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Preliminary Phytochemical Screening and Evaluation of *In vitro* Antioxidant Activity of *Anthocephalous cadamba* by Using Solvent Extracts

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Abstract: The present study was to estimate preliminary photochemical evaluation and *in vitro* antioxidant of leaves and fruit extracts of *anthocephalous cadamba* by using different solvents like pet ether, ethanol, chloroform and water. Preliminary phytochemical analysis reveals the presence of carbohydrates, tannins, saponins, flavanoids and terpenoids. The extract was screened for its potential antioxidant activity using DPPH free radical scavenging activity. The reducing power extract was also determined ascorbic acid was used as a standard and positive control for both leaves and fruit analysis. All the analysis was made by using UV spectrophotometer (Perkin Elmer). The ethanol leaves and fruit extract shown significant shown DPPH (1, 1-Diphenyl-2-picryl-hydrazyl) radical scavenging activity compared to standard antioxidant. The DPPH radical scavenging activity of the extract was increased with increasing concentration. In DPPH free radical scavenging assay IC_{50} values of leaf and fruit extract of *anthocephalous cadamba* was found to be 22 and 18 µg/ml respectively. The results were concluded that extracts have a potential source of antioxidants of natural origin.

Key words: Antioxidant · *Anthocephalous cadamba* · Free Radical · DPPH

INTRODUCTION

Free radicals can have noxious effects on cells and it is believe that free radical damage is a wall in the etiology of several diseases. The radicals are the byproducts of various endogenous processes that can be stimulated by external factors such as irradiation and xenobiotics [1]. Antibiotics protect against the free radical and it is important to balance an enhanced radical production with sufficient supply of antioxidants. There are two basic categories of antioxidants namely synthetic and natural. The most common synthetic antioxidants used in foods are compound with phenolic structures of various degrees of substitution. Whereas natural antioxidants are primarily plant phenolics and Polyphenolic compounds that may occur in all parts of the plant [2]. In the living system free radicals are generated as a part of the bodies normal metabolic process and free radical chain reactions are usually produced in the mitochondrial respiratory chain liver mixed function oxidases, through xanthine oxidase activity, atmospheric pollutants and from transitional metal catalyst drugs and xenobiotics. In addition chemical mobilization of fats stores under various conditions such as lactation, exercise, fever, infection and even fasting can result in increased radical activity and damage [3-6]. Antioxidants may be defined as radical's scavengers which protect the human body against free radicals that may cause pathological conditions such as ischemia, anemia, asthma, arthritis, inflammation, neurodegeneration, Parkinson's disease. Flavones and flavanoids are widely distributed as secondary metabolites with antioxidant and antiradical properties [7-10]. This has attracted a great deal of research interest in natural antioxidants subsequently a worldwide trend towards the use of natural photochemical present in berry crops, tea, herbs, oilseeds, beans, fruits and vegetables have increased. The majority of active antioxidant compounds are flavanoids, isoflavones, flavones, coumarins, catechins, lignans and isocatechins. In addition to above compounds found in vitamin C and Vit E and Tocopherol are known to posess antioxidant potential. The abundant source of unique active components harbored in plants. The present study was taken up on the medicinal plant namely *anthocephalous cadamba* belongs to the family *rubaceae*.

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Anthocephalous cadamba is a deciduous tree of occasionally buttrened up to 37.5 m height found allover India. Leaves are coriaceous broadly oveate elliptic oblong. Fruits are flushy, orange, globose, pseudo carp of compressed angular capsules with persistent calyx. Seeds are small muriculate. The various part of the tree are widely used in ayurveda, siddha and unani system of medicines [11]. The major constituents of leaf and fruits are flavanoids, glycosides, carbohydrates, terpins, terpenoids and saponins. The objectives of this work are to carryout phytochemical screening and evaluation of *in vitro* antioxidant activity of *anthocephalous cadamba* by using different solvent extracts.

MATERIALS AND METHODS

Plant Material: The fresh leaves and fruits of *Anthocephalous cadamba* were collected from the premises of GIET school of Pharmacy of Rajahmundry city of East Godavari district of Andhrapradesh in the month of June to October in the year 2012 and identified by Regional Forest Research Centre in Rajahmundry, East Godavari district andhrapradesh, India.

Preliminary Phytochemical Analysis: Preliminary phytochemical analysis was performed to identify the phyto constituents and to make the further study easy it reveals the following compounds are present.

Preparation of Extracts: Fresh leaves 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 Soxhlet apparatus by the solvent system selected with increasing polarity like pet ether, chloroform, ethanol and water. The extracts was concentrated to a greenish residue by using waterbath. The weights obtained of leaves and fruit extract was tabulated below and they are used for further investigation for potential antioxidant properties.

Antioxidant Assay: The antioxidant activity of plant extracts were determined by *In vitro* method by using DPPH (1, 1-diphenyl-2.picryl-hydrazyl) free radical scavenging assay method.

Table 1: Preliminary phytochemical analysis

TEST	RESULT
Carbohydrates	+
Tannins	+
Glycosides	-
Sapponins	+
Flvanoids	+
Terpenoids	+
Proteins	-
Present indicates	+
Absent indicates	-

Table 2: Weight of Leaves and Fruit extracts of different solvents

SOLVENTS	FRUITS	LEAVES
Pet. ether	3.25 g	2.12g
Chloroform	1.02gms	2.45 g
Methanol	2.02 g	2.31 g
Water	1.75 g	2.63 g

DPPH Radical Scavenging Activity: The DPPH free radical scavenging activity of leaves and fruit extracts of *Anthocephalouscadamba* was determined. DPPH radical scavenging effect was carried out according to the method first employed in Blois [12]. Compounds of different concentration were prepared in distilled ethanol 1ml of each solutions have different concentrations distilled ethanol, 1ml of each compound solutions having different concentrations were taken in different test tubes; 4 ml of a 0.1m ethanol solution of DPPH was added and shaken vigorously. The tubes were then incubated in the dark room at RT for 20 min. A DPPH blank was prepared without compound and ethanol was used for the baseline correction. Changes (decrease) in the absorbance at 517 nm were measured using a UV-visible spectrophotometer and the remaining DPPH was calculated. The percent decrease in the absorbance was recorded for each concentration and percent quenching of DPPH was calculated on the basis of the observed decreased in absorbance of the radical. The radical scavenging activities wereexpressed as the inhibition percentage and were calculated using the formula:

$$\text{Radical scavenging activity (\%)} = [(A0 - A1 / A0) \times 100]$$

where A0 was the absorbance of the control (blank, without compound) and A1 was the Absorbance of compound. The radical scavenging activity of ascorbic acid was also measured and compared with that of the different synthesized compound. For all the Compounds and standards half inhibition concentration (IC50) was calculated.

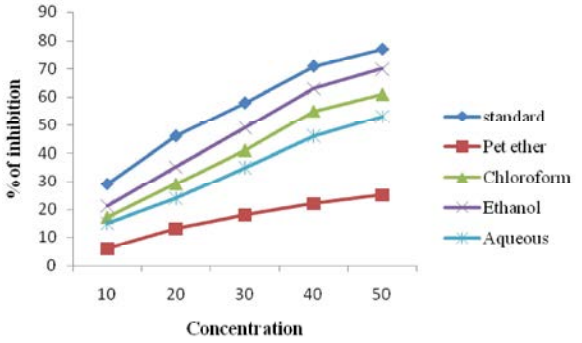

Fig. 1: DPPH radical scanenging activity of fruit extracts of anthocephalus cadamba added to ethanolic solution of DPPH and radical scavenging activity was measured as 517 nm as compared to ascorbic acid.

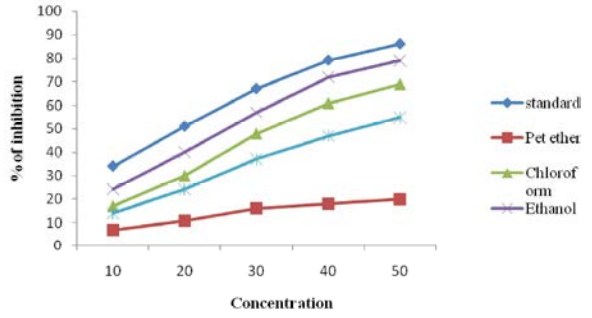

Fig. 2: DPPH radical scanenging activity of leaves extracts of *anthocephalus cadamba* added to ethanolic solution of DPPH and radical scavenging activity was measured as 517 nm as compared to ascorbic acid.

Table 3: IC_{50} values of Leaves and Fruit extracts of different solvents of Anthocephaluscadamba

EXTRACT	FRUITS (µg/ml)	LEAVES(µg/ml)
Standard	17	13
Ethanol	22	18
Chloroform	29	24
Water	41.42	34

RESULTS AND DISCUSSION

DPPH radical scavenging activity of leaves and fruit extract of *Antocephalous cadamba* and ascorbic acid are presented in the Figures 1 and 2 respectively.

In this present study the antioxidant activity of leaves and fruit extract of *Anthocephalous cadamba* investigated by using DPPH scavenging assay of the extract. The ethanol extract of *Anthocephalous cadamba* showed profound activity than chloroform, pet ether and aqueous extract the pet ether reported very less activity when compared to that of standard and reference. The DPPH antioxidant activity is based on the ability of DPPH a stable free radical to decolorize the presence of antioxidants.

The DPPH radical contains an odd electron which is responsible for the absorbance at 570 nm and also for visible deep purple colour. When DPPH accepts an electron donated by an antioxidant compound the DPPH is decolorized which can be quantitatively measured from the changes in absorbance comparison of antioxidant activity of the extract and ascorbic acid to shown in the Figures1 and 2 respectively. The ethanol extracts of fruit and leaves showed significant dose dependent inhibition of DPPH activity than the chloroform, pet ether and water.IC_{50} (concentration providing 50%inhibition) values were calculated as the low IC_{50} value indicates strong antioxidant activity in a sample. The IC_{50} values of leaf and fruit extract were tabulated below

CONCLUSION

It has been reported that reactive oxygen species contribute to various patho physiological conditions and endogenous defense mechanisms have evolve to offer protection in these conditions and increasing antioxidant reserves of organism can reduce oxidative stress and some of the plant derived agents may help to reduce it. Determination of the natural antioxidant compounds of plant extracts will help to develop new drug candidates for antioxidant therapy. The plants are the best sources for obtaining natural antioxidants for various medicinal uses such as aging and diseases related to radical mechanism. The study is to explore the antioxidant principles from natural resource and it is time for us to explore and identify our traditional therapeutic knowledge and plant sources for recent advancement to fight against the oxidative stress.

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