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In Vitro Antioxidant Potential and Phytochemical Study on Flower and Leaves of *Cassia auriculata*

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Abstract

The research work carried out to estimate the total phenolic and flavonoid contents of the leaf and flower extracts of *Cassia auriculata* to innovate an antioxidant potential by *in vitro* method. Detection of alkaloids, flavonoid, tannin, carbohydrates, steroids, saponins and glycosides contents of leaf and flower samples were done by phytochemical analysis. In addition, the antioxidant of leaf and flower extracts of *C. auriculata* was analyzed for its free radical scavenging property. The results of the study investigated that the total antioxidant activity was found to be high in the methanolic extracts of flower and leaf samples of *C. auriculata* and also recorded the total phenolic and total flavonoid contents to be more than the chloroform and ether extracts. Our innovation concluded that the extracts of both flower and leaf of *C. auriculata* showed an excellent antioxidant potential effect and it can be utilized for medicinal purpose to protect the human from several diseases.

Key words: *Cassia auriculata*, antioxidant, phenolic, flavonoids

Introduction

India is one of the richest plant sources possessing medicinal value to the world ensuring health security. There are estimated to be around 25,000 effective plant-based formulations, used in folk medicine which is in practice as home remedies in India and China. Plants and plant product are part of the diet and many of them exhibit medicinal properties, due its medicinal values they are

being used as ayurvedic and siddha medicines for several thousand years. Phytochemicals are responsible for medicinal activity of plants (Savithramma *et al* 2011) these are non-nutritive chemicals that have protected human from various diseases. Since medicinal plants are nontoxic and easily affordable they play a vital role not only for pharmacological research and drug development, but also when plant constituents are used directly as therapeutic agents and as starting materials for the synthesis of drugs (Masood 1972).

The major constituents consists of carbohydrates, amino acids, proteins and chlorophylls while secondary metabolites Some of the most important bioactive phytochemical constituents are the glycosides, alkaloids, flavonoids, tannins, steroids, terpenoids, essential oils and phenolic compounds (Kumar *et al* 2009). Last two decades, there has been an increasing interest in the investigation of different extracts, obtained from traditional medicinal plants, as potential sources of new antimicrobial (Bonjar & Farrokhi 2004) and antioxidative agents (Amakura *et al* 2002). In recent years, multiple drug/chemical resistance in both human and plant pathogenic organisms has developed due to the indiscriminate use of commercial antimicrobial drugs/chemicals in the treatment of infectious diseases.

Cassia belonging to the family Leguminosae which is a large genus of around 500 species of flowering plants and is widely distributed throughout Asia including India, Mauritius, China, East Africa, South Africa, America, Mexico, West Indies and Brazil. *Cassia* species have been of medicinal interest in phytochemical and pharmacological research due to their excellent medicinal values. Plants belonging to *Cassia* species are used extensively in various parts of the world against a wide range of ailments, the synergistic action of its metabolite being probably responsible for the plants beneficial effects. They are well known in folk medicine for their laxative and purgative uses (Verma *et al* 2010)**.**

Besides, there are several researchers have been found to exhibit anti-inflammatory, antioxidant, hypoglycemic, hyperglycemic, antiplasmodial, larvicidal, antimutagenic and anticancer activities. They are widely used for the treatment of wounds (Joshi 2000), skin diseases such as ringworm, scabies and eczema, gastro-intestinal disorders like ulcers, uterus disorders rheumatism, anorexia and jaundice (Pieme *et al* 2006). In the ayurvedic system of medicine this plant is also used for

the treatment of fever, headache, ulcers, leprosy and liver disease. Recently, the antidiabetic, hypolipidemic, antioxidant and hepatoprotective effect of *Cassia auriculata* have been reported by Uma Devi and Udupa (2005), and earlier reports shows that the flower and leaf extract of Cassia contains the antipyretic activity (Vedavathy & Rao 1991). They are also widely used for the treatment of wounds, skin diseases such a ringworm, scabies and eczema, gastro-intestinal disorders like ulcers disorders, rheumatism, anorexia and jaundice. Several natural compounds extracted from medicinal plants have been exhibit antioxidant and/or radical scavenging properties, which protect the human body from chronic diseases.

Materials and Methods

Preparation of the extracts: Twenty-five grams of leaf and flower samples are mixed individually with solvents of methanol (80% v/v), chloroform and petroleum ether in a 250 ml beaker and enclosed with an aluminum foil. The samples were sonicated in a sonicater (42 kHz, 135 W; Branson Ultrasonic Corporation, USA). The solvent surface in the beaker was kept at the level of the water in the ultrasonic bath and the water in the sonicator is circulated and regulated at constant room temperature (27 ± 2^0C to avoid a rise in water temperature caused by the ultrasonic. After the initial extraction, the sample residues were then submitted again for 15 min of sonication twice with 150 ml of 80%, each solvents were taken for the study and then respective liquid extracts were then filtered, final volume was made upto 600 ml and kept at 4^oC at refrigerator in air tight containers for further study.

Preliminary phytochemical investigation: The preliminary phytochemical screening of the extracts was carried out to know the different constituents available in the extracts as per the standard procedures followed by Suseela *et al* (2010).

Determination of total phenol content: The amount of total phenol content was determined by Folin-Ciocalteu's reagent method, 0.5 ml of extract (1 mg/ml) and 0.1 ml Folin-Ciocalteu's reagent (0.5 N) was mixed and the mixture was incubated at room temperature for 15 min. Then 2.5 ml saturated sodium carbonate solution was added and further incubated for 30 min and the absorbance was measured at 760 nm. Gallic acid was used as a positive control and the total phenol values are expressed in terms of garlic acid equivalent (µg/ml of extracted compound).

Total flavonoid content : Plant extracts were dissolved in methanol to obtain a concentration of 500 µg/ml and 1 ml of test sample, 4 ml of distilled water added to a volumetric flask (10 ml capacity) and kept for five minutes, and then added 0.3 ml of 5% NaNO2, 0.3 ml of 10% AlCl3.H2O. After six minutes of incubation added 2 ml of 1M NaOH into the reaction mixture. Immediately the final volume was made up to 10 ml of distilled water. The absorbance was measured at 510 nm. The experiment was performed in triplicate (Suseela *et al* 2010). A calibration curve was constructed using quercetin (100-500 µg/ml) as standard and total flavonoid content of the extract (µg/ml) expressed as quercetin equivalents.

Total Antioxidant activity-Ferric Thiocyanate method: The peroxy radical scavenging activity was determined by thiocyanate method using α- tocopherol (50-800 µg/ml) as standard. Increasing concentration of the fractions (50-800 µg/ml) in 0.5 ml of distilled water was mixed with 2.5 ml of 0.02 M linoleic acid emulsion (in 0.04 M phosphate buffer pH 7.0) and 2 ml phosphate buffer (0.04 M, pH 7) in a test tube and incubated in darkness at 37 ºC. At intervals during incubation, the amount of peroxide formed was determined by reading the absorbance of the red colour developed at 500 nm by the addition of 0.1 ml of 30% ammonium thiocyanate solution and 0.1 ml of 20 mM ferrous chloride in 3.5% hydrochloric acid to the reaction mixture. The percentage scavenging activity was calculated and was compared with α-tocopherol.

Radical scavenging assay-DPPH: The free radical scavenging activity of the fractions was measured *in vitro* by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. Around 0.3 mM solution of DPPH in 100% ethanol was prepared and 1ml of this solution was added to 3 ml of the fraction dissolved in ethanol at different concentrations (25-400 µg/ml). The mixture was shaken and allowed to stand at room temperature for 30 min and the absorbance was measured at 517 nm using a spectrophotometer. The % scavenging activity at different concentrations was determined and compared with that of ascorbic acid, which was used as the standard.

ABTS radical scavenging assay

To the reaction mixture containing 0.3 ml of ABTS radical, 1.7 ml phosphate buffer and 0.5 ml extract was added at various concentrations from 2 to 200 µg/ml. Blank was carried out without drug. Absorbance was recorded at 734 nm. Experiment was performed in triplicate (Sreejayan *et*

al 1996). The statistical analysis of the experimental results was expressed as mean ± standard deviation (SD) of three replicates.

Results and Discussions

Phytochemical screening revealed the presence of phenolics and flavonoids in high levels, which could be mainly responsible for the remarkable antioxidant effect of this plant. Also, the presence of other phytochemical constituents like alkaloids, flavonoid, tannin, carbohydrates, steroids, saponins and glycosides were found to be present in the methanolic extracts of flower and leaf listed (Table-1). Fig: 1 shows that the total phenolic content of standard gallic acid. The flower extract of *C. auriculata* showed highest total phenolic content and it was 107 µg/ml calculated as Gallic acid equivalent of phenols was detected. The total phenolic content exhibited the following order: methanolic extract (107 µg/ml) > chloroform extract (62 µg/ml) > petroleum ether extract (36 µg/ml). In leaf total phenolic content of flower exhibited the following order: methanolic extract *(*88 µg/ml) > chloroform extract (45 µg/ml) > petroleum ether extract (27 µg/ml).

Fig: 2 showed ths tthe total flavonoids content of standard Quercetin. The flower extract of *C. auriculata* showed highest total phenolic content and it was recorded 148 µg/ml calculated as Quercetin equivalent flavonoids was detected and the total flavonoids content exhibited the following order: imethanolic extract (148 µg/ml) > chloroform extract (72 µg/ml) > petroleum ether extract (8.5 µg/ml). The toal flavonoids content in leaf exhibited the following order: methanolic extract (140 µg/ml) > chloroform extract (68 µg/ml) > petroleum ether extract (1.5 µg/ml).

Fig: 3 showed that the total antioxidant activity of standard ascorbic acid. The flower extract of *C. auriculata* showed highest total antioxidant capacity and it was 200 µg/ml calculated as Ascorbic acid equivalents was detected. The total antioxidant activity of flower and leaf extracts exhibited the following order: methanolic extract (Flower 200 µg/ml, leaf 180 µg/ml) > chloroform extract (flower 95 µg/ml, leaf 89 µg/ml) > petroleum ether extract (flower 70 µg/ml, leaf 65 µg/ml). In the study, the percentage of scavenging effect on the DPPH radical was increased consistently with the increase in the concentration of both leaf and flower methanolic extracts from 25 to 400 µg/ml. The percentage of inhibition was varied 35.24 at 25µg/ml to 91.23 at 400 µg/ml for

methanolic leaf extract, chloroform leaf extracts, they were 30.12 at 25 µg/ml and 86.01 at 400 µg/ml and for pet ether extract 16.12 at 25µg/ml to 48.91 at 400 µg/ml (Figure 4).

From the results it is known that the species, *C. auriculata* possess hydrogen donating capabilities for methanolic leaf extract and does scavenging free radicals. In the study revealed that the methanolic flower extract of *C. auriculata* has highest antioxidant activity than that of its leaf extract (Fig-5). The percentage of inhibition was existed from 38.12 at 25µg/ml to 94.26 at 400 µg/ml for methanolic flower extract, chloroform flower extracts, they were 34.06 at 25 µg/ml and 88.19 at 400 µg/ml and for pet ether extract 18.14 at 25µg/ml to 52.56 at 400 µg/ml. The ABTS radical scavenging activity of *C. auriculata* flower and leaf extracts shown in Fig. 6 and 7. It was able to scavenge ABTS radical in a concentration range of 2-200 µg/ml. The presence of chemical compounds in the extracts of *C. auriculata* may inhibit the potassium persulfate activity and hence reduced the production of ABTS•+. This study reports that the methanolic flower extract of *C. auriculata* has highest antioxidant activity than that of its leaf extracts.

Based on the solvent as well as the extraction techniques which determines the elution of phyto compounds present in the plant materials. In the present study polar solvents such as methanol, petroleum ether and chloroform were used as an extracting solvent. Sonication techniques revealed to be one of the simple and fastest methods of extraction with the ability to extract and retain maximum amount of phytocompounds in comparison with the other available conventional extraction techniques. Sonication causes efficient tissue disruption and penetration of solvent into the solvent matrix of the plant material (Annegowda *et al* 2010).

The bioactive principles such as alkaloids, phenolic compounds, flavanoids and tannins etc present in the medicinal plants may be responsible for self defence of plants from pathogens and pest and also to cure various ailments caused by microorganisms (Sukumaran 2011). Depends on the phenolic content of the pants the antioxidant potential may be concludes, the correlation between antioxidant content and antioxidant potential of pants has been proved by many researchers (Dorman *et al* 2014).

Presence of phenolic content in plants determines the biological activity such as antioxidant and antibacterial etc. All plants possess phenolic compounds but the amount may vary. Each phenolic group has their own spectra characterizes, presence of functional derivatives and the sub

constituents, positioning of hydroxyl group in the aromatic ring determines the activity of the compound. Redox properties, which plays an important role in neutralizing free radicals, quenching single and tripet oxygen, or decomposing peroxidase these action is be lived to be influenced by the phenolic compounds present in the plants (Itagaki *et al* 2009). From reviewing many reports it has been proved that *C. auriculata* of stem extract from various solvents possess maximum antioxidant activity and phenolic content (Baravalia *et al* 2009).

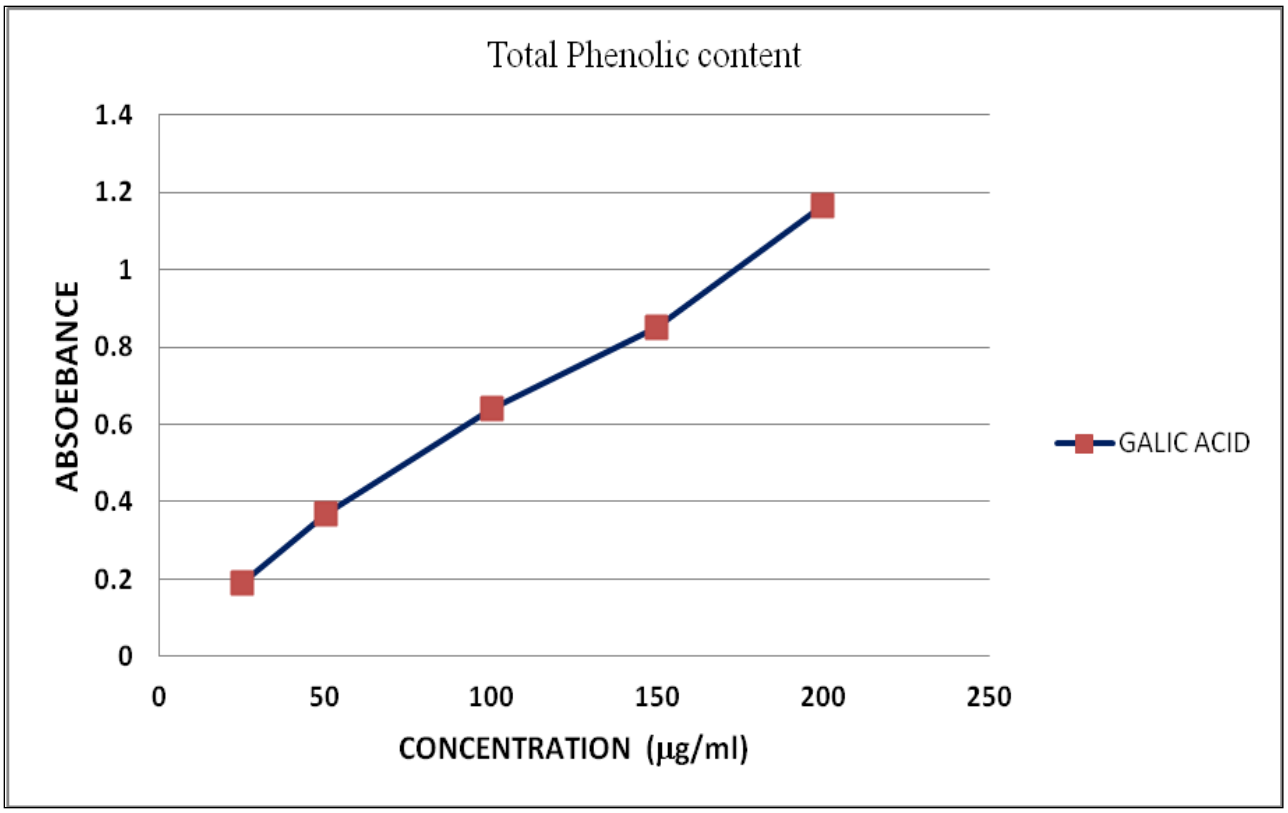

Fig. 1: Total phenolic content

DPPH is a stable free radical containing nitrogen in centre, upon reduction of electrons or hydrogen donating ability of the extract containing antioxidant compound shows the reduction of violet colour to yellow colour (Oliveira *et al* 2009). The degree of decolourisation shows the potential of the extracts in terms of hydrogen donating ability. The reactive oxygen species such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrile which leads to oxidative stress and causes cellular damage (Burlon & Ingod 1984). Oxidative stress may lead to degenerative diseases such as cancer, aging, atherosclerosis, inflammation, ischemic injury. The secondary metabolites present in the plant extracts help to protect against these diseases by contributing antioxidant vitamins and enzymes.

Table 1: Phytochemical content in leaf and flower extract of *Cassia auriculata*

Components	Methanolic extract		Chloroform extract		Petroleum ether extract	
	Flower	Leaf	Flower	Leaf	Flower	Leaf
Alkaloids	+	-	-	-	-	-
Flavonoids	+	+	-	-	+	+
Tannins	+	+	+	+	-	+
Carbhohydrates	+	+	+	+	-	-
Steroids	+	-	-	-	-	+
Saponins	-	-	+	+	-	-
Glycosides	+	+	+	+	-	+

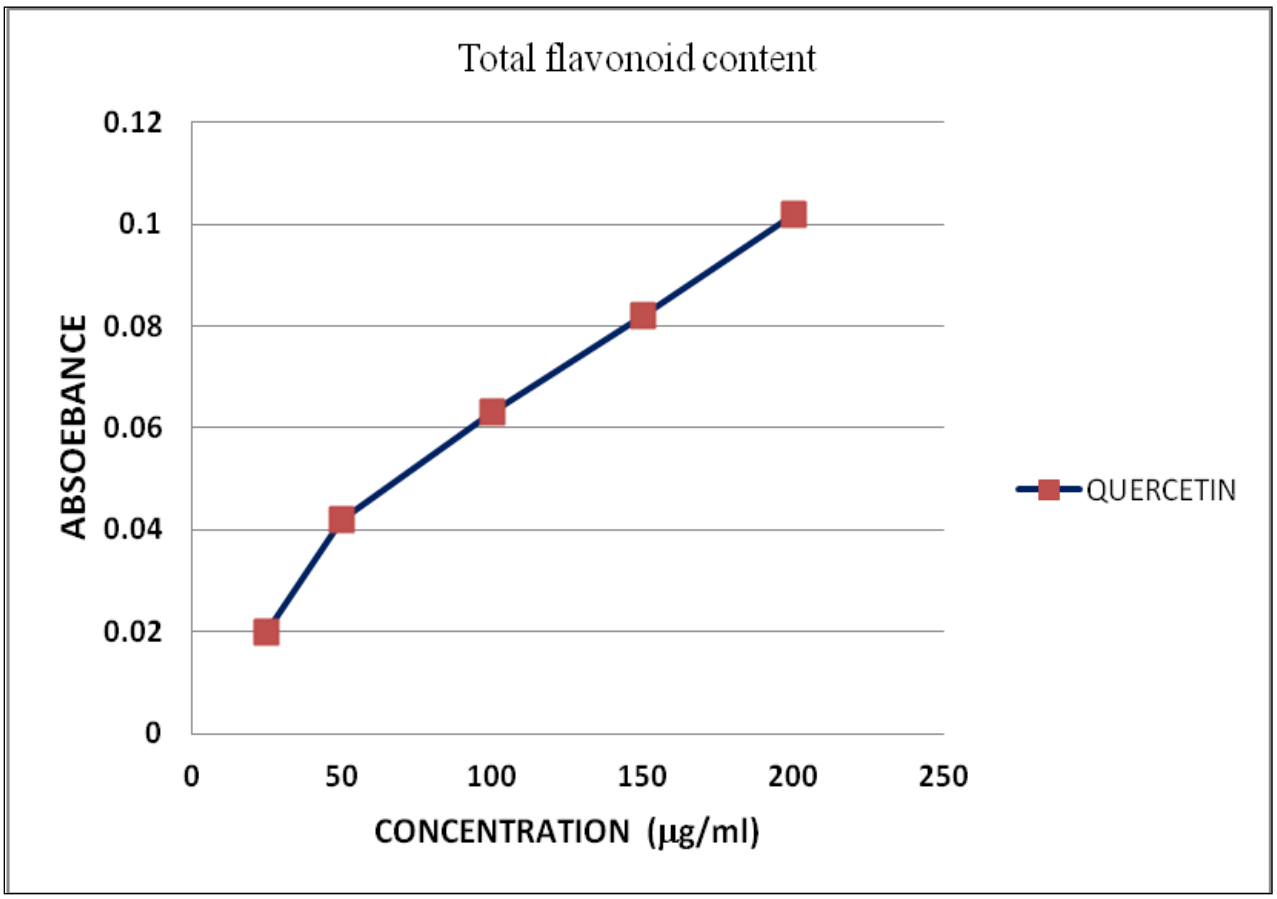

Fig. 2: Total flavonoid content

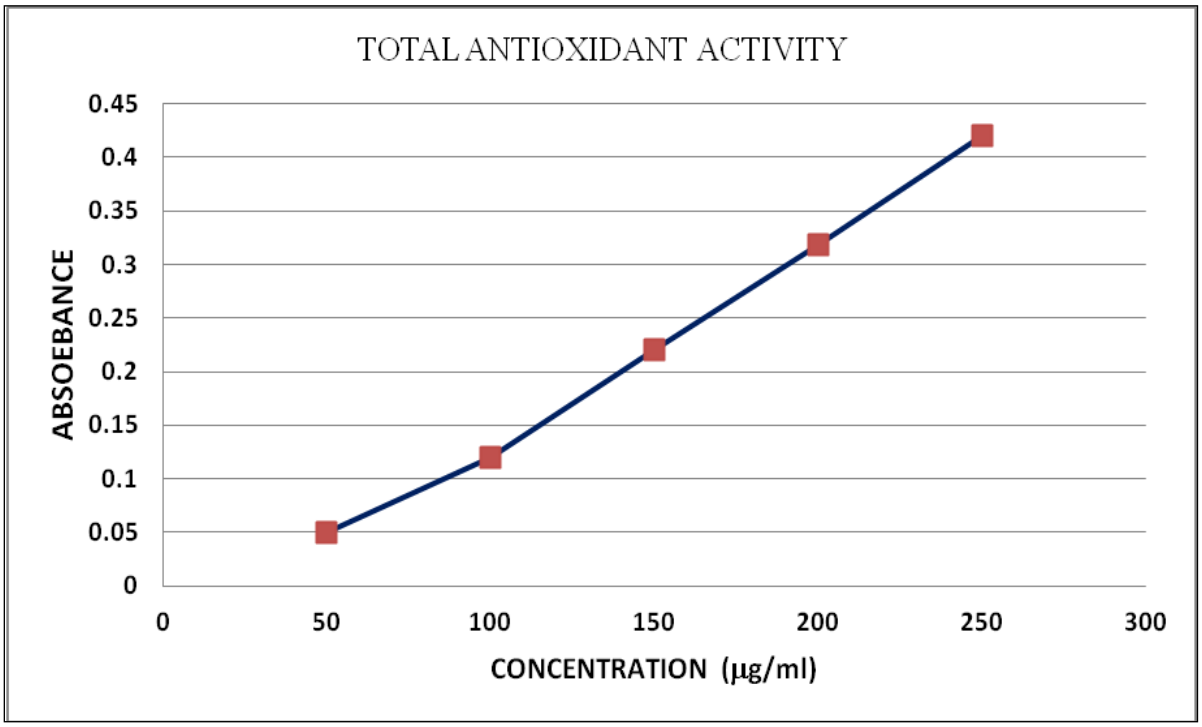

Fig. 3: Total antioxidant activity.

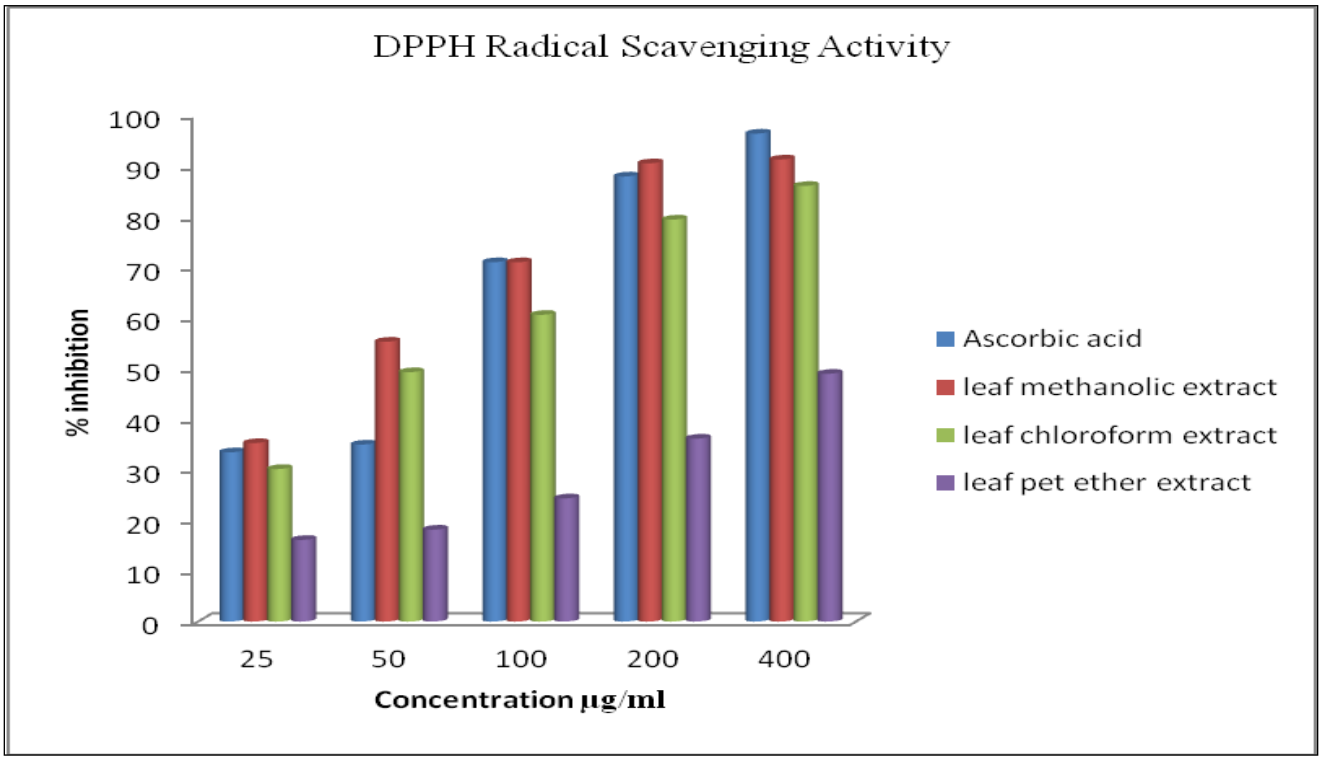

Fig. 4: Comparative DPPH radical scavenging activity of *C. auriculata* leaf extract

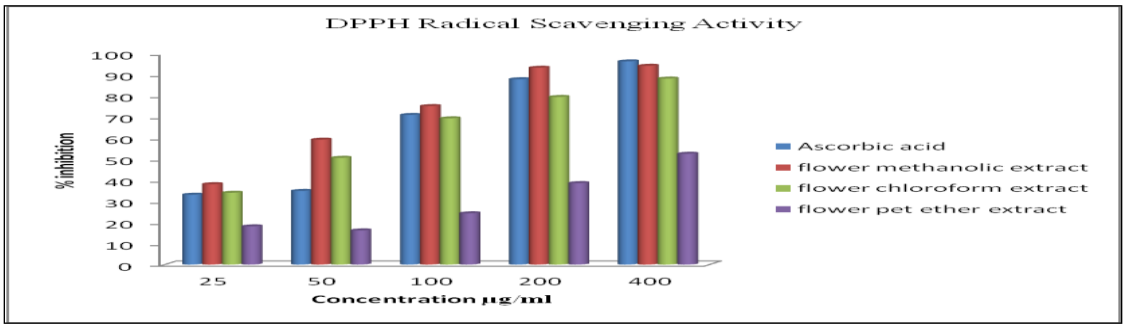

Fig.5: Comparative DPPH radical scavenging activity of *C. auriculata* flower extract

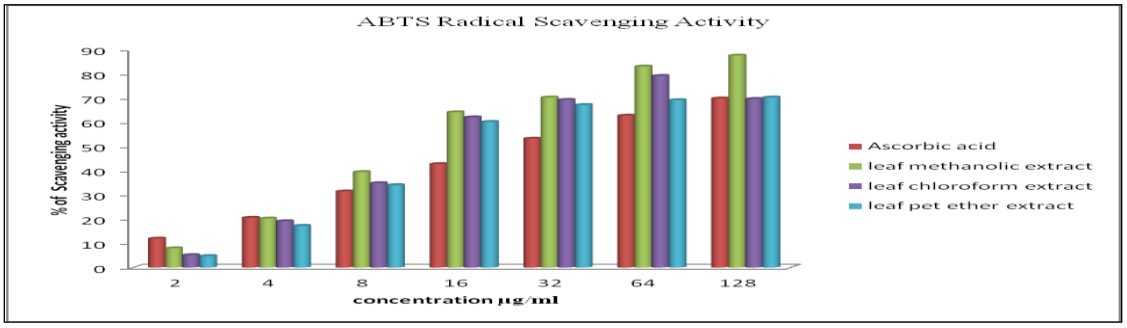

Fig. 6: ABT radical scavenging activity of *Cassia auriculata* leaf extract

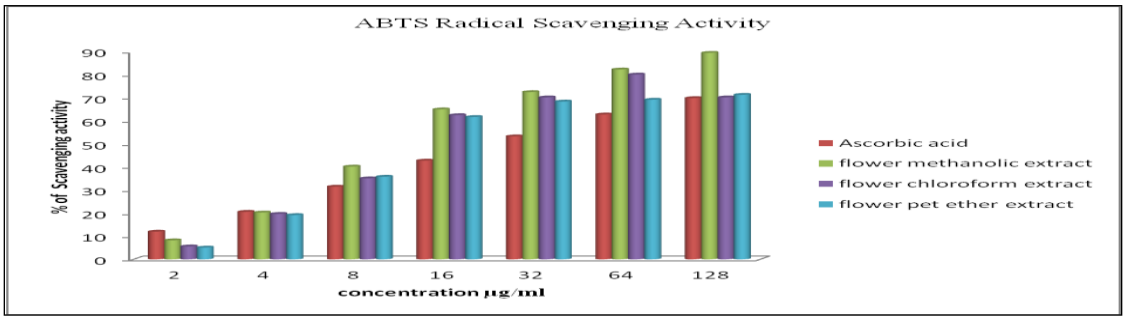

Fig.7: ABT radical scavenging activity of *Cassia auriculata* flower extract

Conclusion

The results of the present study showed that the phytochemical analysis and the antioxidant activity of *C. auriculata* plant revealed that the presence of biologically active compounds. The resourcefulness of this plant is opened for pharmaceutical industries for further investigations and to explore the antioxidant active principles which can be used in drug industries to cure several diseases human being.

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