

Evaluation of Antihelminthic Activity of Leaves Extract of Methanolic and Aqueous Extracts of *Neolamarckia cadamba* Leaves

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ABSTRACT

Herbal medicines include herbs, herbal materials and finished herbal products that contains active ingredients from parts of plant .In last few decades there has been an exponential growth in the field of herbal medicines. *Neolamarckia cadamba*, commonly called KADAM, is an ever green, tropical tree. The genus name honours French naturalist Jean Baptiste Lamarck. The species has widely but incorrectly called ANTHOCEPHALUS CHINESIS. It has scented orange flower in dense globe shaped cluster. Kadam features in Indian religions and mythologies. The microscope 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, phloem in the form of ring and oil globules. The traditional use of leaf of *NEOLAMARCKIA CADAMBA* as an anti-helminthic has been conformed as the leaf extract displayed profound anti-helminthic activity in the study. Further it would be interesting to isolate the possible phytoconstituent and characterize the active constituents which may be possible for the anti-helminthic activity and to possible the mechanism of action.

INTRODUCTION

PHARMACOGNOSY DEFINITION

"Pharmacognosy" derives from two Greek words, "Pharmakon" or drug, and "gignosis" or knowledge. While representing a classical field of sciences, Pharmacognosy has undergone significant change in recent years and today represents a highly interdisciplinary sciences which is one of five major areas of pharmaceutical education.

Its scope includes the study of the physical, chemical, biochemical and biological properties of drugs substances, or potential drugs or drug substances of natural origin as well as the search for new drugs from natural sources .Research problems in pharmacognosy include studies in the areas of phytochemistry, microbial chemistry, biosynthesis, biotransformation, chemotaxonomy and other biological and chemical sciences.

IMPORTANCE OF HERBAL DRUGS

Herbal medicines include herbs, herbal materials and finished herbal products that contains active ingredients from parts of plant .In last few decades there has been an exponential growth in the field of herbal medicines. It is

getting popularized in developed countries owing to its natural origin and lesser side effects. Ayurvedic medicines emphasis the relation ship between man and plants throughout the development of human culture. Due to toxicity and side effects of allopathic medicines the use of herbal medicines has lead to sudden increase in the number of herbal drugs manufacturer.

DESCRIPTION

Neolamarckia cadamba, commonly called KADAM, is an ever green, tropical tree. The genus name honours French naturalist Jean Baptiste Lamarck. The species has widely but incorrectly called ANTHOCEPHALUS CHINESIS. It has scented orange flower in dense globe shaped cluster. Kadam features in Indian religions and mythologies.

- A fully mature kadam tree can reach up to 45m in height.
- It is a large tree with a broad crown and straight cylindrical bole.
- It is quick growing with a broad spreading branches and grows rapidly in the first 6-8 years.
- The trunk are 13-32 cm long.
- Flowering usually begins when the tree is 4-5 years old.

- Kadam flowers are sweetly fragrant, red to orange in colour.
- The fruit of n.cadamba occurs in small, fleshy capsule packed closely approximately 8000 seeds.

Botanical features

- Leaves are glossy green, opposite, simple more or less sessile to petiolate, ovate to elliptical with dimensions of 15-50 cm by 8-25 cm.
- Flowers are bisexual, 5merous, calyx tube funnel shaped, corolla gametophyllous saucer shaped with a narrow tube, the narrow lobes imbricate in bud.
- Stamens 5, inserted on the corolla tube, filamentous short, anthers basifixed.
- Ovary inferior, bilocular, sometimes 4-locular in the upper part, style exerted and spindle shaped stigma.
- Fruitlets numerous with their upper parts containing 4 hollow or solid structure
Fruit and seed description:
The fruits are small capsules, packed closely together to form a fleshy ,yellow or orange, coloured inflorescence containing approx. 8,000 seeds .The small capsules split into four parts

MICROSCOPY

The microscope 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 ,phloem in the form of ring and oil globules. The leaves of neolamarckia cadamba having methyl salicylate aroma when crushed by hands. The bark consists of thin walled, rectangular cells, phloem fibres some cells consists of chloropyll and prismatic crystals of calcium oxalate.

ANALYTICAL PARAMETERS OF NEOLAMARCKIA CADAMBA

S. No.	PARAMETERS	RESULTS
1	FOREIGN MATTERS	NMT 2 %
2	TOTAL ASH	8-9%
3	ACID INSOLUBLE ASH	0.6-1.5 %
4	WATER SOLUBLE ASH	2-2.5 %
5	SULFATED ASH	4-4.5 %
6	ALCOHOL SOLUBLE EXTRACTIVE VALUE	4-6 %

7	WATER SOLUBLE EXTRACTIVE VALUE	4-4.5 %
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PHYTOCHEMISTRY OF NEOLAMARCKIA CADAMBA

Neolamarckia cadamba primarily consists of indole alkaloids, terpenoids, saponins, saponins Terpenes, steroids, fats and reducing sugar.

- The bark consists of tannins; cadambagenic acid (18alpha -olean -12ene -3beta -hydroxy 27,28-dioic acid), quinovic acid and beta -sitosterol two novel triterpenoid saponins.

-phenasin A
-phenasin B

Leaves consists of Glycosydic indole alkaloids
-cadambine (C₂₇H₃₂N₂O₁₀)

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-3alpha-dihydrocadambine (C₂₇H₃₄N₂O₁₀)

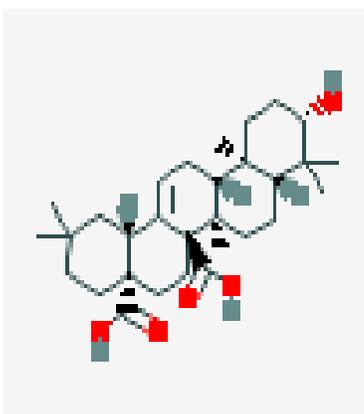
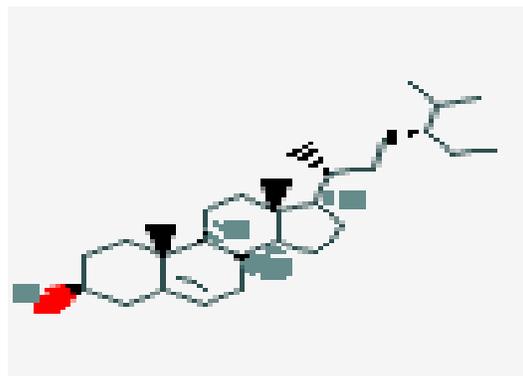
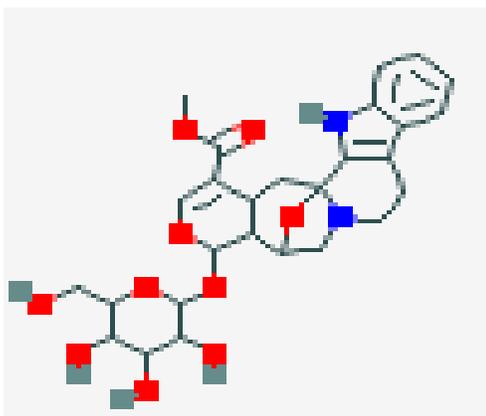
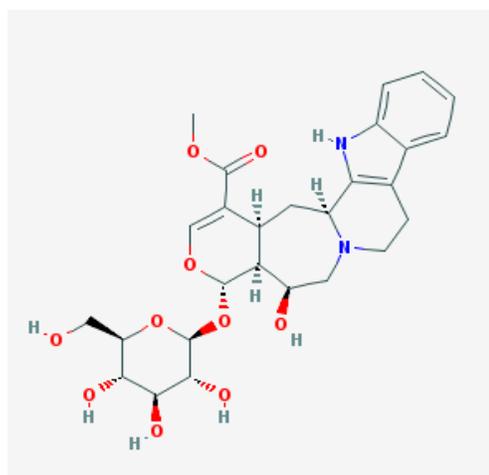
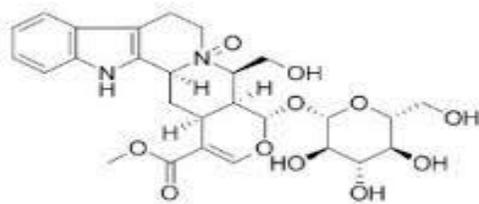
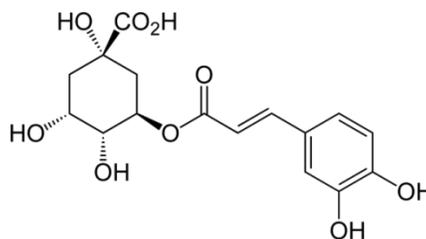
-isodihydrocadambin (C₃₇H₄₄N₂O₁₅)

- Leaves consists of two non glycosidic alkaloids

-cadambine

-isocadambine

Two novel monoterpenoid indole alkaloids, aminocadmabine A(C₂₄H₂₇N₃O₅) and aminocadmabine B (C₂₅H₂₉N₃O₅) obtained from leaves of neolamarckia cadamba. The flowers of Neolamarckia cadamba yield an essential oil and the main constituents of oils are linalool, geraniol, geranyl acetate, linalyl acetate, alpha seline, 2 nonanol, beta phellandrane, alpha bergamottin, p-cymol, curcumene, terpenolene, camphene and mycrene. The seeds of anthocephalous indicus composed of water soluble polysaccharides D-xylose, D-mannose and D-glucose in the molar ratio 1:3:5. A new saponin named saponin B(C₄₈H₇₆O₁₇) reported from NEOLAMARCKIA CADAMBA. NEOLAMARCKIA CADAMBA also contain an acid called chlorogenic acid. Three monoterpenoid glucoindole alkaloids, 3 beta - isodihydrocadambine, cadambine and 3alpha dihydrocadambine isolated from NEOLAMARCKIA CADAMBA. Eight different alkaloids also obtained from neolamarckia cadamba named cadambine CFJ 83, isomalindan, cadamine, 2 derives. HFP34, GZPM 28, malindan, dihydrocadmbin 2 derives. GPX 71, GPX 73, isomalindan, isodihydrocadambine, 2derives. GPX 51, GPX53, malindan.

**CADAMBAGENIC ACID****BETA SITOSTEROL****CADAMBINE****3 ALPHA DIHYDROCADMBINE****ISODIHYDROCADMBINE****CHLOROGENIC ACID**

EXPERIMENTAL WORK**PRELIMINARY CHEMICAL SCREENING FOR EXTRACTS OF NEOLAMARCKIA CADAMBA**

The extracts prepared were tested for the type of chemical constituents present by known qualitative tests, The results are given as follows.

S. NO.	NAME OF THE TESTS	METHANOL EXTRACTS	AQUEOUS EXTRACTS
1	Steroids :	–	–
	A) Salkowski test		
2	B)Liebermann buchard Test	–	–
	Triterpenes :	+	+
3	A)Salkowski test :		
	B)Liebermann buchard Test :	+	+
4	Saponins :		
	A) Foam test :	+	+
5	B)Haemolysis Test	+	+
	Alkaloids :		
6	A)Mayers Test	–	–
	B)Dragondroffs Test	–	–
7	C)Wagners Test	–	–
	D)Hagers Test :	–	–
8	Carbohydrates :		
	A)Fehlings Test :	+	+
9	B)Molischs Test :	+	+
	C)Barfoed's Test :	+	+
10	D)Benedicts Test :	+	+
	Flavonoids Test :		
11	A)Schinoda Test :	+	+
	B)Ferrichloride Test:	+	+

Anti helminthic activity**Animals**

Indian adult earthworms (*Pheretima posthuma*) were used to study anti helminthic activity. The earth worms were collected from moist soil and washed with normal saline to removal all faecal matter. Earthworms are 3-5 cm in length and 0.1 to 0.2 cm in width were used for all experimental protocol.

Drugs and Chemicals

Albendazole (Glaxo Smithline), methanol (Merck chemicals) are used during experimental protocol.

Anti-helminthic activity

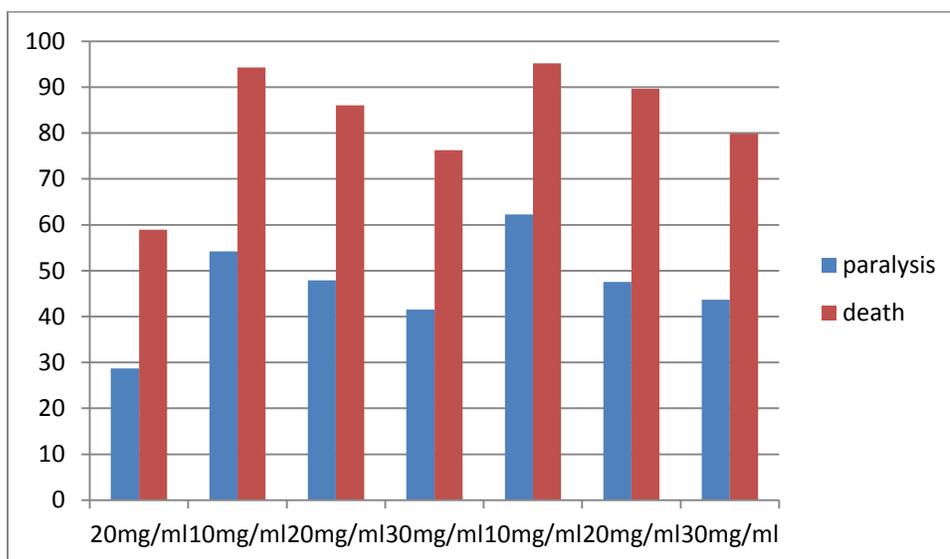
The anti helminthic assay was carried out as per the method of Ajaiyeoba et al (2001) with minor modifications. The assay was performed on the adult Indian earthworm *Pheretima posthuma* due to its anatomical and physiological resemblance to the human intestinal round worm parasite (Vidarthi, 1967; Chatterjee, 1967). Due to their ready availability, earthworms have been used widely for the initial evaluation of anti helminthic compounds in vitro (Sollman, 1918; Jain et al., 1972; Dash et al., 2002). The earthworms were divided into different groups, each group

containing six worms. Five different concentrations of methanolic extracts of (10,20,30,40, and 50 mg/ml in distilled water) were prepared. The time of paralysis was noted when no movement of any sort could be observed except when the worms were shaken

vigourously. The times of death of the worm were recorded after ascertaining that worms neither moved when shaken vigourously or when dipped in warm water (50). Albendazole (25mg/ml) was used as reference standard while water served as a control.

Table for invitro antiheminthic activity of methanolic activity of NEOLAMARCKIA CADAMBA

Group	Extract (Mg/ml)	Paralysis (P)	Death (D)
Control (0.5%CMC)	-----	-----	-----
Stanadard (Albendazole)	20mg/ml	28.71+1.86	58.90+6.85
Methanolic extracts of leaves	10mg/ml	54.22+2.95	94.3+2.76
	20mg/ml	47.90+2.59	86.03+2.25
	30mg/ml	41.54+1.54	76.25+2.36
Aqueous extracts of leaves	10mg/ml	62.25+8.10	95.2+11.97
	20mg/ml	47.55+2.05	89.66+4.69
	30mg/ml	43.67+6.83	79.88+3.86



Comparison between standard and treated groups

CONCLUSION

In the light of the results of the present study, it can be concluded that the leaves of *NEOLAMARCKIA CADAMBA* possess remarkable anti-helminthic property. The findings of the observation also provide future support to and reinforce the traditional use of the plant in different medical disorders. The results of the phytochemical screening revealed that the methanolic and aqueous extracts of the *NEOLAMARCKIA CADAMBA* contains triterpenoid, saponins, flavonoids and tannins. These compound are responsible for the bioactivities but quite difficult to describe the observed activities to any specific group of compounds. Hence further studies are suggested to be undertaken to pinpoint the compounds found in the extracts of *NEOLAMARCKIA CADAMBA* and to better understand the mechanism of such action scientifically.

The traditional use of leaf of *NEOLAMARCKIA CADAMBA* as an anti-helminthic has been conformed as the leaf extract displayed profound anti-helminthic activity in the study. Further it would be interesting to isolate the possible phytoconstituent and characterize the active constituents which may be possible for the anti-helminthic activity and to possible the mechanism of action.

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