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ANTIOXIDANT ACTIVITY AND PHYTOCHEMICAL STUDIES OF SIX THERAPEUTIC PLANTS WITH TOTAL PHENOLIC CONTENTS

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ABSTARCT
These free radicals produced by the biochemical reaction in human body and cause the structural and functional damage to the neuron, protein, lipids, nucleic acid and cellular molecules The present study intended to evaluation the occurrence of preliminary phytochemicals and free radical scavenging potentials of leaves extracts of therapeutic plants like *Annona squamosa* L. (AS) *Argemone Mexicana* L. (AM) *Calotropis gigantia* Br. (CG) *Cassia auriculata* L. (CA) *Ficus religiosa* L. (FR) *Jatropha curcas* L. (JC) and their correlation with the total phenolic contents (TPCs). The free radical scavenging activity of ethanolic extracts were assessed by 2, 2-diphenyl-1-picrylhydrazyl assay and total phenolic contents by Folin-Ciocalteu method. The percentage radical scavenging activities of samples were compared to the standard BHA. The leaf extract of *Cassia auriculata* L. showed marked quantity of phenolics and promising antioxidant activity followed by other extracts. The reductive ability of the extracts was found to increase in a concentration dependent. The results suggested that the phenolic compounds in extracts are most likely to be responsible for the pragmatic free radical scavenging of antioxidant activity.

KEYWORDS: Therapeutic plants, antioxidant activity, 2, 2-diphenyl-1-picrylhydrazyl assay,phytochemal, total phenolics.

INTRODUCTION

The involvement of oxidative stress in the pathogenesis of atherosclerosis, inflammation, cancer and neurodegenerative disorder like Alzheimer, Parkinsonism and Convalescent. Oxidative stress is mainly induced by the reactive oxygen species. This species include both oxygen radical such as peroxyl, superoxide anion, hydroxyl, peroxynitrite and nitric oxide radical and non radical derivatives of oxygen like hydrogen peroxide, hypochlorous acid and singlet oxygen (Luis MM et al., 2008). These free radicals produced by the biochemical reaction in human body and cause the structural and functional damage to the neuron, protein, lipids, nucleic acid and cellular molecules (Kumaran A et al., 2006).

In recent years, many plants extracts and different types of secondary metabolite have been shown not only free radical scavenging and antioxidant activities but also anti inflammatory, hepatoprotective (Wei F et al., 2010), antiproliferative (Hana K et al., 2010), neuroprotective (Alessandra F et al., 2012) and cardio protective activities(Carlos LC et al., 2008).

Flavonoids are typical phenolic compounds and rich in plants. The common feature of flavonoids is hydroxyl group substituted flavon moiety. These characteristic chemical functionalities are necessary for the flavonoids to scavenge free radical and prevent the oxidation of biological molecules by converting the more reactive oxygen species by donating hydrogen atom into inactive species. Therefore antioxidant and free radical scavenger could be more beneficial in the prevention and treatment of oxidative stress induced disorder and disease.

In the present study was undertaken to evaluate the selected medicinal plants (Wealth of India, 1959, Watt G et al., 1992, Asholkar LV et al., 1992, Yoganarasiman SN et al., 2000) for major phytoconstituents and their antioxidant properties of ethanolic (leaf) extract of plants of *Annona squamosa* L. (AS) *Argemone Mexicana* L. (AM) *Calotropis gigantia* Br. (CG) *Cassia auriculata* L. (CA) *Ficus religiosa* L. (FR) *Jatropha curcas* L. (JC).

MATERIAL AND METHOD(S)

Collection of Plant Materials

The plants as leaves of selected six plants were collected around Gulbarga regions Karnataka. The plant materials were washed thoroughly with distilled water and shade dried at room temperature. The dried leaves were ground well and stored in airtight containers. Authenticated voucher specimens of the selected plants were deposited in the Herbarium of Department of Botany, Gulbarga University, Gulbarga, and Karnataka.

Preparation of plant extracts

The selected plants material of *Annona squamosa* L. (AS) *Argemone mexicana* L. (AM) *Calotropis gigantia* Br. (CG) *Cassia auriculata* L. (CA) *Ficus religiosa* L. (FR) *Jatropha curcas* L. (JC) were subjected to solvent extraction by Soxhletion method (Harbarne JB et al., 2002). Hundred (100) grams of the powdered plant sample was successively extracted with 250 mL of ethanol solvent. All extract were filtered through a watman No-1 filter paper (Harbarne JB et al., 2002). and evaporated to dryness under reduced pressure controlled temperature (40-50º C). All the extracts were stored at 0^0c in airtight containers until need for further studies of phytochemical analysis and antioxidant activity.

Phytochemical screening

All the crude extracts were subjected to phytochemical screening for the presence of alkaloids, terpenoids, carbohydrates, glycosides, flavonoids, saponins and phenols using the standard method of harborne (Singh RP et al., 2002) and their results are tabulated in table-1.

Determination of Antioxidant capacity

DPPH Radical Scavenging Activity (Singh RP et al., 2002)

Initially different concentrations (100µg, 200 µg, 400µg, 600 µg and 800 µg) of test sample and Butylated hydroxyl anisole (BHA) were taken in different test tubes. 1 milliliter of 0.1mM methonolic solution of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) was added to these tubes. The volume was adjusted to 5 ml by adding methanol and shaken vigorously. The tubes were allowed to stand at room temperature for 30 min. The control was prepared as above without any extract. The absorbances of the sample were measured at 517 nm.

Radical scavenging activity was calculated using the following formula.

Radical scavenging activity (%) = $A_{control} - A_{sample} / A_{control} \times 100$

Where $A_{control}$ is the absorbance of the control (DPPH + methanol) and A_{sample} is the absorbance of the sample (DPPH + methanol + sample).

Determination of IC $_{50}$ values (Ebrahimabadi AH et al., 2010)

IC $_{50}$ values were calculated from the linear regression of the percentage antioxidant activity against concentrations of extracts used. IC $_{50}$ values were defined as the concentrations of samples required for the conversion of the half of the DPPH radicals to their more stable molecular counterparts 2, 2-diphenyl-1-picrylhydrazines.

Quantitative estimation of phenolic compounds:

Quantitative estimation of phenolic compounds by Folin-ciocIteu method. (Malick and singh.1980)500mg of the plant material was homogenated using pestle and mortar in 80% ethanol. The homogenized solution was centrifuged at 10,000 rpm for 20 minutes. The supernatant was retained and the extraction was repeated with the residue for 5-7 times. All the supernatants were mixed and evaporated to dryness. The residue thus obtained was dissolved in 5ml of distilled water and used for the estimation of total phenols. 1ml of the extract was mixed with 1ml of Folin-Ciocalteu reagent and 2ml of Sodium carbonate solution. Shaken the tube and heated in a boiling water bath for 1 min. and then cooled under running tap water. Diluted the solution to 25ml by adding distilled water and measured the absorbance at 650nm. With the help of standard curve obtained using different concentrations of catechol, calculated the amount of phenol present in the sample.

RESULTS AND DISCUSSION

DPPH Radical scavenging activity

The stable DPPH radical model is a widely used, relatively quick and precise method for the evaluation of free radical scavenging activity. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. Antioxidant on interaction with DPPH both transfer electron or hydrogen atom to DPPH and thus neutralizing its free radical character and convert it to 1-1, diphenyl-2- picryl hydrazine and the degree of discoloration indicates the scavenging activity of the drug.

The reduction capacity of DPPH radical is determine by the decrease in its absorbance at 517 nm induced by antioxidants (Cotran RS et al., 1999). The decrease in absorbance of DPPH radical caused by antioxidants because of the reaction between antioxidant molecules and radical progress which results in the scavenging of the radical by hydrogen donation. It is visually noticeable as a change in color from purple to yellow. Hence DPPH is usually used as a substance to evaluate the antioxidant activity (Blois MS et al., 1958). Table- 2 shows DPPH radical scavenging activity of standard BHA of selected plants. The extract of *Cassia auriculata* leaf was showed highest DPPH scavenging activity and compared with BHA as standard (28 µg/ml). The antioxidant activity of selected plants extracts were showed in following order shown in table 3.

Total phenolic content

Many plant extracts have been reported to have multiple biological effects, including antioxidant properties due to their phytoconstituents including phenolics. The antioxidant activity of phenolics is mainly due to their redox properties, which can play an important role in

adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides. The extracts of *Cassia auriculata*.L leaf showed highest total phenolic content as Catechol equivalent of phenols was detected. Table- 3 shows the total phenolic content of ethanolic extracts of selected plants exhibited.

Table -1: Preliminary Phytochemical screening of ethanolic extract of selected plants.

Sl. No.	Plant Name	Secondary metabolites					
		Ster	Alka	Flavo	Phen	Glyco	Sap
1.	AS	+	+	+	+	+	-
2.	AM	+	+	+	+	+	-
3.	CG	+	+	+	+	+	+
4.	CA	+	+	+	+	+	-
5.	FR	+	+	-	-	+	-
6.	JC	+	+	-	+	+	+

+ Indicate presence, --Indicate absent

Annona squamosa L. (AS) *Argemone mexicana* L. (AM) *Calotropis gigantia* Br. (CG) *Cassia auriculata* L. (CA) *Ficus religiosa* L. (FR) *Jatropha curcas* L. (JC).

Table -2: Results of DPPH radical scavenging activity of ethanolic extracts of selected plants.

Sl. No.	Conc. (µg/ml)	% of radical scavenging activity.						
		AS	AM	CG	CA	FR	JC	Standard
1.	100	30.89	22.29	20.11	49.20	39.73	37.16	56.22
2.	200	38.39	29.88	22.47	52.26	42.98	40.87	64.86
3.	400	40.39	30.79	23.19	55.93	44.35	43.56	68.36
4.	600	49.86	35.71	25.79	57.11	45.28	46.92	72.21
5.	800	52.36	40.10	27.85	66.26	47.22	47.76	74.99

Annona squamosa L. (AS) *Argemone mexicana* L. (AM) *Calotropis gigantia* Br. (CG) *Cassia auriculata* L. (CA) *Ficus religiosa* L. (FR) *Jatropha curcas* L. (JC).

Table 3: Results of $IC_{50}$and TPCs value of ethanolic extracts of selected plants.

Sl.No.	Plant Name	IC_{50} µg/ml.	TPCs (mg /g)
1.	AS	70	0.214
2.	AM	360	0.047
3.	CG	100	0.202
4.	CA	270	0.074
5.	FR	360	0.056
6.	JC	431	0.086
7.	Standard	22	-

Annona squamosa L. (AS) *Argemone mexicana* L. (AM) *Calotropis gigantia* Br. (CG) *Cassia auriculata* L. (CA) *Ficus religiosa* L. (FR) *Jatropha curcas* L. (JC).

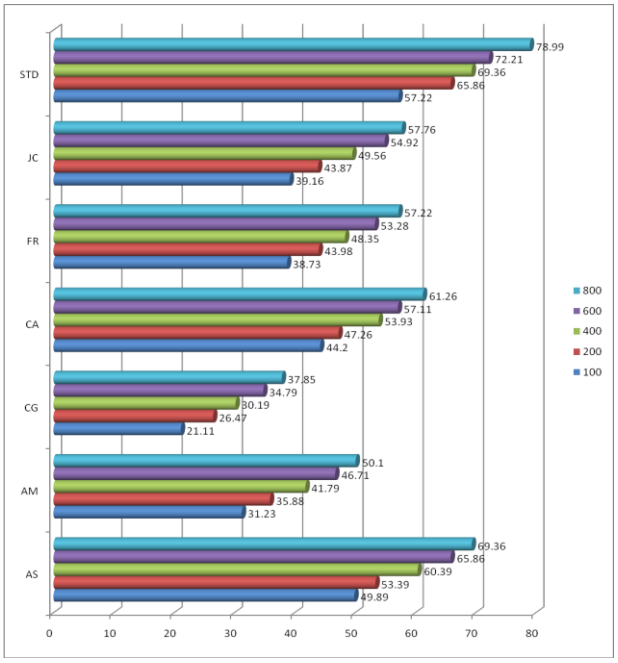

Fig -1: Results of DPPH radical scavenging activity of ethanolic extracts of selected plants.

Annona squamosa L. (AS) *Argemone Mexicana* L. (AM) *Calotropis gigantia* Br. (CG) *Cassia auriculata* L. (CA) *Ficus religiosa* L. (FR) *Jatropha curcas* L. (JC).

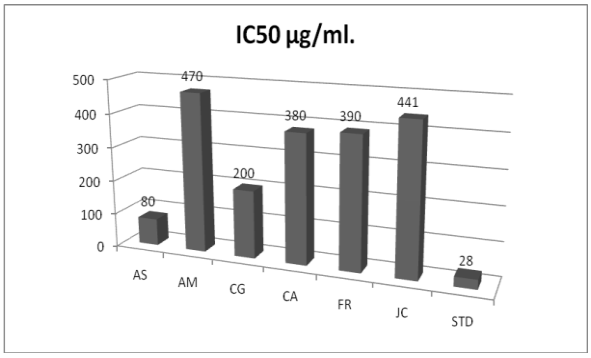

Fig -2: Results of IC_{50} value of ethanolic extracts of selected plants.

Annona squamosa L. (AS) *Argemone mexicana* L. (AM) *Calotropis gigantia* Br. (CG) *Cassia auriculata* L. (CA) *Ficus religiosa* L. (FR) *Jatropha curcas* L. (JC).

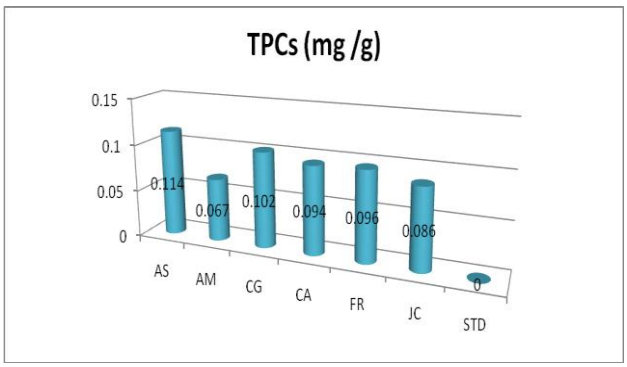

Fig -3: Results of TPCs value of ethanolic extracts of selected plants.

Annona squamosa L. (AS) *Argemone mexicana* L. (AM) *Calotropis gigantia* Br. (CG) *Cassia auriculata* L. (CA) *Ficus religiosa* L. (FR) *Jatropha curcas* L. (JC).

CONCLUSION

The objective of the study was to determine the free radical scavenging potential of the whole plant, *Cassia auriculata*.L showed and also to provide a comparative analysis between the other selected plants and their ethanol extracts of the parts as a free radical scavenger to specify the extract with a better scavenging potentials. According to the findings, the ethanol leaf extract showed in fig no-2 and 3, the highest amount of phenolics, and free radical scavenging activity as compared to the seeds and root. As we know that free radicals are important contributors to several severe pathological conditions, the findings suggest that the extracts of the whole plant is equally useful as a source of natural antioxidants with subsequent health benefits. Hence, further investigation is required to isolate and elucidate the active principles, and to evaluate pharmacological properties.

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