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PHARMACOGNOSTIC EVALUATION AND PHYTOCHEMICAL SCREENING OF *ANTHOCEPHALUS CADAMBA*

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ABSTRACT

The present work deals with Pharmacognostic evaluation and phytochemical screening for hydroalcoholic extract of leaves of medicinal plant *Anthocephalus cadamba (Roxb.)Miq.* The scientific parameter is necessary to identify the exact plant material and to find its quality and purity. The present study deals with various, physical, chemical evaluation and preliminary phytochemical screening of the alkaloidal content of stem bark of the title plant has been determined. These studies indicated the possible information for correct identification and standardization of this plant material for its Antifungal action.

KEYWORDS

Anthocephalus cadamba (Roxb.)Miq, Pharmacognostic evaluation and Phytochemical screening and Antifungal action.

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INTRODUCTION[1-10]

Anthocephalus cadamba (Roxb) Miq.Syn. *Anthocephalus chinensis* (Lamk) (Rubiaceae) is widely distributed throughout the greater part of India, especially at low levels in wet places. In traditional system of medicine warm aqueous extract of *Anthocephalus cadamba* leaves have been used to alleviate the pain, swelling and for cleansing and better wound healing. Recently, *Anthocephalus cadamba* has been reported to possess wound healing, antioxidant, antimalarial and hepatoprotective activity. Herbal medicine is the earliest scientific tradition in medical practice, and it

remains an important part of medicine to this day. *Anthocephalus cadamba* (Syn. *A. chinensis* (Lam.).

A (Rich. ex Walp.) is a medium sized tree belonging to the family Rubiaceae. It is found all over India and grows in North and Eastern Bengal and Western Ghats. It is also known as 'wild cinchona' in English and *kadamba* in Hindi.

The present study was undertaken to screen the antibacterial activity of the leaves of *Anthocephalus cadamba*. It is used as a folk medicine in the treatment of fever, anemia, uterine complaints, blood diseases, skin diseases, leprosy, dysentery, and for improvement of semen quality. The leaves are recommended as a gargle in cases of stomatitis.

The traditional healers of Bastar region use Kadamba bark in treatment of eye diseases. It is also used in case of stomatitis. The traditional healers of Chhattisgarh Plains prefer the decoction of leaves in place of bark for same purpose. The fruit juice is given to children to treat gastric irritability.

A decoction of the leaves is good for ulcers and wounds the fruits are edible. The timber is used for making pulp and paper, boxes, crates and furniture. The wood is also used as fuel. Throughout human history, people have relied on natural products and plants in particular, to promote and maintain good health and to fight sickness, pain and disease. The past 200 years have witnessed not only acceleration in the rate of extinction of plant and animal species, but also the erosion of traditional knowledge related to the medicinal properties and uses of plants and other natural products.

The bark is said to be pungent, bitter, sweet, acrid, saline, aphrodisiac, cooling, indigestible, galactogogue, astringent to the bowels, vulnerary, alexiteric, good in uterine complaints, blood diseases, burning sensation, in snake bite1 and for improvement of semen quality. The antimicrobial, anti-inflammatory and anti-hepatotoxic activities have been reported by earlier workers.

Triterpene glycoside few triterpenoids, saponins and alkaloids were also been reported. Due to the medicinal importance of the plant and the absence of recent investigation into the chemistry of this medicinal bark, it was thought worth to investigate the photochemistry again.

There are thousands year old traditions and records of popular healing methods that have maintained their importance despite new developments and progress in the field of chemistry, pharmaceutics and medicine. The role of natural products in the development of drugs used in modern medicine is unsurpassed even when synthetic chemistry has been developed beyond expectations. Indeed, interest in herbal drugs is increasing.

Anthocephalus cadamba is commonly known as Kadamba-vriksha and is a genus of trees, distributed throughout the Indo-Malaysian region. A medium sized tree attaining 2 m girth and 18 m height, branches spreading horizontally and slightly enlarged at their junction with the main stem.

Antifungal activity on the growth and physiology of human pathogenic yeasts or filamentous fungi either *in vitro* or *in vivo*. Furthermore, its direct therapeutic use either in superficial or systemic infections due to bacteria or fungi has not been clearly established.

In the present study, we examined the antifungal properties of organism oil and its major chemical constituent carvacrol against *Candida albicans*, a dimorphic yeast-like fungus. *C. albicans* which resides as commensal in the mucocutaneous cavities of skin, vagina and intestine of humans can cause infections under altered physiological and pathological conditions such as infancy, pregnancy, diabetes, prolonged broad spectrum antibiotic administration, steroidal chemotherapy as well as AIDS.

We have demonstrated that origanum oil inhibits the growth of *C. albicans in vitro* as well as *in vivo.* We conclude that the daily oral administration of origanum oil may be highly effective in the prevention and treatment of candidiasis.

Morphology of Plant[11-15]

Figure No.1 and 2: Picture of cadamba plant (*Anthocephalus cadamba miq.)*

Description

Name: *Cadamba*

Biological source: *Anthocephalus cadamba*

Family: *Rubiaceae*

Other Names: Wild cinchona, Kadamba,
Habital: All over India
Parts Used: Fruits, Leves and Bark

Ayurvedic texts

Vrttapuspa, Kadambari, Nipa, Karnapuraka, Sindhu puspa, Madadhya, lalanapriya etc.

Sage Caraka has categories

- Vedanasthapana – analgesic,
- Vamanopaga – adjunct to emesis and its fruit pulp
- Sukrasodhana – purifier of seminal fluids.
- Visaghna – detoxifier
- Stambhana – anti diarrhoeal
- Dhuli kadamba – which blooms in the spring and
- Bhumi kadamba – which has small flowers.

Chemical constituent

- Isolation and structure elucidation of cadambine and 3 dihydrocadambine.
- A glycosidic alkaloid –3 isodihydro cadambine – isolated and characterised.
- Indole alkaloids cadambine, 3 a-dihydrocadambine, cadamine, isocadamine.
- A new triterpenic acid cadambagenic acid – and along with quinovic acid and sitosterol isolated. 3 – isodihydrocadambine isolated as acetates from leaves.
- The leaves and bark contained 0.2 and 0.18% of alkaloids respectively and hentriacontanol, and two non-glycoside.
- Barks are triterpenes, tripernoid glycosides, saponins.
- A polysaccharide composed of xylose, mannose and glucose in ratio 1:3:5 isolated from seeds (Figure No.3).

Properties

It is cultivated all over India. Leaves are about 7.5-18 X 4.5-16 cm in size, flower head globes, yellow, solitary, terminal, 3.7 cm in diameter consisting of small, yellow or orange colored, scented flowers; fruits are fleshy, orange, globes pseudo carp of compressed angular capsules with persistent calyx; seeds small, muriculate.

The leaves are used in hydrocoele and in Pyorrhea. The decoction of leaves is good for ulcers, wounds and metrorhea.

Kadamba is bitter, pungent and astringent in taste, pungent in the post digestive effect and has cold potency. It alleviates all the three dosas, predominantly kapha and pitta. It possesses light and dry attributes. By its special potency, it acts as vedanasthapana – analgesic and visaghna – detoxifies the toxins.

In Raja Nighantu, three kinds of kadamba are mentioned namely, dhara kadamba that is the kadamba described above, Dhuli kadamba – which blooms in the spring and Bhumi kadamba – which has small flowers. Figure No.3: Flower of cadamba plant (*Anthocephalus cadamba miq.*).

Medicinal Uses

The roots, fruits, leaves, bark skin is used from medicinal purposes. Externally, the wounds and ulcers are dressed with its leaves slightly warmed to alleviate the pain, swelling and for cleansing and better healing of wounds. The decoction of the leaves is also used for this purpose. The paste of its bark skin is benevolent in conjunctivitis, as an external application.

Internally, the decoction of bark skin is an effective remedy for diarrhea, dysentery and colitis. The juice of bark skin combined with cumin seeds and sugar alleviates vomiting. The excessive thirst in fevers is quenched with its fruit juice. Kadamba is the best panacea for raktapitta, edema and cough. The decoction of roots is salutary in urinary ailments like dysuria, urinary calculi and glycosuria. Menorrhagia is effectively controlled with the fresh juice of its leaves or their decoction.

The fruit juice augments the quantity of breast milk in lactating mothers and also works well as a lactodepurant. Kadamba is rewarding in skin diseases as it improves the complexion of the skin. In burning sensation of the body and fever, the bark skin is commonly used. The bark skin and the fruits are salubrious in general debility.

Natural antibiotics are used for several infectious diseases, mostly bacterial and fungal infections. Phytochemistry of A. cadamba and its application in

the treatment of various ailments like diabetes mellitus, diarrhoea, fever, inflammation, haemoptysis, cough, vomiting, wounds, ulcers, debility and antimicrobial activity.

Botanical features of cadamba plant

1. Leaves glossy green, opposite, simple more or less sessile to petiolate, ovate to elliptical (15-50 x 8–25 cm).
2. Flowers inflorescence in clusters; terminal globose heads without bracteoles, sub sessile fragrant, orange or yellow flowers; Flowers bisexual, 5-merous, calyx tube funnel-shaped, corolla gamopetalous saucer-shaped with a narrow tube, the narrow lobes imbricate in bud.
3. Stamens 5, inserted on the corolla tube, filaments short, anthers basifixed. Ovary inferior, bi-locular, sometimes 4-locular in the upper part, style exerted and a spindle-shaped stigma.
4. Fruit lets numerous with their upper parts containing 4 hollow or solid structures. Seed trigonal or irregularly shaped.

MATERIALS AND METHODS[16-30]

Plant Material

The leaves of *Anthocephalus cadamba (Roxb.)Miq.* was collected from Hadapsar, Pune.

Preparation of Extract

The leaves of *Anthocephalus cadamba (Roxb.)Miq.* shaded dried, and then these are made into coarsely powdered form using dry grinder. The powdered leaves of the plant (250gm.) was packed in soxhlet apparatus and continuously extracted with 20% ethanol till complete extraction, after completion of extraction the solvent was removed by distillation and then concentrated extract obtained was dried under reduced pressure using rotatory evaporator at temperature not exceeding 40^0C and then give moderate heating on water bath.

Disks of 5mm in diameter were punched out from a sheet of whatman filter. Disks were placed in Petri plate, allowing a distance of 2-4 mm between each disk. Steilized in a hot air oven at 160^oC for 1 hour. After sterilization, disks were allowed to cool aseptically.

Culture Media

Sabouraud dextrose agar medium (SDA) - Table No.1.

Extraction of crude drug

After the collection of crude drug i.e. leaves of crude drug from land, drying of crude drug is carried out. The drying of crude is carried out at room temperature in shade for about one month. The leaves of the crude drug plant after complete drying are subjected to grinding. The grinding is performed by means of mixer grinder.

Maceration Processes

After grinding of leaves, the powder mixture is subjected to soaking in the solvent of extraction like Ethanol, water, clove oil mixture in 4 days. During soaking the small quantity of active constituents comes into solution. Then filtered in simple filter paper used in filtering mixture.

Then after completed the filtration the extraction of solvent is evaporated in heating on water bath up to the concentration of extract in powdered form it after complete the evaporating of solvent.

Microbial standardization

The following test was carried on the media before or in parallel with the test for sterility.

To maintain sterility of working processes

To maintain sterility of formulation following processes are done.

Fumigation

Aseptic room was fumigated with mixture of 500 ml of formaldehyde with potassium permanganate. Effectiveness of sterilization was tested using nutrient agar and sabouraud dextrose agar plate by incubation at 30-37^{o}C and 20-25^{o}C respectively for 7 days after 2 hours exposure. Frequency of testing was twice in a week.

Formaldehyde has is generated by heating a concentrated solution of formaldehyde with potassium permanganate. Formaldehyde in aqueous solution is known as formalin and contains 37-40% of formaldehyde. Vaporization of formaldehyde either from formalin or para formaldehyde is used to sterilized an enclosed area. Formaldehyde is generated by adding 150gm of potassium permanganate to 280 ml of formalin for every 1000

cubic feet room volume after 70% of relative humidity and 22^{o}C temperature gives best sterilization result.

Mechanism of action

Formaldehyde is an extremely reactive chemical. It combines readily with vital organic nitrogen compound such as proteins nucleic acid. It is bactericidal agent with poor penetrating power. It kills both vegetative cells an spotes. Formaldehyde in gases from can be used for disinfection and sterilization of enclosed area. The exterior platform surface was cleaned with 95% alcohol.

Sample dilution

The surface of sample container was disinfected with water. The container was opened and transferred 1 gm of semisolid water extract with aseptically from its container to a sterile volumetric flask containing 1 ml of sterile distilled water respectively to get a dilution of 1:1. The volumetric flask of each was shaken nicely up to complete dissolution.

Antifungal activity of *Anthocephalus Cadamba*[31-42]

Plating method

Sterilized nutrient agar medium and sabouraud dextrose agar medium was prepared and poured aseptically in the separate petri plates and allowed to solidify under aseptic condition.

Antifungal activity

Antifungal testing was carried out by disk diffusion technique. In this technique antifungal diffuses from disk into medium Following overnight incubation, the medium is examined for area of no growth around the disk (zone of inhibition). The zone is measured and compared against a control solvent zone (alcohol and water respectively). The incubation zones a produced by control solvent against the same organism vary in size due to differences in antifungal molecular structure.

A large zone of inhibition is produced by an antifungal that diffuses rapidly and smaller zone by one that diffuses more slowly. Zones size is also affected by the degree of bacterial growth. Other factors which influences disk diffusion test include volume of sample, pH of agar medium and constituents of agar medium. Candida albicans was used for testing antifungal activity.

Sterilized media were poured into their respective 90mm diameter sterile Petri dishes to a depth of 4mm (25 ml/plate) and then allowed to solidify.

Nutrient agar medium was used to test the antifungal activity to the sabouraud dextrose agar medium Colonies of organisms were emulsified in a small volume of sterile nutrient broth for bacteria and sabouraud dextrose broth for fungi. With the help of sterile pipette 1 ml of emulsified organisms was added to their respective Petri plates containing suitable media to the center position. With the help of sterile glass spreader, emulsion was spreader uniformly on the surface of the media.

The inoculated plates were then allowed to dry for few minutes. With the help of sterile forceps antifungal disks dipped in different formulation concentration were placed on the surface of their respective media at particular corner, which were previously labeled. In each plates ones corner was used for keeping the antifungal disk dipped in the control antifungal solution (control solution). This control antifungal solution was alcohol. The disk was then passed down on the medium.

Plates were then kept in refrigerator for 15 minutes to effect the diffusion of test samples. The plates were removed from refrigerator and incubated at 30-37^{o}C for 24 hours for bacteria and at 20-25^{o}C for 24 hours for fungi. After 24 hours, diameter of the inhibition zone was measured from one edge to other edge of zone. The n point of inhibition was where growth starts.

The zone of inhibition was observed and results was reported by the type of reaction shown by samples on test organisms as-

- Sensitive (S) - Zone diameter wider than 8 mm.
- Intermediate (I) - Zone diameter 5-8 mm.
- Resistant (R) - Zone diameter smaller than 5 mm or no zone of inhibition.

RESULTS AND DISCUSSION

The Petri plate method was used to determine the inhibition zones of A. cadamba extracts (aqueous). The plant leaves does not showed significant antifungal activity against candida albicans (Table No.2 and Figure No.4).

Table No.1: For fungus to test the antifungal activity

S.No	Ingredients	Quantity
1	Dextrose	40g
2	Mixture of equal parts of peptic digest of animal tissue and pancreatic digest of casein	10g
3	Aagar	20g
4	Distilled water to	1000ml
5	pH after sterilization	5.6
Organisms		
1	Candida albicans - No.10231	

Table No.2: Chemical tests for extract

S.No	Chemical Test	Observation (Water Extract)
1	**Test for Sterols**	
	Salkowski's test	+ve
	Sulphar test	+ve
	Liebermann Burchards's test	+ve
2	**Test for Triterpenoids**	
	Salkowski's test	-ve
	Libermann Burchards's test	- ve
3	**Test for Gum and Mucilage**	
	Drug + Ruthenium red	- ve
	Drug + Water	-ve
	Drug + Aqueous Potassium hydroxide	-ve
4	**Test for Amino Acids**	
	Ninhydrine test	-ve
5	**Test for Cardiac Glycoside**	
	Baljet's test	+ve
	Keller- Killiani test	+ve
	Raymond's test	+ve
	Bromine water test	+ve
	Legal's test	+ve

6	**Test for Anthraquinone Glycoside**	
	Borntrager test	+ve
	Modified Borntrager test	+ve
7	**Test for Saponin Glycoside**	+ve
	Foam test	+ve
	Haemolysis test	+ve
8	**Test for Cytogenetic Glycoside**	
	Guignard reaction or sodium picrate test	-ve
9	**Test for Coumarine Glycoside**	
	Alcoholic extract + Alkaline	+ve
10	**Test for Carbohydrates**	
	Molish's Test	-ve
11	**Test for Alkaloids**	
	Mayer's test	-ve
	Wagner's test	-ve
	Hager's test	+ve
12	**Test for Flavonoids**	
	Lead acetate test	+ve
	Shinoda test	-ve
	Test soln.+ Excess NaOH	-ve
13	**Test for Tannins and Phenolic Compounds**	
	5% Ferric Chloride test	-ve
	Gelatin test	-ve
	Lead acetate	+ve
	Bromine water	+ve
	Acetic acid solution	+ve
	Potassium dichromate	-ve
	Dilute Iodine sol	+ve
	Dilute Nitric acid	+ve
	Dilute Ammonium hydroxide + Potassium ferrocyanide solution	+ve
	Ammonium hydroxide + Excess 10% Silver nitrate, Heat	
14	**Test for Proteins**	+ve
	Biuret test	+ve
15	**Test for Fat and Fixed Oils**	
	Spot test	-ve
	Saponification test	+ve

Figure No.1: Picture of cadamba plant (*Anthocephalus cadamba miq.)*

Figure No.2: Antifungal activity without tested against Candida albicans

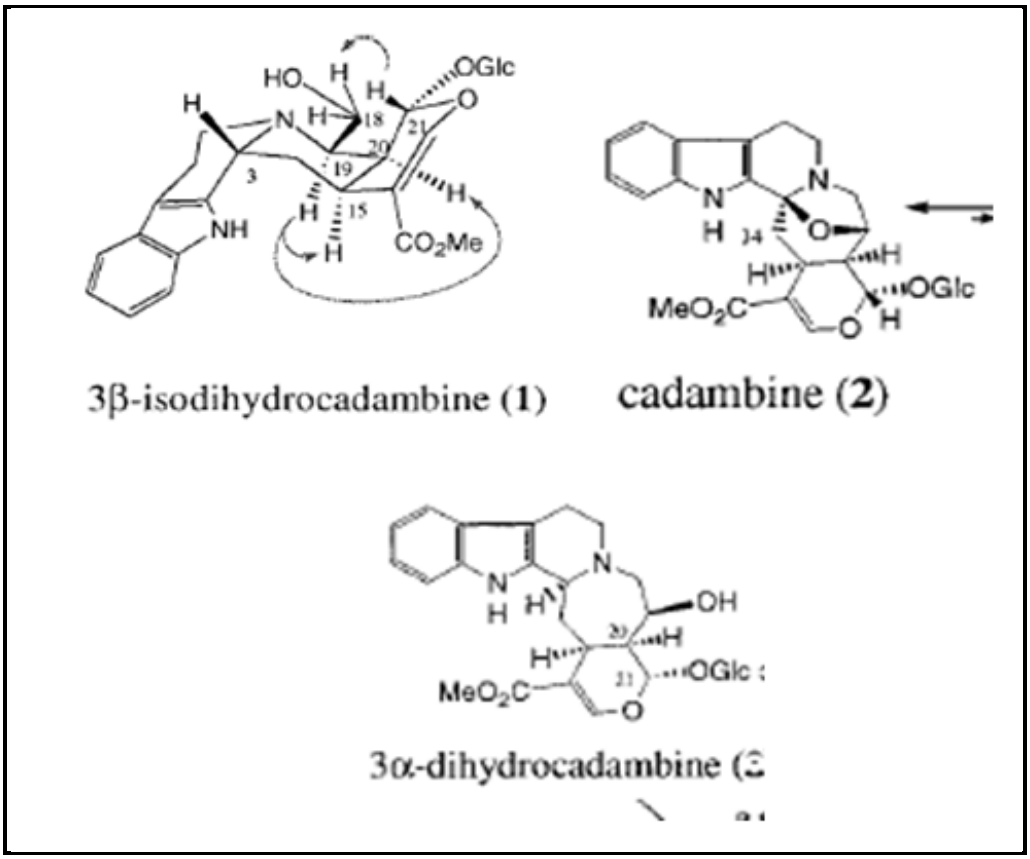

Figure No.3: Chemical constituent

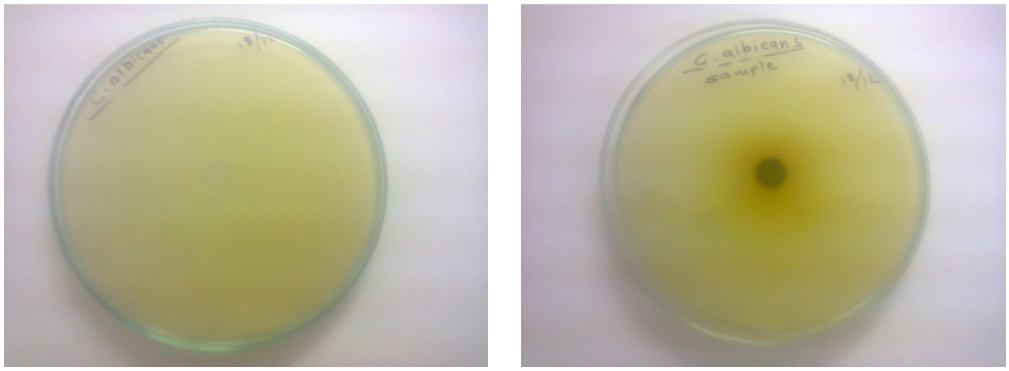

Figure No.4: Antifungal activity tested against *Candida albicans*

CONCLUSION

In the present study, leaves of *Anthocephalus cadamba Roxb*. were collected and shade dried. It was reduced to required particle size and then subjected to the successive extraction with the various solvents like Ethanol, Distilled water according to the heating metal. Some part of the various extracts was subjected to Pharmcoganostic Evaluation for the identification of various Phytoconstituents and rest of extracts were utilized for pharmacological screening for assessment of Antifungal activity, using following method. 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. The Antifungal activity tested against Candida albicans for the same plant.

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