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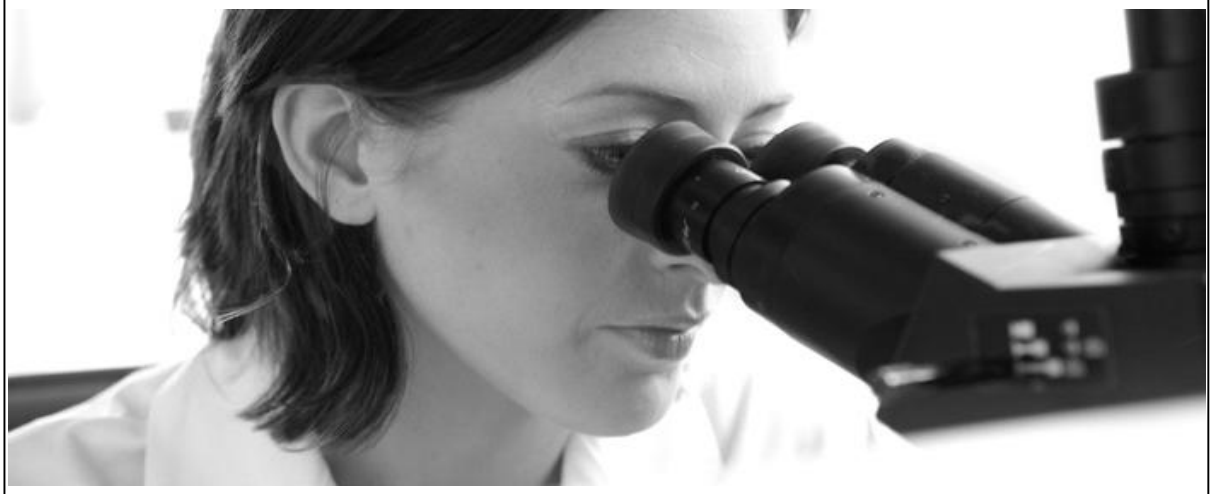

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RESEARCH ARTICLE

PRELIMINARY PHYTOCHEMICAL SCREENING OF LEAF EXTRACTS OF ANTHOCEPHALUS CADAMBA

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ABSTRACT

All plants have bioactive compounds which are used for curing of various human diseases and also play an important role in healing. Phytochemicals have two categories i.e., primary and secondary constituents. Primary constituents have chlorophyll, proteins sugar and amino acids. Secondary constituents contain terpenoids and alkaloids. Medicinal plants have antifungal, antibacterial and anti-inflammation activities. The leaves of the Anthocephalus cadamba were washed, air dried and then powdered. The aqueous extract of leaf samples were used for the phytochemical analysis to find out the phytochemical constituents in the plants. The main objective of the research work was to check the presence or absence of the phytochemical constituents in the leaves of Anthocephalus cadamba.The results of the phytochemical analysis showed that the terpenoids, tannins, reducing sugar, steroids, phenolic, flavonoids and alkaloids were found to be present. The phytochemical analysis of the plants is very important commercially and has great interest in pharmaceutical companies for the production of the new drugs for curing of various diseases. It is expected that the important phytochemical properties recognized by our study in the indigenous medicinal plant Anthocephaluscadamba of will be very useful in the curing of various diseases of Humans.

INTRODUCTION

Phytochemical are bioactive chemicals of plant origin. They are naturally synthesized in all parts of the plant body; bark, leaves, stem, root, flower, fruits, seeds, etc. i.e. any part of the plant body may contain active components (P. Tiwari *et al*, 2011).

Phytochemical have been recognized as the basis for traditional herbal medicine practiced in the past and currently en vogue in parts of the world (T. P.Lalitha *et al*,2012) In the search for phytochemical that may be of benefit to the pharmaceutical industry, researchers sometimes follow leads provided by local healers in a region(K. Das *et al*,2010). Following such leads, plant parts are usually screened for phytochemicals that may be present. Then it can be used as the basis for a new pharmaceutical product. Anthocephalus cadamba (Syn A. chine sis (Lamk) A. Rich (Rubiaceae) is widely distributed throughout India and 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 and also cure diabetes mellitus, diarrheao, inflammation, haemoptysis, cough, vomiting, wounds, ulcers, debility and antimicrobial activity. The leaves are recommended as a gargle in cases of stomatitis (Slkar *et. al*., 1996). Some scientific studies have been carried out to reveal its antimalarial (Sianne and Fanie 2002) and antihepatotoxic activities (Kapil, *et. al*., 1995). In the present study Methonalic leaves extract of Anthocephalus cadamba samples were used for the phytochemical analysis to find out the phytochemical constituents in the plants. The main objective of the research work was to check the presence or absence of the phytochemical constituents in the leaves of Anthocephalus cadamba.

MATERIALS AND METHODS

Collection of plant materials

The plant materials of *Anthocephalus cadamba* (Rubiaceae) and *Cymbopogan citratus* (for synthesis of silver nanoparticles) were collected from in and around Palani hills, Palani,

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Tamilnadu, India. The plants were authentified at BSI (Botanical Survey of India) and the specimens were deposited at Zoology Department, Bharathiar University, and Coimbatore, India.

Preparation of plant extracts

The plant materials of *Anthocephalus cadamba* leaves washed with tap water, shade dried at room temperature and powdered by an electrical blender. From each sample, 100g of the plant materials were extracted with 300ml of organic solvent methanol for 8hrs in a soxhlet apparatus (Vogel, 1978). The crude plant extracts were evaporated to dryness in rotary vacuum evaporator. Phytochemical screening of seaweed extracts: Seaweed extracts were subjected to various qualitative chemical tests to screen for phytochemical constituents.

Detection of alkaloids

Preparation of filtrate solvent free extract (50 mg) is stirred with 2 ml of dilute hydrochloric acid and filtered. To a 1 ml of filtrate a drop of Mayer's reagent was added by the side of tube and then observed for a white creamy precipitate (Evans, 1997).

Detection of carbohydrates

The extract (100 mg) is dissolved in 5 ml of water and filtered. To 2 ml of filtrate two drops of alcoholic solution of "-naphtha were added, the mixture is shaken well and 1 ml of concentrated sulfuric acid was added slowly along the sides of the test tube and allowed to stand, then observed for the formation of violet ring (Molish's test). To 0.5 ml of filtrate 0.5 ml of Benedict's reagent was added. The mixture was heated on a boiling water bath for 2 min and after that observed for characteristic red colored precipitate formation (Benedict's test).

Detection of proteins and amino acids

The extract (100 mg) was dissolved in 10 ml of distilled water and filtered through what man no.1 filter paper and the filtrate is subjected to tests for proteins and amino acids. To 2 ml of filtrate, few drops of Million's reagent were added and were observed for white precipitate (Millon's test).

Detection of phenolic compounds

The extract (50 mg) was dissolved in 5 ml of distilled water. To this few drops of neutral ferric chloride solution was added and observed for a dark green coloration (Mace, 1963).

Detection of flavonoids

To 5 ml of dilute ammonia solution a portion of the aqueous filtrate of each algal extract followed by addition of concentrated sulfuric acid. There was observed for a yellow coloration. The yellow coloration disappears on standing (Harborne, 1973; Sofowara, 1993).

Detection of steroids

Two milliliters of acetic anhydride was added to 0.5 g ethanol extract of each sample with 2 ml of sulfuric acid and this was observed for color change from violet to blue or green in some samples.

Test for cardiac glycosides (Keller-Killani test)

5 ml of each extract was treated with 2 ml of glacial acetic acid containing 1 drop of ferric chloride solution. This was underplayed with 1 ml of concentrated sulfuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring while in the acetic acid layer a greenish ring may form just gradually throughout thin layer.

FT-IR Spectral Analysis

FT-IR spectral (λ_{max} in cm^{-1}) analysis was carried out for the active column chromatographic fractions of A3 and E4 extract of *Anthocephalus cadamba* leaves detect the functional group of the bioactive compounds. It was recorded in KBr pellet on a FT/IR-8400s – SHIMADZU.

RESULTS AND DISCUSSION

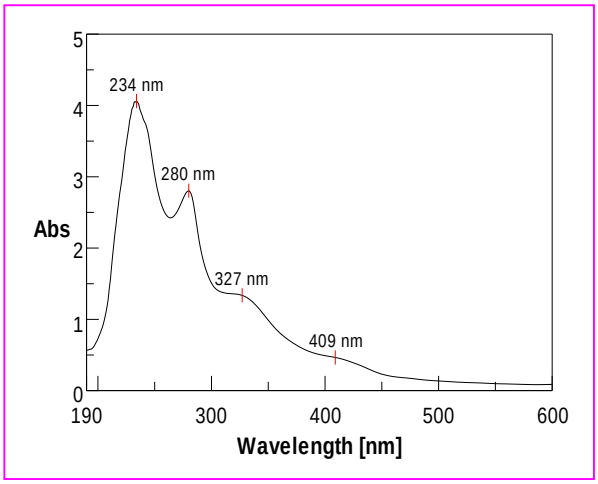
Phytochemical analysis of methanolic extract of Anthocephalus cadamba

Tests	Methanolic extract
Alkaloids	+
Carbohydrates	+
Proteins	-
Flavonoids	+
steroids	+
Glycosides	+
Phenolic compounds	+

(+)Positive (-)Negative

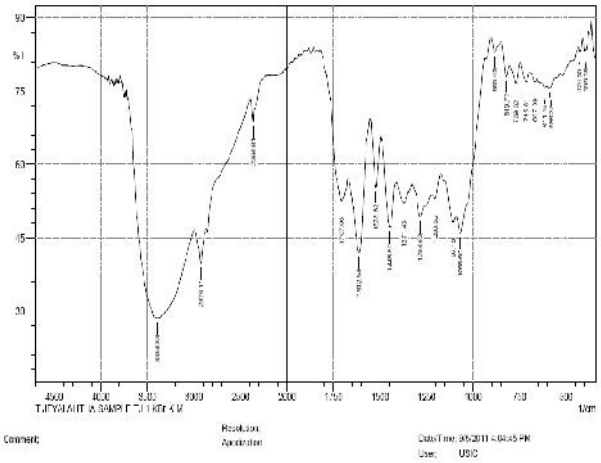
Based on the color reactions, the possibility of the isolable compounds in the crude Methanol extract of *Anthocephalus cadamba* plant are Phenolic compounds, Flavonoids, Steroids, alkaloids and oils. This shows that leaves offers a wider array of phytochemicals.

UV/Vis. absorption spectra



The crude methanol extract of *Anthocephalus cadamba* is further analyzed by the UV/Vis. absorption spectra. Crude extracts shows the three absorption bands are 234 nm, 280 nm, 327 nm and 409 nm (Fig. 1). The absorption range between 200-400 nm indicates the presence of the flavonoids as a major constituent in the crude methanol extract of *Anthocephalus cadamba*. Flavonoids are used as anti-inflammatory, anticancer, antiviral properties (Umachigi .s.p. *et al*) it is the safest non-immunogenic drug.

FT-IR spectra



List of phytochemicals shown in FT-IR spectrum of crude extract of *Anthocephalus cadamba*

S.NO.	Wave Number	Functional Groups
1	3394.83	Phenols & Alcohols
2	2926.11	Carboxylic Acid
3	2364.81	Nitriles
4	1707.06	Aldehyde
5	1612.54	Amine
6	1523.82	Nitro Groups
7	1448.59	Secondary Amine
8	1371.43	Nitro Groups
9	1284.63	Aromatic amines
10	1203.62	Aliphatic Amines
11	1107.18	Ketones
12	1068.60	Amides
13	883.43	N-H Primary Secondary Amines
14	819.77	N-H Primary Secondary Amines
15	769.62	N-H Primary Secondary Amines
16	715.61	N-H Primary Secondary Amines
17	667.39	S-O stretch – Sulfonates
18	611.45	C-Cl stretch alkyl halides C-Br stretch alkyl halides C-Cl stretch acid chlorides
19	586.38	C-Cl stretch alkyl halides C-Br stretch alkyl halides C-Cl stretch acid chlorides
20	424.35	C-Cl stretch alkyl halides
21	399.28	C-Cl stretch alkyl halides

The crude methanol extract of *Anthocephalus cadamba* is further analyzed by the FT-IR spectra to find out the functional groups. Fig. 2 shows the FT-IR spectra of crude methanol extract of *Anthocephalus cadamba*. The observed stretching frequencies of crude methanol extract of *Anthocephalus cadamba* show bands are 3394.83, 2926.11, 2364.81, 1707.06, 1612.54, 1523.82, 1371.43, 1203.62, 1448.59, 1371.43, 1203.62, 1107.18, 1068.60, 819.77, 769.62, 715.61, 667.39, 611.45, 586.38, and 424.35. The bands around 3394.83, 2926.11, 2364.81, 1707.06, 1612.54 manifest major IR bands, While the minor bands at 1523.82, 1371.43, 1203.62, 1448.59, 1371.43, 1203.62, 1107.18 and 1068.60.

The band around 1371 cm^{-1} can be assigned to methyl groups. To a large extent, the band at 1068 cm^{-1} might be contributed by the –C=O groups of the polyphenols such as flavones, terpenoids and the polysaccharides present in the crude methanol extract of *Anthocephalus cadamba*. The bands around 1734 cm^{-1} can be assigned to C=O stretching vibrations of the carbonyl functional groups. Presence of OH group has got the ability of forming hydrogen bounding capacity. So this extract has the higher potential towards inhibitory activity against microorganism.(Ashok Kumar &Ramaswamy, 2013)Presence of peptide bounds also inhibits the growth of bacteria (KimA.Brogden,2014)

CONCLUSION

Phytochemicals found present in leaves of Anthocephalus cadamba indicates their potential as a sources of principles that may supply novel medicines. Hence it could be the source for the industrial manufacture of drugs useful in the chemotheraphy of some microbial infection. Furthermore, isolation purification and characterization of the phytochemicals found present will make studies interesting.

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