rf (retardation factor) depends on the following parameters:

1. Solvent system
2. Absorbent (grain size, water content, thickness)
3. Amount of material spotted
4. Temperature

The chromatograms were developed at room temperature in a 10x10cm twin trough chamber using solvent systems Toluene: Ethyl acetate in a ratio of 6:4 for the ethanolic extract of *Neolamarckia cadamba*. 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 10min. These chromatograms were scanned and evaluated under wave lengths of 254nm & 366nm using a camag TLC to get graphical representation of finger prints. From the TLC finger printing of the ethanolic extract drugs, presence of the saponins, alkaloids, glycosides, steroids, flavonoids as the principle chemical compounds were identified.

UV Visible spectrophotometer

Preparation of sample 5 gm of powder of each *Neolamarckia cadamba* were extracted with 100 ml Ethanol. From the filtrate 3ml of extract is treated with centrifuged.

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 alkaloids, glycosides , steroids and sterols, saponins, flavonoids, phenolic compounds and volatile oils 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. *N. Cadamba* is often confused with other species due to their relative similarities. The species has been taxonomically described as distinct species by earlier workers. The Leafs finds its application in several other traditional and folklore systems of medicine around the globe that have been previously described and surprisingly no pharmacopoeia 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 [14-21].

Conclusion

In the present study, leafs of *Neolamarckia Cadamba* Roxb. Pharmacognostical Evaluation for the identification of various Phytoconstituents and rest of extracts were utilized for pharmacological screening. The various extracts after the Pharmacoganostics Evaluation have shown the presence of following active principles. Distilled water extract: Steroids, Glycosides, Alkaloids, Tannins, Phenolic compounds, Flavonoids. From the ongoing studies, it can be concluded that the above macroscopic and microscopic studies together may be used as a tool for identification of *Neolamarckia Cadamba* with its Pharmacognostical characteristics, discriminating it from its other species diversity.

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