Phytochemical Evaluation of *Anthocephalus Cadamba* and Invitro Cytotoxicity Studies

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Abstract: - *Anthocephalus cadamba* is a medium sized tree, belonging to family Rubiaceae, is found all over India. It is crucially significant as it has the largest number of phytochemicals and secondary metabolites having pharmacological and biological properties. The roots, leaves, fruit and bark of the plant is employed as ethno-drug for various health benefits. The aim of this study was to perform the radical scavengenging activity along with invitro cytotoxicity studies. Comparative analysis of phytochemicals in various extracts of *A.cadamba.* Leaves, fruits and bark of the plant were collected and various aqueous extracts were prepared. Extracts were screened for various phytochemicals qualitatively and quantitatively. Investigations revealed the presence of alkaloids, flavanoids, terpenoids, saponin, glycosides in various parts of the plant. Among various extracts, the total phenolic content and total flavanoids content were significantly higher in fruit extract while total alkaloid content was higher in stems extract. Results confirmed that *A.cadamba* (fruits, stems, leave) contains important phytochemicals-alkaloids, saponins, glycosides, flavanoids, terpenoids, phenolic content etc. which possess various biological and therapeutic activities.

Key Words: — ***Anthocephalus cadamba, Cytotoxicity studies, Phytochemical, fruit extract.***

I. INTRODUCTION

The use of herbal and medicinal plants to alleviate human suffering is as old as the evolution of human civilization. Medicinal virtues of herbal plants are already mentioned in the epics like Ramayana and Mahabharata [1]. From all over the worlds millions of people continue to use plants as their primary source of medicines. Much of this traditional information passed down through generations [2]. The diversity of medicinal plants in various biogeography regions in India is shortlisted. The estimation of West Himalaya is 1560 species of medicinal plants, Andaman & Nicobar has 3000, Western Ghats 3400and Eastern Ghats 1540[3].It has also been reported that Karnataka has 14900 medicinal species, Tamilnadu has 1500, Kerala has around 1500 and Andhrapradesh use to have 1100 species [4]. 21st century is currently acclaimed as the Century of Biology. The advancement made in sciences if applied properly can able to transform bio resources of nations to economic power. Centuries of traditional wisdom using species of plants for medicines for the prevention of different kinds of hazardous disease by ethical communities known as ethno medicine [5].

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Biodiversity representation is generally of two kinds .One is it a resources with so many actual uses and potent value of humanity.

Another one is a complex self-sustaining ecological system that generally helps to maintain the integrity and resilience of biosphere [6].Medicinal plants play an important role in the health maintenance of the largest portion of the world population. Since ancient times, herbal plant medications have been used to cure several types of diseases In spite of the great advances observed in modern medicine in recent decades; plants still need to make an important contribution to health care.

There are number of plants which are for medicinal purpose in several Countries such as China, India, and Egypt are well known for the active usage of medicinal plant in the treatment of incurable diseases. India is one of the largest producers of medicinal herbs in the world due to which it is also known a botanical paradise [7].

The current generation is unaware of its properties and its importance .Surprisingly, the natives and traditional healers do not have enough knowledge about therapeutic properties and its uses. As a result, the natural population of A.cadamba is decreasing and in near future, one can see it only in old pictures [8].The traditional healers of different regions of the world use the A.cadamba bark for the treatment of hoarseness

of throat. It is considered as one of the most effective remedies. A.cadamba bark plays a major role in the treatment of eye diseases. It is also effective in cases of stomatitis. Recently, various kinds of plant extracts supported biological activity and also helps in the inhibition of free radicals which are generated as a part of the body's normal metabolic process [9].

II. Materials And Methods

A. Plant Materials

The fresh leaves, fruits and stems of Anthocephalous cadamba were collected from the Nursery of jiit Noida, Uttar Pradesh in the month of June to October in the year 2018.

B. Preparation of Extracts

Fresh leaves ,stems and fruits of Anthocephalous cadamba was cutting to small pieces and are dried under sun for above two weeks then they are subjected to small scale blender and turned into coarse powder then the plant materials are extracted with Rotatory evaporator.

The ethanolic extracts were concentrated to a greenish residue by using water bath.

III. Qualitative Estimation

Following standard protocols were used for qualitative analysis of samples to check for the presence of several phytochemicals.

Test for Flavonoid:

2 ml of each extract was added with few drops of sodium hydroxide, few drops of dilute hydrochloric acid were added and yellow colour indicates the presence of flavonoid.

Test for Alkaloid:

3ml of each extract was added with few drops of Wagner's reagent, red brown colour indicates the presence of alkaloid.

Test for Saponin:

1ml of aqueous extract is diluted by distilled water up to 10 ml and shaken for 15 min. There is formation of layer of froth indicates the presence of saponin.

Test for Tannin:

2ml of extract was taken in test tube and added 2ml of Ferric chloride solution. Deep brown green colour of the solution was formed which shows the presence of Tannin.

Test for Phenol:

2 ml of each extract was added with 2 ml of aqueous ferric chlorides were added with few drops of alcohol; formation of blue colour indicates the presence of phenol.

Test for Protein:

2 ml of each extract was added with 2% of ninhydrin solution and dissolve in 10 ml of ethanol; formation of blue or violet colour indicates the presence of proteins.

Terpenoid:

1 ml of extract of each solvent and add some drops of chloroform followed by a few drops of concentrated sulphuric acid; formation of reddish brown precipitate indicates the presence of terpenoids in the extract.

IV. Quantitative Estimation

A. Determination of Total Phenolic Content (TPC)

Total phenolic content (TPC) was estimated by using Folin Ciocalteu method [McDonald et al., 2001]. For preparation of standard plot, 25 µl varying concentration of gallic acid (0.2, 0.4, 0.6, 0.8, 1.0 mg/ml) were mixed with 1.25 ml of Folin Ciocalteu reagent (0.2 N) and 1ml 7.5% (w/v) sodium carbonate.

The solution was incubated for minimum 3 h at room temperature. Absorbance was measured at 765nm. TPC of herbal formulation extracts were analyzed using similar procedure and results were expressed as mg gallic acid equivalent (GAE) /g herbal formulation.

B. Determination of Total Flavonoid Content

Aluminum chloride colorimetric method was used to determine flavonoid. Each plant extracts (0.5 ml of 1:10 g/ml) in ethanol were separately mixed with 1.5 ml of ethanol, 0.1 ml of 10% aluminum chloride 0.1 ml of 1 m Potassium acetate and 2.8 ml of distilled water.

The solutions were kept at room temperature for 30 min; the absorbance of the mixture was measured at 415 nm with a UV/ Visible spectrophotometer. The calibration curve was prepared by using quercetin solution at concentration 20 to 100 µg/ ml in ethanol.

C. Determination of Total Alkaloid Content

Accurately measure aliquots (10 to 30 µg/ml) of curcumin standard solution and transfer each to different separator funnels. Then, add 5 ml pH 4.7 phosphate buffers and 5 ml BCG Solution and shake a mixture with chloroform.

The extracts were used to get collected in a 10 ml volumetric flask and then diluted to adjust volume with chloroform. The absorbance of the complex in chloroform was measured at 470nm with a UV/Visible spectrophotometer against blank prepared as above but without curcumin.

V. DPPH Radical Scavenging Activity

The DPPH free radical scavenging activity of leaves, stem and fruit extracts of Anthocephaluscadamba was measured in vitro by 2, 2'-diphenyl-1- picrylhydrazyl (DPPH) assay. The stock solution was produced by dissolving 24 mg DPPH with 100 ml methanol and stored at 20º C. The working mixture was prepared by diluting DPPH solution with methanol to attain an absorbance of about 0.98±0.02 at 517 nm using the spectrophotometer.

A 3 ml aliquot of this solution was mixed with 100 µl of the sample at various concentrations (20-100 mg/ml).The reaction mixture needs to be shaken well and incubated in the dark for minimum 15 min at room temperature.

Then measure the absorbance at 517 nm. The controls were prepared as above without any sample. The scavenging activity was measured based on the percentage of DPPH radical scavenged as the following formula;

DPPH Radical scavenging activity (%) = [(control absorbance – sample absorbance / control absorbance) ×100]

The radical scavenging activity of ascorbic acid were measured and compared with that of the different synthesized compound.

VI. Antimicrobial Activity

Antimicrobial activity was tested using various microorganisms using the Gentamycin (50µg/ml) as standard by cup plate agar diffusion method. The organisms selected for antibacterial activity were Bacillus subtilis, Bacillus licheniformis, Micrococcus luteus and Pseudomonas putida was observed around area inoculated with the extract which depicts the antimicrobial activity of Anthocephalus cadamba (ethanolic extract of leaves,stem , fruits) by zone inhibition method.

The organisms selected for antifungal activity were Aspergillus niger, Aspergillus orchrace, Trichoderma, Rhizopus orchayae.

VII. Results

A. Qualitative Estimation

PHYTHOCHEMICALS	*A. Cadamba* leaves	*A.Cadamba* fruits	*A.Cadamba* stems
Tannin	+	+	-
Saponin	+	+	+
Steroid	-	-	-
Terpenoid	+	+	+
Flavonoid	+	+	+
Alkaloid	+	+	+
Proteins	+	-	+

Phytochemical Tests of *A. cadamba.*

Where, (+): positive (-): negative

B. Quantitative Estimation

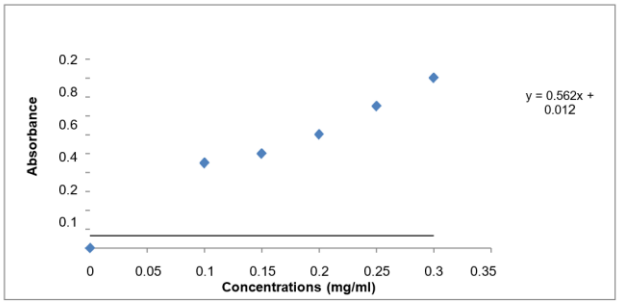

Fig.1. Standard curve of curcumin

The total content of alkaloid estimated in test samples. The standard curve of curcumin.

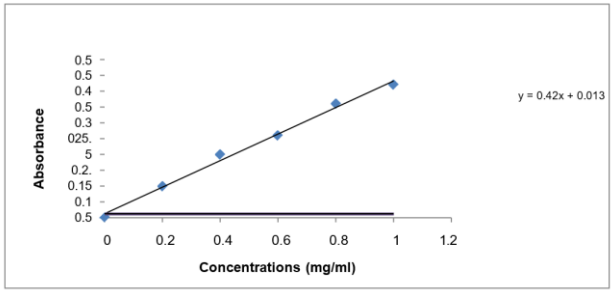

Fig.2.Standard curve of gallic acid

After plotting the standard graph of total phenolic with gallic acid, the ethanolic extract of Anthocephalus cadamba was tested for the same content of total phenolic concentrations.

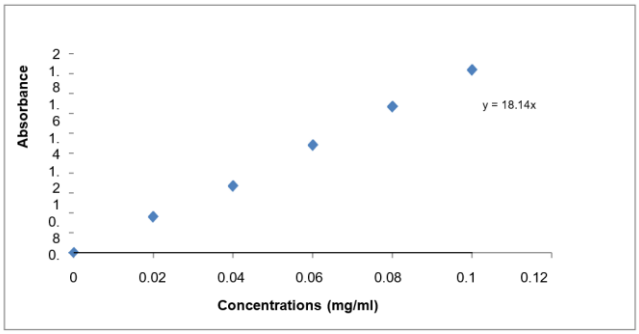

Fig.3. Standard curve of quercetin

Types of *A.cadamba* Extracts	**Total flavonoid content (mg QTE*/extract)**	**Total phenolic content (mg GAE/g extract)**	**Total alkaloid content (mg CC/g extract)**
Ethanolic extract of leaves	24.27 ± 0.48	21.7 ± 0.16	15.3± 0.11
Ethanolic extract of stems	26.38 ± 1.13	32.23 ± 0.58	24± 0.44
Ethanolic extract Of fruits	42.86 ± 1.31	48.2 ± 0.44	17.6± 0.34

The total flavanoids estimation in the test samples. After plotting the standard graph of total flavanoids content with gallic acid, the ethanolic extract of *A.cadamba* was tested for the same content of total phenolic concentration.

C. *Antioxidant Activity*

The antioxidant activity was plotted against various concentrations (20-100mg/ml) of test samples of leaves, stems, and fruits of *Anthocephalus cadamba*. Therefore, it was reflected that the stems of *Anthocephalus cadamba* has a maximum antioxidant activity followed by the extract and the standard of AA.

Conc.(mg/ml)	20	40	60	80	100
%SCV of leaves	54.21	55.98	63.3	71.96	73.39
%SCV of fruits	25.25	33.83	39.73	54.28	59.46
%SCV of stems	58.66	61.6	69.64	71.42	79.19
%SCV of Ascorbic acid	83.12	84.28	85.17	86.51	87.34

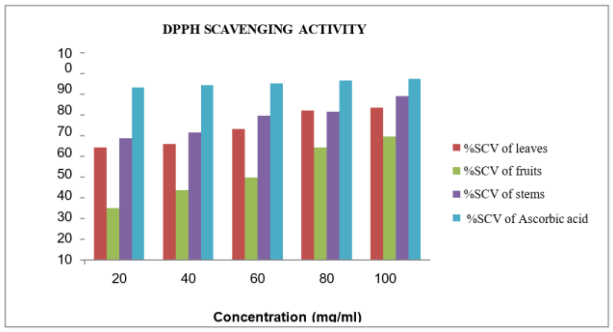

Fig.4. Graphical representation of DPPH radical scavenging activity

D. *Antimicrobial Activity*

Zone of inhibition *A.cadamba* (mm)						
Bacterial Species	Antibiotic	Leaves Extract	Stem Extract	Fruit Extract	Ethanol	Distilled Water
B.Licheniformis	19	15	11	12	11	0
B.subtilis	20	13	12	10	10	0
Micrococcus luteus	17	13.5	13	12	10	6
Pseudomonas putida	22	14.5	11	12.5	12	0

When the samples were loaded in the agar plates, there occurs the diffusion of the sample into the well which leads to the inhibition of growth of microbes in and around the wells loaded with samples. These inhibitions are clearly seen in the plates after incubation of 24- 72 hours for anti-bacterial and antifungal activity.

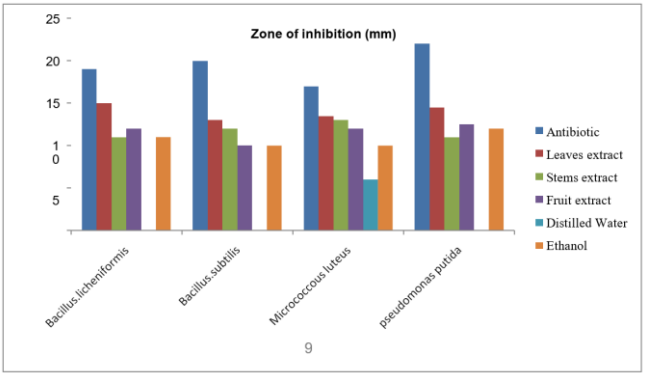

Fig.5. Graphical representation of Antibacterial –Zone of inhibition

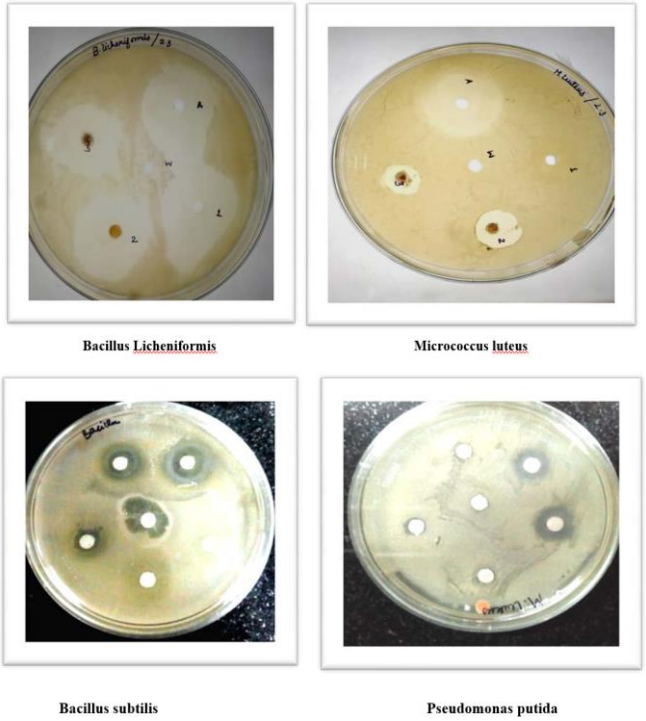

Fig.6. Bacterial strains showing zone inhibition

Fig.7. Fungal stains showing zone inhibition

Zone of inhibition *A.cadamba* (mm)						
Fungal Species	Antibiotic	Leaves Extract	Stem Extract	Fruit Extract	Ethanol	Distilled Water
Aspergillus niger	17	10	11	10	6	0
Aspergillus orchraceus	20	11	12	10	8	0
Rhizopus oryazae	18	8	8	12	6	6
Trichoderma	15	10	14	10.5	5	0

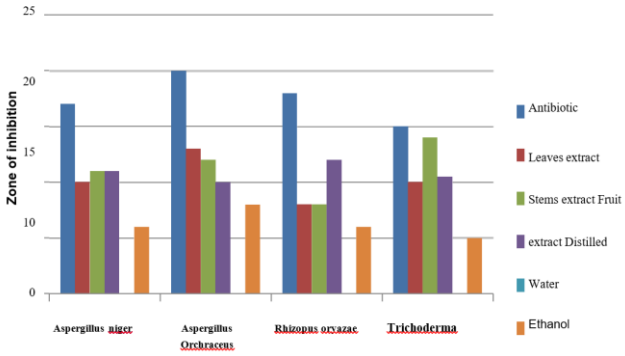

Fig.8. Graphical representation of zone inhibition of fungal strains

VIII. CONCLUSION

Among various extracts, the total phenolic content and total flavanoids content were significantly higher in fruit extract while total alkaloid content was higher in stems extract. Results confirmed that A.cadamba (fruits, stems, leave) contains important phytochemicals- alkaloids, saponins, glycosides, flavanoids, terpenoids, phenolic content etc. which possess various biological and therapeutic activities. The antioxidant activity significantly higher in stem extracts. The antibacterial activity is higher in leaves extracts and antifungal activity significantly higher in stems extract.

A.Cadamba is an indigenous flowering plant. In the Context of the above discussion, it can be concluded that the A.Cadamba (fruits, stems, leaves) contain important phytochemicals- alkaloids, saponins, glycosides, flavanoids, terpenoids, phenolic content etc. which possess various biological activities, which indicate that A.Cadamba play an important role in drug research.

Therefore, the plant is a worthy contender for systemic, chemical and biological studies to determine the active principle.

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