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In vitro evaluation of free radical scavenging potential of *Cassia auriculata* L.

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ABSTRACT

Free radicals are well documented for playing a dual role in our body as both deleterious and beneficial species. Excess production of free radicals or decrease in antioxidant level leads to oxidative stress. It results in pathological manifestations such as atherosclerosis, cancer, inflammatory condition, diabetes, Alzheimer's disease and Parkinson's disease etc. A variety of synthetic medicines employed in the treatment of different diseases that are capable to generate free radicals in body which may cause another disease. In Ayurveda, the plant sources are rich of antioxidants. Phyto-constituents are capable to terminate free radical reactions and prevent our body from oxidative damage with less side effects and compatible to body physiology. Considering the above facts present work deals with evaluation of free radical scavenging potential of Cassia auriculata L plant.the extracts of leaves, stems and fruits were screened in-vitro, for their possible radical scavenging antioxidant activity by employing 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) and nitric oxide (NO) reducing power with ascorbic acid as a standard. The results of the present study revealed that the acetone extract of leaves, stem and fruits can be a potential source of natural antioxidant, which can be applied as new source of Antioxidant Activity Index (AAI)which may complete demand of modern era to use such phytoconstituents or phytomedicines.

Key words: *Cassia auriculata L* , DPPH radical ,NO,Ascorbic acid, extracts of plant parts.

INTRODUCTION

Antioxidants are any substance that delay or inhibits oxidative damage to a target molecule. Antioxidants prevent cell and tissue damage as they act as scavenger. Antioxidants can terminate

or retard the oxidation process by scavenging free radicals.Overproduction of the free radicals can be responsible for tissue injury. Cell membranes are made of unsaturated lipids and these unsaturated lipid molecules of cell membranes are particularly susceptible to free radicals[1].Oxidative damage can direct to a breakdown or even hardening of lipids. Breakdown or hardening is due to lipid peroxidation leads to death of cell or it becomes unfeasible for the cell to properly get its nutrients or get signals to achieve another. Environmental agents also initiate free radical generation leads different complications in body.[2,3]. Anti-oxidants are substances capable to mop up free radicals and prevent them from causing cell damage. Free radicals are responsible for causing a wide number of health problems which, include cancer, aging, heart diseases , gastric problems etc. Antioxidants cause protective effects by neutralizing free radicals which, are toxic byproducts of natural cell metabolism [4].Increasing the antioxidant intake can prevent diseases and lower the health problems. Fruits, vegetables and medicinal herbs are the richest sources of antioxidant compounds[5].Herbs are staging a comeback and herbal 'renaissance' is happening all over the world. The herbal products today symbolize safety also compatible with human normal physiology.Natural products, mainly obtained from dietary sources provide a large number of antioxidants. Phytoconstituents are also important source of antioxidants and capable to terminate the free radical chain reactions [6]

Cassia auriculata Linn.is found throughout central and southern India, cultivated in Punjab, Haryana, Uttar Pradesh and West Bengal.The shrub usually occurs on roadsides, waste line, and railwaybankments.[7] Leaves are anthelmintic and used to treat ulcers,skin diseases and leprosy. An aqueous extract of leaves possesses hypoglycemic activity[8]. Comparative spectrogram of different parts of the plant and different extracts of leaves was reported for their phenol flavonoid contents.[9] The plant *C. auriculata* has a wide range of pharmacological actions. Its leaves constituted polysaccharides, flavonoids, anthracene derivatives and dimeric procyanidins[10]. The plant constitutes bioactive molecules such as pyrrolozidine alkaloids, flavonoid glycosides, tannins , fatty oils consisting of palmitic, oleic and linoleic acids.[11].The reports reveled that ethyl acetate extract of leaves exhibits significant radical scavenging activity[12]**.**

In present work detailed systematic evaluation of various extracts of different parts such as leaves, stems and fruits were studied for the radical scavenging potentials by employing 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) and nitric oxide(NO) reducing power with ascorbic acid as a standard.

EXPERIMENTAL SECTION

Plant Material:

Cassia auriculata L was collected from Western Pune, Maharashtra, India. The taxonomic identification is accomplished with the help of flora of Bombay Presidency [13] and Flora of Maharashtra [14] for identification. Authentification was commited by Botanical Survey of India, Pune (Maharashtra), its number is BSI/WC/Tech/2009/95.

Materials

1,1-diphenyl-2-picrylhydrazyl (DPPH), sulphanilamide, naphthyl ethylenediamine dihydrochloride were obtained from Sigma Chemicals Co., USA. All other chemicals and reagents were of analytical grade.

UV Spectrophotometer (UV-VIS1700Pharma Spectrophotometer Schimadzu) was used to measure the absorbance at various concentrations of the extracts under study.

Preparation of extracts:
Air shade dried, finely pulverized and exactly weighed plant material was utilized to prepare extracts with measured volumes of solvents like acetone, ethanol, methanol and distilled water. The freshly prepared extracts were analysed to prevent any degradation.Solvents were removed under reduced pressure to get the crude mass of extracts. Weighed amounts of dried extracts were dissolved in known volume of methanol .Various aliquots of each extract were prepared for the DPPH and Nitric Oxide assays.

DPPH radical scavenging activity [15-18]:
DPPH is converted to 1, 1-diphenyl -2-picryl hydrazine when it reacts with antioxidants. A change in colour from purple to yellow is observed. Aliquots of extracts were made to total volume of 3ml using methanol. 0.15ml of freshly prepared DPPH solution (98μg/ml) was added, stirred and left to stand at room temperature (27^0C) for 30 minutes in dark. The control contains only DPPH solution in methanol while methanol served as the blank (negative control).The reduction capability of DPPH radical was determined by the decrease in its absorbance. Absorbance was noted at 517nm by using UV-VIS spectrophotometer.

Nitric Oxide scavenging activity [18-20]: In this spectrophotometric method the absorbance of chromophore formed during the diazotization of the nitrile with sulphanilamide and the subsequent coupling with naphthyethylenediamine dihydrochloride was measured. Sodium nitroprusside (SNP-5mM) in phosphate-buffer saline was mixed with an equivalent amount of methanol to get the control. Methanol served as blank. Methanol was added to test solutions at different concentrations to make up a volume of 3ml and incubated at room temperature (27^0C) for 90 minutes. This incubated solution (1.5 ml) was added to 1.5 ml of Greiss Reagent. Absorbance at 546 nm was noted using UV–VIS spectrophotometer.

In both methods the capacity of scavenging free radicals was calculated as follows:

Scavenging activity (%) = {(Control Abs.—Sample Abs.)/Control Abs} × 100

Each experiment was carried out in triplicates and results were recorded as mean % antiradical activity ± SD.

IC_{50} values were calculated from the plotted graph of scavenging activity against the concentrations of the samples. IC_{50} is defined as the total antioxidant necessary to decrease the initial DPPH radical by 50%. IC_{50} was calculated for all the extracts based on the percentage of DPPH radicals scavenged. Ascorbic acid was used as the reference compound (positive control) with concentrations 50 to 500 μg/ml for both the above spectroscopic methods.

RESULTS AND DISCUSSION

Freshly prepared extracts of the dried plant material were subjected to screening for their possible antioxidant activities. For this purpose, DPPH free radical scavenging activity and Nitric

Oxide scavenging methods using UV- VIS spectrophotometer were employed. DPPH radical scavenging test is based on the exchange of hydrogen atoms between the antioxidant and the stable DPPH free radical. DPPH is a stable free radical at room temperature which accepts an electron or hydrogen radical to form a stable diamagnetic molecule. DPPH radical is reduced to the corresponding hydrazine, a colour change of the solution from violet to yellow is observed and that is monitored spectrophotometrically. More reduction of DPPH radical is related to the high scavenging activity of the particular extract [21]. The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm, which is induced by antioxidants.The significant decrease in the concentration of the DPPH radical is due to the scavenging ability of *C. auriculata*

Fig 1, 2: DPPH radical scavenging activity of plant parts

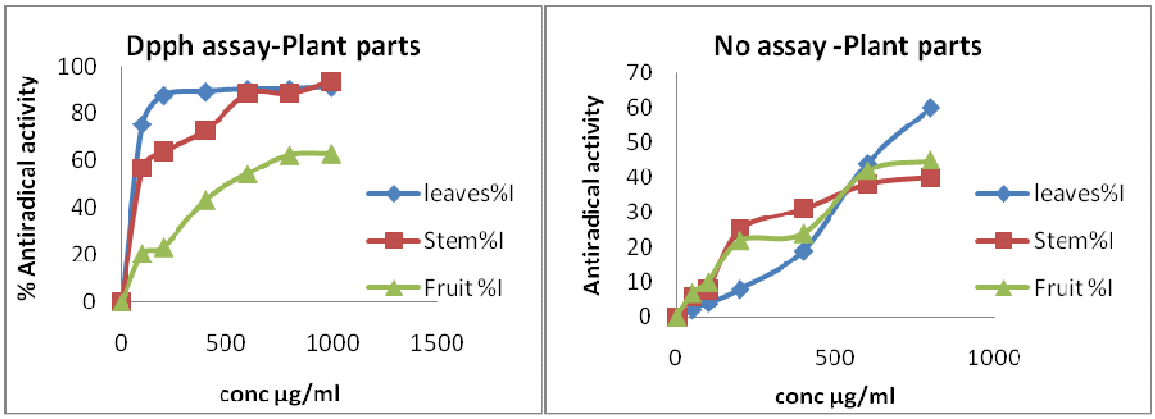

Fig3: DPPH assay of extracts of plant parts

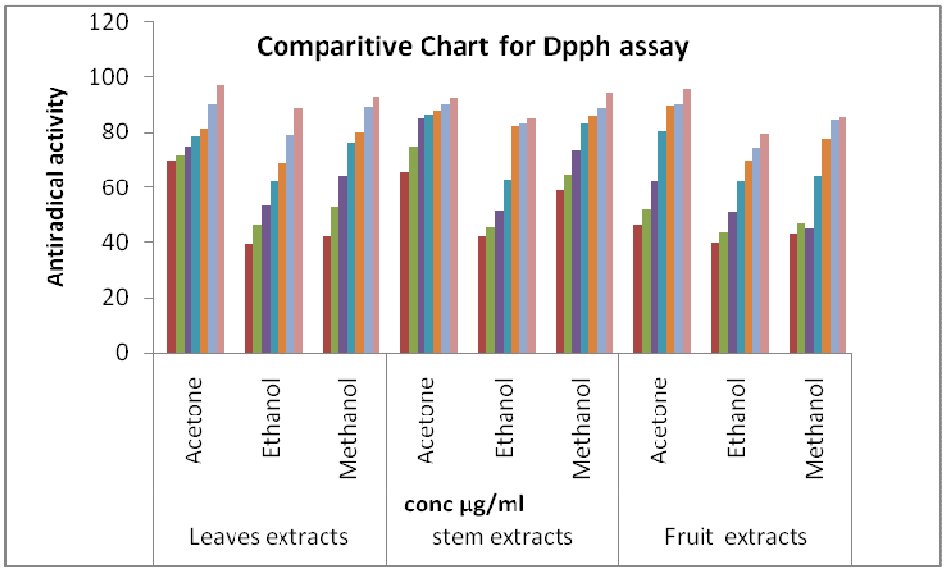

Fig4: NO assay of extracts of plant parts

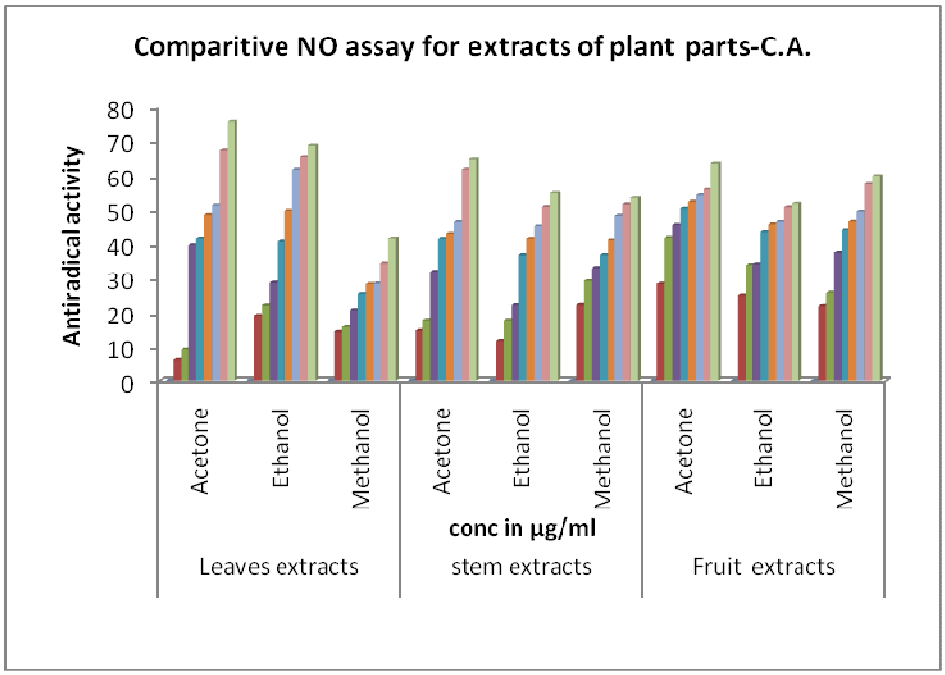

Table: 1- IC_{50} values of plant parts

Plant parts	Dpph assay	No assay
Leaves	333±0.03	707 ±0.04
Stem	526±0.01	886 ±0.09
Fruit	615±0.04	827 ±0.03

Table2: - IC_{50} values of various extracts

	IC_{50} values								
	Extracts of leaves			Extracts of stem			Extracts of fruits		
Method	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**
DPPH assay	19±0.03	21±0.02	52±0.08	45±0.09	67±0.33	71±0.05	114±0.04	214±0.93	280±0.04
Nitric oxide assay	86±0.06	86 ±0.05	180±0.04	109±0.07	105±0.01	90±0.07	110±0.03	84±0.03	91±0.02

A : Acetone Extract B: Ethanol Extract C: Methanol Extract
Each value represents mean ±SEM (n=3)

Nitric Oxide (NO) is a diffusible free radical that plays many effective roles in diverse biological systems including neuronal messenger, vasodilatation, antimicrobial and antitumor activities[18]. Nitric oxide is generated from the decomposition of SNP and measured by Greiss Reagent.SNP in aqueous solution at physiological pH spontaneously generates NO, which interacts with oxygen to produce nitrite ions that can be measured by the use of Greiss reagent.All the extracts for both these methods analysed at the same range of concentration.(50-1000 µg/ml) A significant decrease in the NO radical is due to the scavenging activity of the extracts. At the range of concentrations under study, ascorbic acid exhibited 90.16% inhibition; acetone extracts

of plant parts exhibited higher radical scavenging activity than all other extracts by DPPH assay and by Nitric oxide method but it is lower than ascorbic acid. IC_{50} values were calculated from plotted graphs of scavenging activity against the concentrations of samples. The values of IC_{50} for each extract are reported in Table-1.

Statistical analysis

Results are expressed as the standard error mean of three independent experiments. Student's *t* test was used for statistical analysis; P values < 0.05 were considered to be significant.

CONCLUSION

Comparative assay for the plant parts shows the leaves exhibit higher radical scavenging activity than stem and fruit. The semipolar to polar extracts of these plant parts were analysed further. All extracts of exhibit higher range of the radical scavenging activity but acetone extract shows highest radical scavenging activity among these three plant parts. It means these extracts are rich in flavonoids as well as phenolic compounds which along with other polyphenolics in the plant material may be responsible for the antioxidant activities of these extracts. Further work is under way to confirm the anti-oxidative effect of these promising plant extracts by using other methods.

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