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PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF LEAF AND BARK EXTRACTS OF ANTHOCEPHALUS CADAMBA

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Abstract: Anthocephalus cadamba is a holy plant that belongs to the Rubiaceae family and consists of several chemical constituents which are synthesized in all parts of the plant. The present study carried out for phytochemical screening and antimicrobial testing of leaf and bark extracts. Fresh leaves and bark of the plant were collected and subjected for shed drying, ground to a coarse powder, and used for soxhlation process using various solvents like petroleum ether, ethanol, distilled water, etc. the extract obtained was further used for phytochemical screening of chemical constituents. Petroleum ether extract consists of pigments, ethanolic extract consists of alkaloids, indole alkaloids, glycosides, flavonoids, triterpenoids, whereas aqueous extract consists of saponin glycosides. Ethanolic extracts of leaf and bark were subjected to antimicrobial activity testing against both gram-positive and gram-negative bacteria using a nutrient agar medium. Both leaf and bark extracts exhibited a broad spectrum of antibacterial activity against both gram-positive and gram-negative bacteria.

Index Terms: **Kadamba tree, Anthocephalus Cadamba, soxhlation, phytochemical screening, antimicrobial activity**

I. INTRODUCTION

Anthocephalus cadamba (Roxb.) Miq. Syn A. chinensis (Lamk) A. Rich (Rubiaceae) is a widely distributed plant that is used in traditional medicine to treat a variety of conditions, including fever, anemia, uterine complaints, blood disorders, skin diseases, dysentery, leprosy, and dyspepsia. Many potential natural antibiotic sources have been employed in recent years to treat a variety of infectious disorders, most commonly bacterial and fungal infections. The phytochemistry of A. cadamba and its use in the management of a number of illnesses, including inflammatory diseases, hemoptysis, diabetes mellitus, diarrhea, fever, wounds, ulcers, debility, and antibacterial activity. In conditions of stomatitis, the leaves are advised to be gargled [1](Slkar et al., 1996). Its antihepatotoxic [2] (Kapil et al., 1995) and antimalarial [3] (Sianne and Fanie 2002) properties have been demonstrated by a few research investigations. According to Niranjan et al. (2000), Kitagawa et al. (1996), Mahato and Garai (1998), Brown and Chapple (1976), triterpenes, tripernoid glycosides, saponins, and indole alkaloids—cadambine, 3 a-dihydrocadambine, cadamine, isocadamine, and isodihydrocadambine—are the main components of bark [4-7].

The plant's bark is used to cure fever and eye irritation because it is said to have tonic, bitter, pungent, sweet, acrid, astringent, febrifugal, anti-inflammatory, digestive, carminative, diuretic, expectorant, constipating, and antiemetic qualities. Vegetables are made from the flowers. Although the leaves have an unpleasant taste and a mildly aromatic scent, their infusion is beneficial for metorrhea, ulcers, and sores. It is also helpful in the management of snakebite. This herbal preparation, known as nygrodhadi kvatha churna, is commonly used in powder form [8-11] (Kirtikar KR and Basu BD (1999), Nandkarni's KM (2002), Patel D and Kumar V (2008), Shantha TR. et al (2008).

Warm aqueous extract of Anthocephalus cadamba leaves has been used in traditional medicine to reduce pain and swelling, as well as for cleansing and promoting faster wound healing. It has recently been found that Anthocephalus cadamba exhibits hepatoprotective, antioxidant, antimalarial, and wound-healing properties. The oldest scientific tradition in medicine is herbal medicine, which is still a significant aspect of healthcare today.

II. METHODS

2.1 Plant material : The stem bark of Anthocephaluscadamba (Roxb.) Miq. Was collected from the tree. They are washed and cleaned thoroughly and subjected for shade drying.

Figure 1: Dried leaves and bark of Anthocephalus cadamba

2.2 Preparation of extract :

The stem bark of Anthocephaluscadamba (Roxb.) Miq. Shaded dried. After few hours the dried bark taken into descicators by filling calcium chloride at bottom of the desicator and filter paper above the calcium chloride.Thedesicator was closed with help of lid. After two days the bark was removed from the desicator and allowed to dry.

2.2.1 Water extraction :

The dried bark is crushed into small pieces by manually and small amount of bark is taken into a beaker. Slowly added boiling water to the beaker with continuous stirring until the contents are soaked and kept a side with proper covering for two days. After two days the sample was exposed to heat for 5minutes. Later the extract is filterated by using muslin cloth. The obtained is again subjected to heat until the sample get concentrated to 20ml and stored for 2days. Fungus was observed after 3days so it was discarded.

2.2.2 Extraction process by using petroleum ether :

The dried bark is made into coarsely powdered form using dry grinder. 50gm of stem bark is taken in a beaker, to that 300ml of petroleum ether is added and subjected to imbibition process. After two days the stem bark of plant was packed in soxhlet apparatus and continuously extracted with petroleum ether till complete extraction for 3days.

After completion of extraction the solvent was removed and subjected for evaporation by proper covering. After two days the solvent get evaporated and sedimented at the bottom of petridish.

Figure 2: Soxhalate extraction of Bark, leaves of Anthocephalus Cadamba

2.2.3 Ethanolic extraction :

Approximately 50g of powdered stem bark is taken in a beaker. To that 100ml of ethanol is added and subjected to imbibition process. After 2days, it was filtered by using muslin cloth and subjected for evaporation by proper covering. The solvent get evaporated and the contents are sedimented at the bottom of the beaker. (Same procedure is repeated for leaves also and the same is observed).

2.3 Phytochemical screening of Anthocephalus cadamba:

2.3.1 Test for triterpenoids :

a)Salkowski test : Chloroform solution of the extract is shaken with concentrated H2SO4. Lower layer turns to yellow on standing.

b)Libermannburcharnd test : Chloroform solution of extract is added with few drops of acetic acid and 1ml of concentrated H2SO4. Deep red colour is observed at the junction of 2layers.

2.3.2 Test for alkaloids :

Wagner's test : To the extract add few ml of wagner's reagent. Reddish brown precipitate is observed.

Composition of Wagner's reagent : 1.3g iodine +2g KI in 20ml water + water upto 100ml KI3(Potassium tri iodide solution)

Dragendroff's test : To the extract add dragendroff's reagent. Orange red precipitate is observed.

Mayer's test : To the extract add mayer's reagent. White or cream colour precipitate is observed.

Test for Indole alkaloids :

Nitric acid test : To the extract add HNO3. Yellow colour is observed.

2.3.4 Test for Glycosides :

a)Libermann's test : 2ml of extract + 2ml of CHCl3 + 2ml CH3COOH. Violet to blue to green colouration is observed.

b)Saponin glycosides : Foam test : A small amount of extract is shaken with little quantity of water and persist for 10mins it leads to formation of foam.

2.3.5 Test for flavonoids :

Lead acetate test : Alcoholic extract is mixed with few drops of 10% lead acetate. Yellow colour precipitate is observed.

Zinc HCl test : Alcoholic extract + zinc dust + concentrated HCl. Red colour is observed after few minutes.

Shinoda test : Alcoholic extract + Mg turnings + concentrated HCl drop wise. Pink scarlet, crimson red (or) occasionally green to blue colour appears in few minutes.

Feeric chloride test : Alcoholic solution of extract is mixed with few drops of neutral ferric chloride test. Green colour is observed.[12,13]

2.4 Testing of Antimicrobial activity:

2.4.1 Preparation of culture media or nutrient agar medium :

Nutrient agar is used as a general purpose medium for the growth of a wide variety of nonfastidious microorganisms. It typically contains (mass/volume):[14]

It consists of peptone, beef extract and agar. This relatively simple formulation provides the nutrients necessary for the replication of a large number of non-fastidious microorganisms.

Nutrient Agar/broth is used for the cultivation and maintenance of non-fastidious organisms as well as enumeration of organisms in water, sewage, dairy products, feces and other materials.

Composition of Nutrient Agar

BeefExtract 3.0g

Peptone 5.0g

Agar…15.0 g

Sodiumchloride 5.0g

Distilled Water… 1000 ml

Final PH adjusted 6.8+/_0.2

2.4.2 Preparation of Nutrient Agar

Nutrient agar and broth are available commercially in powdered (free-flowing, homogeneous) form. Dissolved the dehydrated medium in the appropriate volume of distilled water i.e., 28 gm dehydrated nutrient agar (see the manufacturer instruction) in 1000 ml distilled water. Heated with frequent agitation and boil for 1 minute to completely dissolve the powder. Sterilized the medium by autoclaving (121°C for 15 min). Then they are cooled to around 50 °C (122 °F) and poured into Petri dishes which are covered immediately. Once the dishes hold solidified agar, they are stored upside down and are often refrigerated until used.

2.4.3 Testing of antimicrobial activity for bark extract:

The nutrient agar medium tubed were heated on water bath for melting. The molten medium was cooled to 55^{o}C and inoculated with gram positive bacteria (strepto cocci) and poured into sterile petri plates and allowed to solidity. Wattman filter paper disc was placed in ethanolic extract of Neolamarckia cadamba bark and allowed for 5min. Placed the disc at the centre of the petri place and incubated at 37oC for 24hrs in inverted position. The same procedure was repeated with gram negative bacteria (E. Coli), and amoxicillin is used as a control for antimicrobial activity testing and above procedure was repeated for that also.

III. RESULTS AND DISCUSSION

Preparation of extracts :

Aqueous extract : The prepared aqueous extract of kadamba bark and leaves were subjected for evaporation for two days. After 2days the microbial growth was observed. Hence the extracts was discarded.

Petroleum ether extract : The petroleum ether extraction of kadamba bark and leaves were prepared by soxhlation and subjected for evaporation. The extracts was thicken after 2days. This extract consists of only pigments.

Alcoholic extract : Alcoholic extraction of bark and leaves obtain by simple maceration process. The obtained extracts were subjected for various phytochemical constituents by using various tests and determination of anti microbial activity by using strains of gram positive and gram negative organisms.

Test for triterpenoids :

The ethanolic extract was given positive test with salkowski's test(lower layer turns to yellow on standing) and libermannburchard's test (deep red colour is observed at the junction of two layers).

Test for alkaloids :

The ethanolic extract was given positive test with wagner's test (reddish brown ppt isobserved), dragandroff's test (orange ppt is observed), mayer's test (white or cream colourppt is observed).

Indole alkaloids :

The ethanolic extract was given positive test with Nitric acid test (yellow colour is observed)

Test for glycosides :

The ethanolic extract was given positive test with Libebmann's test (violet to blue to green colouration is observed)

Saponin glycosides :

The ethanolic extract was given positive test with foam test (foam is produced)

Test for flavonoids:

The ethanolic extract was given positive with lead acetate test (yellow colourppt is observed), zinc HCl test (red colour is observed), shinoda test (pink scarlet, crimson red or occasionally green to blue appears), ferric chloride test (green colour is observed).

Anti microbial activity determination:

The alcoholic extracts of leaf and bark of neolamerca cadamba were tested for their antimicrobial activity against gram positive and gram negative bacteria. the antimicrobial activity was determined by measuring inhibition zone diameter.

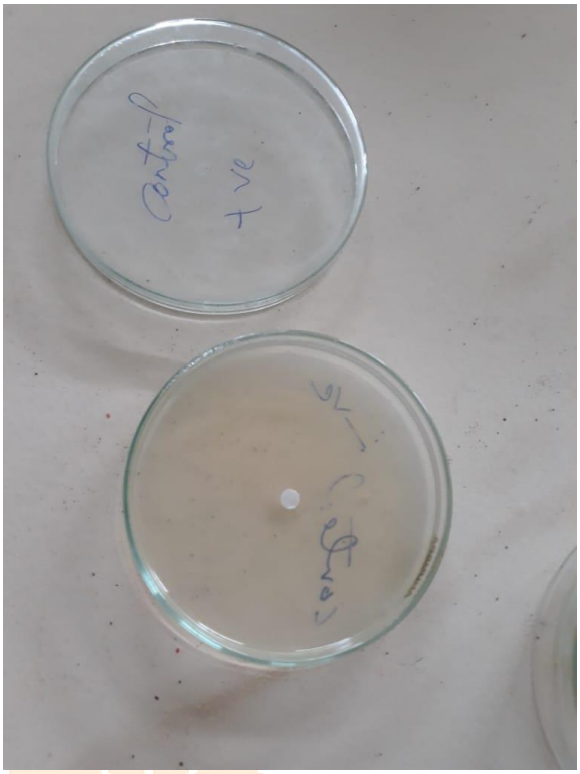

Figure 3: Anti-microbial activity of extracts of Anthocephalus Cadamba

The incubated petridishes are subjected for inhibition zone determination and it was shown that there is no growth of micro organism was observed throughout the plate. From the literature survey it was shown that the extracts of leaf and bark given the inhibition zones of around 22cm. In regular laboratory we used normal petriplates so the inhibition zone diameter was not measured perfectly but it is shown that there is no growth of organism in case of both gram positive and gram negative organisms.

IV. CONCLUSION

Phytochemical screening of anthocephaluscadamba was carried out by using various solvent extracts. Alcoholic extract consists of alkaloids, triterpenoids, flavonoids, glycosides, saponine glycosides. From the study of determination of antimicrobial activity of leaf and bark extracts of neolamerca cadamba, its showed that the alcoholic extracts of leaf and bark of neolamarca cadamba exhibited antimicrobial activity against gram positive and gram negative bacteria. From the results that are obtained by the study its concluded that leaf and bark extracts of neolamerca cadamba is having broad spectrum of antimicrobial activity.

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