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Research

Phytochemical Screening & Comparative study of different extracts on Antifungal Activity of *Neolamarckia cadamba leaf*

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Abstract

Natural compounds can be a lead compounds, allowing the design and rational planning of new drugs, biomimetic synthesis development and the discovery of new therapeutic properties not yet attributed to known compounds. The present study has made an attempt to evaluate the Phytochemical screening and antifungal activity of *Neolamarckia cadamba Roxb.* leaf extract (NCLE). Antifungal activity of *Neolamarckia cadamba* was conducted on fungal organism on *Aspergillus niger*. From phytochemical screening of the crude aqueous, ethanolic & cyclohexane extract of the leaves of showed the presence of Alkaloids, Tannins, Saponins, Steroids and Glycosides. In biological evaluation, the antifungal activities of aqueous, ethanolic & cyclohexane extract of *Neolamarckia cadamba* under the varying concentration of 25, 50, 75, 100 µg/ml tested against *Aspergillus niger*. Zone of inhibition of aqueous, ethanolic & cyclohexane extracts were compared with that of standards like Flucanazole in which the aqueous extract of NCLE showed a better antifungal activity next to the standard. The aim of the current research investigation was to perform the extraction, preliminary phytochemical screening and to evaluate the *invitro* antifungal activity of fruit of *Neolamarckia cadamba.*

Keywords: *Neolamarckia cadamba*, antifungal activity, agar plate method, phytochemicals.

INTRODUCTION

Fungal infections are one of the deadliest infections accountings in excess of 1.5 million deaths annually worldwide. The major reason that makes fungal infections more life threatening because they are been neglected by the society. Though in last 20 years there are many developments in the diagnosis and treatment of fungal

disease but still majority of population are devoid of the benefits of these developments [1]. Among all the fungal diseases, infection of skin hold the 4th position and it accounts for the majority of death also [2].

Plant kingdom has always been a hub for many natural compounds with novel structure and this keep the investigators interested in doing research about many plants' species till today. Results of new researchers showed that plants are enrich of many bioactive secondary metabolites such as saponins, alkaloids and terpenoids which characterized by antifungal property. Depending on that, these plants can be considered as a potent future source for anti-fungal drugs [3]. When recent scenario regarding fungal diseases and antifungal drugs are taken into consideration it has been seen that the development of resistance of fungus towards the presently used antifungal drugs has increased. [4, 5, 6, 7, 8, 9, 10, 11] With the challenges like morbidity and mortality there always lies difficulty in antifungal treatment for patients receiving therapy for AIDS, diabetes, chemotherapy or organ transplant as some of the molecular processes of fungus are similar to humans, so toxicity to fungal cells could affect human cells too. [12] Therefore, it become an oblige for the researcher to discover and produce a new, efficient, and safe anti-fungal treatments from new sources like plants.

The fungus *Aspergillus niger* is a type of mould, which can sometimes be attributed to the cause of some cases of pneumonia. It is also the causative agent of 'black mould' on the outsides of certain foods, such as apricots, onions, grapes, etc - therefore making *Aspergillus niger* a food 'spoilage' organism. These are classed as 'Conidiophores' - an organism which forms filaments or hyphae, otherwise known as conidia (the a-sexual method of fungal reproduction). The name '*Aspergillus*' comes from the Latin word 'aspergillum', which roughly translates to 'holy water sprinkler', referring to the shape of these sprinklers being very similar to how these fungi appear when viewed under a microscope. The organism causes a condition aspergillosis which shows a symptoms like fever and chills haemoptysis, shortness of breath, chest or joint pain, headaches or eye symptoms, skin lesions.

Fig 1: *Neolamarckia cadamba* tree

Neolamarckia cadamba Roxb. (NT) belongs to the family Rubiaceae, commonly known as kadam in Sanskrit as Kadamba, Priyanka, Neepa; in Bengali as Kadam, Neepa; in kannada as Kadava; in Hindi as Kadam; in English as Common bur- flower; in Assam as Roghu, Kadam; in Tamil as Arattam, Vellaikkatampu and in Telugu as Kadambamu, Kadimi. is a species of tropical tree, which is an indigenous plant of South Asia and Southeast Asia. Kadam is known to be one of the most commonly planted trees in the tropics. Morphologically it is a tree up to 45 m tall, without branches for more than 25 m. Diameter up to 100 (160) cm but normally less, sometimes with buttresses. The crown is umbrella shaped and the branches are characteristically arranged in tears. The leaves are green in colour with glossy (upper), rough (lower). The powder is usually odourless and tastes as acrid, bitter. Flowers are small, numerous in compact, scented terminal spherical heads of 4-5 cm diameter, on slender peduncle. Each flowers contain fruits which are inserted on the fleshy head and forms a composite fruit which becomes yellowish or brownish when ripe; Seeds are minute.

It is an ornamental tree commonly found in roadside, gardens and parks and is planted commonly to obtain match stick and paper pulp. Timber of kadam tree is mainly utilized in plywood industry. Flowers are used for making perfume. Ancient medicinal systems such as Ayurveda, Siddha and Unani medicine recommended the use of various parts of kadam tree. NT is one of such Ayurvedic plant that has been narrated in many Indian Ayurvedic medicinal books. it is used in the treatment of various diseases like diabetes mellitus, fever, inflammation, haemoptysis, cough, wounds, vomiting, diarrhoea, ulcers and antimicrobial activity. [13]

Fig 2: *Neolamarckia cadamba* leaves and fruit

Different parts of *cadamba* have shown to contain various groups of chemical constituents viz. alkaloids, saponins, terpenoids, flavonoids, triterpenoid glycosides-selinene, 2-nonalol, αphellandrene, α- steroids, fats and reducing sugars. Recently, the presence of indole alkaloids, Phytoesterols other glycosides have been identified as a major constituent from various parts of a Cadamba. Tannin and an astringent principle which is due to the presence of an acid similar to cincho-tannic acid and α-sitosterol has also been isolated from the bark of the tree. Cadambine ($C_{27}H_{32}N_2O_{10}$), 3α-dihydrocadambine ($C_{27}H_{34}N_2O_{10}$), iso dihydrocadambine ($C_{37}H_{44}N_2O_{15}$) and 2 non glycosidic alkaloids isocadamine and cadamine were isolate d from leaves of the tree. Three terperpenoids viz. α-amyrincaprylate, lupeol and nor α-amyrin wer first time purified from the petroleum ether fraction of bark ethanolic extract. Two novel triterpenoids saponin, phelasin A and phelasin B were isolated from the bark of cadamb tree. Linalool, geraniol, geranyl acetate, linalyl acetate, α-selininene, 2-nonanol, α-phellandrene, αbergamottin, p-cymolmcurcumene, terpinolene, camphene and myrcene were isolated from the flowers of cadamba. [14-19]

Plant Profile

Heartwood: pinocembrin, chrysin, naringenin, kaempferol, aromadendrin, quercetin, taxifolin, dihydrowogonin, Dihydrotectochrysin.

Stem: Padmakastein, amygdalin, prunetin, sakuranetin, puddumetin.

Root: Ursolic acid, stigmasterol, prunetinoside, glucogenkwanin.

Seed: Naringenin-5-Oα -L-rhamnopyranoside, 4′-O-methylliquiritigenin -7-Oα -L-rhamnopyranoside, naringenin 4′-methylether 7-xyloside, β -sitosterol-3O-D-galactopyranoside.

Leaves: Quercetin-3-rhamnoglucoside, kaempferol.

Fruit: Essential oil and thus the most constituents of oils are linalool, geraniol, linalyl acetate, α-selinene, 2-nonanol, β-phellandrene, α-bergamottin, terpinolene, camphene and myrcene

Commercially available: Cadambagenic acid, quinovic acid, β -sitosterol, cadambine, cadamine.[20-28]

From literature survey it was found that the almost all parts of the plant *Neolamarckia cadamba* is used in the treatment of various diseases. Decoction of leaves is used as gargle in aphthae or stomatitis and in the treatment of ulcers, wounds, and metorrhea. Bark of the plant is used in fever, inflammation, cough, vomiting, diarrhoea, diabetes, burning sensation, diuresis, wounds, ulcers and in the treatment of snake-bite,[29,30] Antidiabetic activity, [31,32,33] Analgesic, Antipyretic and Anti-inflammatory activities, [34]. Antidiarrhoeal activity, [35] Diuretic and Laxative activity. [36] Antihepatotoxic effects, [37] Hypolipidemic activity, [38] Antioxidant activity, [39] Antimicrobial and wound healing activity, [40] Anthelmintic activity, [41] Toxicological studies.[42,43] The present study was undertaken for the preliminary phytochemical screening using three different solvents aqueous, ethanol and cyclohexane followed by antifungal activity of *Neolamarckia cadamba.* A comparision study was done to determine the activity based on the solvent used to extract the phytochemicals of NT leaf.

MATERIALS & METHODOLOGY

Collection of Plant Materials

Fresh leaves of *Neolamarckia cadamba* were taken from the Dundigal area of Hyderabad, Telangana, India. The fresh leaves were collected in bulk, washed with potable water to remove adhering dirt followed by rinsing with distilled water, and then shade dried and powdered. [44]

Fig 3: Fresh leaves of *Neolmarckia cadamba*

Fig 4: Dried leaves of *Neolamarckia cadamba*

Preparation of Extracts

The extracts were prepared based on procedures by Patel et at. (2011). The fresh leaves were washed two to three times on tap water and distilled water. Then, it was sterilized with 90% ethanol. The leaves were dried under shade and powdered.[45] Approximately 30 gram of pulverized plant material was extracted for 3 days by soaking it in100 mL autoclaved water. The extracts were then filtered through filter paper, after which the filtrates were left to dry at 40-55°C. The concentrated extracts were then weighted (3.396 g). the same method is applied with ethanol and cyclohexane taking as the solvent.

Fig 5: Powder of *Neolamarckia cadamba* leaves

Fig 6: Ethanol and Cyclohexane extraction by cold maceration

Phytochemical Evaluation

Test for steroidal Triterpenes

Salkowski Test: Few drops of concentrated sulphuric acid was added to the chloroform extract, shaken and on standing, lower layer turns red in colour

Tests for Saponins: Foam Test: Small amount of extract is shaken with little quantity of water; the foam produced persists for 10 minutes. It confirms the presence of saponins.

Tests for Alkaloids

Dragendroff's Test: (potassium bismuth iodide): The acid layer with few drops of Dragendroff's reagent gives reddish brown precipitate. Mayer test (Potassium-mercuric-iodide solution): Alkaloids give cream colour precipitate with this reagent. Wagner test (iodine-potassium-iodide solution): Alkaloids give Brown colour precipitate with this reagent. Hager reagent test (Saturated solution of picric acid): Alkaloids give yellow colour precipitate with this reagent.

Test for carbohydrates

Molish's Test: The extract is treated with molish's reagent and concentrated sulphuric acid along the sides of the test tube, a reddish violet ring shows the presence of carbohydrate. Benedict Test: The extract on heating with Benedicts reagent, brown precipitate indicates the presence of sugar. Shinoda test: The alcoholic solution with few fragments of magnesium ribbon and concentrated hydrochloric acid produced management color after few minutes.

Test for Tannins

Ferric Chloride Test: 5% solution of ferric chloride in 90% alcohol was prepared. Few drops of this solution was added to a little of the above filtrate. If dark green or deep blue color is obtained, tannins are present. Lead Acetate Test: A 10% w/v solution of basic lead acetate in distilled water was added to the test filtrate. If precipitate is obtained, tannins are present. Potassium Dichromate Test: If on an addition of a solution of potassium dichromate in test filtrate, dark color is developed, tannins are present.

Test for Glycosides

Keller-Kiliani Test: A solution of glacial acetic acid (4.0 ml) with 1 drop of 2.0% $FeCl_3$ mixture was mixed with the 10 ml aqueous plant extract and 1 ml H_2SO_4 concentrated. A brown ring formed between the layers which showed the entity of cardiac steroidal glycosides.

Legal Test: The test is employed for digitoxose containing glycosides. The extract of drug is dissolved in pyridine, sodium nitroprusside solution is added to it and made alkaline, pink or red color is produced.

Baljet Test: To the extract, sodium picrate solution is added. It shows yellow to orange colour. [46]

Fig.7: phytochemical evaluation

Study of Antifungal Activity

- Composition and preparation of Potato-Dextrose-Agar medium
- Peeled potato 50.0 gm
- Dextrose 5.0 gm
- Agar-agar 4.0 gm
- Distilled water up to 200 ml

The potato dextrose agar (PDA) was prepared by dissolving 39 grams of PDA into 1000ml distilled water. The media were sterilized by autoclaving at 15 lbs pressure at 121°C for 20 minutes. [47]

Preparation of Fungal Inoculum

At the centre of the PDA pour plates, 5-7 days old fungi were transferred and incubated at 25°C for 5-7 days. After 5-7 days, young and active growing colonies of fungi were ready to be used.[48]

Antifungal Activity of Leaf Extracts

Each test compound (5 mg) was dissolved in 2.5% DMSO (5 ml) to give a concentration of 1000 µg/ml. Fluconazole solution was also prepared at a concentration of 1000 µg/ml in sterilized distilled water. The pH of all the test solutions and control was maintained at 2 to 3 by using concentrated HCl. The extract was tested at dose levels of 25 µg (0.02 ml), 50 µg (0.04 ml), 75 µg (0.06 ml), and 100 µg (0.08 ml) and DMSO used as a control. The solutions of each test compound, control and reference standards (0.05 and 0.1 ml) were added separately in the cups and the plates were kept undisturbed for at least 2 hrs in a refrigerator to allow diffusion of the solution properly into the PDA medium. Petri dishes were subsequently kept at room temperature for 48 hrs. After that, the diameter of zone of inhibition in mm surrounding each of the cups was measured with the help of an antibiotic zone reader.[49,50]

Evaluation of Antifungal Activity

All the experiments were carried out in triplicate for each of the extracts and control. The average of the replicates was taken as the colony diameter of the fungus. The percentage of mycelia growth inhibition were calculated as below:

$I = (C-T)/C \times 100$

Where,

I = Percentage of mycelial growth

C =Diameter of fungal colony (mean) in control

T= Diameter of fungal colony (mean) in treatment

RESULTS & DISCUSSION

Preliminary Phytochemical Screening

The preliminary chemical tests were performed and the test results were noted in the table 2. Aqueous extract shows positive results for all the tests including carbohydrates, glycosides, saponins, tannins, flavonoids, steroidal triterpenes, alkaloids. Ethanol extract shows positive results for all the tests including carbohydrates, glycosides, saponins, tannins, flavonoids, steroidal triterpenes, alkaloids. Cyclohexane showing positive results for all the tests in table 2 except for saponins.

Table 1: Results of Preliminary phytochemical screening of Aqueous, Ethanol and Cyclohexane extract of *Neolmarckia cadamba*

S.No	Test	Aqueous	Ethanol	Cyclohexane
1	Carbohydrates	+	+	+
2	Glycosides	+	+	+
3	Saponins	+	+	-
4	Tannins	+	+	+
5	Flavanoids	+	+	+
6	Steroidal triterpenes	+	+	+
7	Alkaloids	+	+	+

(+) Indicates Presence, (-) Indicates Absence

Determination of Antifungal Activity

Antifungal activity was performed for test and standard samples. *Neolmarckia cadamba* leaf extract was tested for its antifungal activity. Diverse concentrations were employed for the current investigation. In this study, Puncture the plates with the help of a borer aseptically and make 4 wells with concentrations of test sample. Inoculate the plates by swabbing with *Aspergillus niger*. Carefully keep the plates in upright position for incubation at 27°C for 1-week. Observe the plates for clear zone of inhibition around each well. Standard drug was used for comparison with test results. The outcomes were displayed in tables 2,3,4 & 5.

Table 2: Antifungal activity of Aqueous extract of *Neolmarckia cadamba* by well diffusion method against *Aspergillus niger.*

Compound name	Zone of Inhibition (mm)			
	25µL	50µL	75µL	100µL
Aqueous extract	2.6	3.8	4.9	6.2

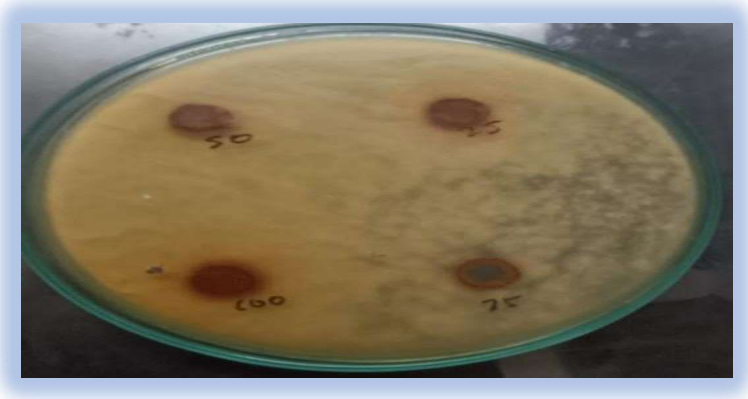

Fig 8: Zone of inhibition of Aqueous extract (Neolmarckia cadamba) against Aspergillus niger

Zone of inhibition of aqueous extract was determined by using the different concentrations (µL) of the extract for 25 µL the zone of inhibition was 2.6mm. By increasing the concentration to 50 µL the zone of inhibition was 3.8mm, for 75 µL the zone of inhibition was 4.9mm and for 100 µL the zone of inhibition was 6.2mm.

Table 3: Antifungal activity of Ethanol extract of *Neolmarckia cadamba* by well diffusion method against *Aspergillus niger.*

Compound name	Zone of Inhibition (mm)			
	25µL	50µL	75µL	100µL
Ethanol extract	2.1	3.4	4.5	5.9

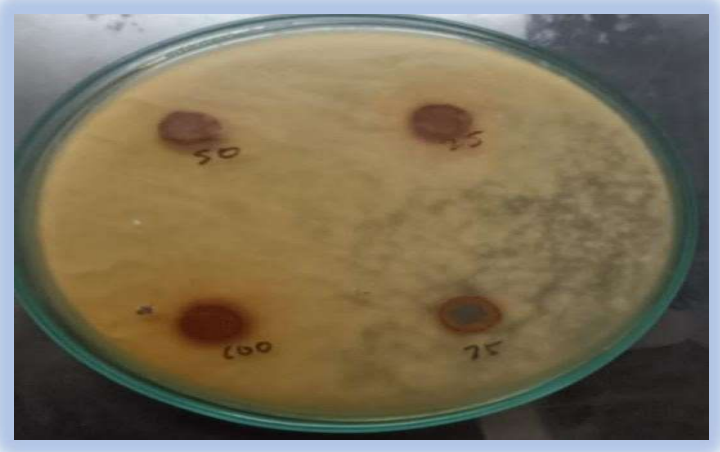

Fig 9: Zone of inhibition of Ethanol extract (*Neolmarckia cadamba*) against

Zone of inhibition of ethanol extract was determined by using the different concentrations (µL) of the extract for 25 µL the zone of inhibition was 2.1mm. By increasing the concentration to 50 µL the zone of inhibition was 3.4mm for 75 µL the zone of inhibition was 4.5mm and for 100 µL the zone of inhibition was 5.9mm.

Table 4: Antifungal activity of Cyclohexane extract of *Neolmarckia cadamba* by well diffusion method against *Aspergillus niger*

Compound name	Zone of Inhibition (mm)			
	25µL	50µL	75µL	100µL
Cyclohexane extract	1.1	2.2	3.4	4.3

Zone of inhibition of cyclohexane extract was determined by using the different concentrations (µL) of the extract for 25 µL the zone of inhibition was 1.1mm. By increasing the concentration to 50 µL the zone of inhibition was 2.2mm for 75 µL the zone of inhibition was 3.4mm and for 100 µL the zone of inhibition was 4.3mm.

Fig 10:- Zone of inhibition of Cyclohexane extract (*Neolmarckia cadamba*) against Aspergillus niger

Table 5: Evaluation of Antifungal activity by Standard drug

S.NO	Test organism	Standard drugs	Diameter of zone of Inhibition (mm)
1.	*Aspergillus niger*	Fluconazole	3.3mm

Determination of antifungal activity by zone of inhibition of the standard drug (Flucanazole) was found to be 3.3mm.

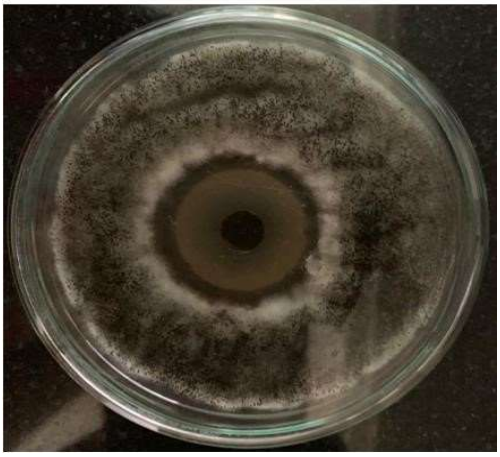

Fig 11: Zone of inhibition of Fluconazole(10mg/ml) against *Aspergillus*.

CONCLUSION

Phytochemical investigation of *Neolamarckia cadamba* revealed the presence of potent bioactive compounds such as alkaloids, flavonoids, tannins, and phenolic compounds, indicating its rich medicinal potential. Furthermore, our antifungal activity assays demonstrated promising inhibitory effects against *Aspergillus niger*, suggesting its possible application as a natural antifungal agent. These findings underscore the importance of further exploration and development of *Neolamarckia cadamba* as a valuable source of novel pharmaceuticals. Future research could focus on isolating and characterizing specific bioactive compounds responsible for its antifungal activity and elucidating their mechanisms of action for potential therapeutic use. *Neolmarckia cadamba* Aqueous extract had shown superior antifungal activity in comparison to that of ethanol &cyclohexane extract. The plant extracts had exhibited a significant antifungal activity when compared to that of standard drug fluconazole.

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