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Phytochemical Analysis And Antioxidant Potential Of Various Fractions Of Cassia Auriculata Flowers

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ABSTRACT -
Cassia auriculata Linn. belongs to family Fabaceae. In the present study, the flowers of Cassia auriculata were shade dried, powdered and extracted with pet ether by Soxhlet extraction and remaining marc were again subjected for Soxhlet extraction with ethyl acetate and then followed by methanol and water respectively and got CAPE, CAEA, CAME and CAWA fractions. The phytochemical screening of all fractions revealed the presence of steroids, flavonoids, tannins and terpenoids etc. Total phenolic content and concentration of flavonoids of four different fractions were determined using spectrophotometric method and in-vitro antioxidant activity of CAPE, CAEA, CAME, CAWA fractions were determined by DPPH free radical scavenging activity. It was found that the CAEA, CAME and CAWA extracts has potent antioxidant activity.

KEYWORDS: Antioxidant activity, DPPH free radical scavenging, phytochemical screening, Cassis auriculata.

I. INTRODUCTION:

Cassia auriculata L., sometimes called "Avaram" in Tamil, is a shrub with lovely yellow blossoms that is significant to ethnobotany. The plant's aerial portions are used in traditional medicine to cure conditions including diabetes, liver issues, rheumatism, conjunctivitis, leprosy, and astringent.

Few researches have been done on the pharmacological properties of the plant, despite the fact that it is widely utilized in traditional medicine. Plants are abundant in phenolic and flavonoid chemicals, which serve as scavengers of free radicals and antioxidants. An antioxidant is any material that reduces, stops, or neutralizes oxidative damage to a target molecule. Due to their capacity to scavenge free radicals, natural antioxidant agents have garnered a lot of attention. Consequently, research is being done on the antioxidant potential of medicinal plants, and there is a growing need for natural food preservatives and antioxidants. An unpaired electron is what makes a free radical a very unstable and reactive species. It is the outcome of cellular metabolism and affects our bodies in a variety of ways. Numerous illnesses, including aging, neurological diseases, disorders associated to the central nervous system, mutagenic disorders, etc., are caused by the formation of free radicals.

Antioxidants prevent severe damage to lipids, carbohydrates, amino acids, proteins, enzymes, RNA, DNA, and other biological materials by trapping free radicals, also known as reactive oxygen and reactive nitrogen species, before they attack. Consequently, it is critical to explore the antioxidant potential of plants and develop new, secure natural sources of antioxidants.

The present study was designed to investigate the total phenolic, total flavonoid content and antioxidant activity of various fractions of Cassia auriculata flowers.

II. MATERIAL AND METHODS

2.1 CHEMICALS

All solvents chloroform, ethyl acetate, absolute ethanol, n-hexane, HPLC grade methanol used were of analytical grade and ordered from Sd-fine chemicals. The standards gallic acid, Rutin, and 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) and aluminium chloride (AlCl3),Folin-Ciocalteu's phenol reagent, ascorbic acid were obtained from

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Merck Co. (Mumbai, India). All chemicals used were of analytical grade.

2.2. Collection and Authentication

In the present study, the flower of Senna auriculata Linn. were collected from Sangamner, Ahmednagar, India in the month of October and November 2015 and identified from the Department of Botany, S. N. Arts, D. J. Malpani Commerce & B.N. Sarada Science College, Sangamner. A voucher specimen (SSD-1) of the same was deposited at the herbarium department. Also the plant specimen was authenticated by Botanical Survey of India, Pune, Maharashtra.

The flowers were thoroughly washed with distilled water. The cleaned flowers were kept for drying under shade at room temperature for ten days. The well dried flowers were grinded in grinder and stored in air tight glass bottles for further use.

2.3. PREPARATION OF EXTRACTS

The 500 g shade dried grinded CA flowers were subjected for Soxhlet extraction with pet with pet ether by soxhlet extraction and remaining marc were again subjected for soxhlet extraction with ethyl acetate and then followed by methanol and water respectively and got CAPE, CAEA, CAME and CAWA fractions. Then filtered the fractions through cotton plug and then through Whatman No.1 filter paper. The filtrate was concentrated using rotary evaporator under reduced pressure.

2.4. Determination of total phenolic content

The total content of phenol in different fractions of CA was determined by Folin-Ciocalteu reagent using reported method with slight modification TPC of various fractions was determined from a gallic acid calibration curve. Calibration curve was prepared by mixing 0.5 ml aliquots of 10, 20, 30, 40, 50, 60, 70,80, 90, 100 µg/ml methanolicgallic acid solutionwith 2.5 ml Folin-Ciocalteu reagent and 2.5 ml (7.5 g/100ml) sodium carbonate. All mixtures were kept for incubation at RT for 30 min and absorbance was measured at 765 nm using spectrophotometer (Systronic UV-Visible -1203). Methanol was used as blank and gallic acid as a standard. A similar procedure was conducted for all fractions. All determinations were carried out in triplicates. TPC was determined from linear equation of standard calibration curve produced with GA and was expressed as gallic acid equivalent per milligrams (µg GAE/mg) of extracts.

2.5. Determination of total flavonoid content

The total flavonoid content of different fractions was determined by using aluminium chloride colorimetric method TFC of various extracts was determined from standard-rutin calibration curve. The solution of rutin of 100 µg/ml concentration was prepared in 80% methanol and further diluted to 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 µg/ml. 0.5 ml aliquots of above concentrations were separately mixed with 1.5 ml of 95% methanol, 0.1 ml of 10% aluminium chloride. 0.1 ml of 1M potassium acetate and 2.8 ml distilled water. All mixtures were kept for incubation at RT for 30 min. Then absorbance of light pink coloured reaction mixture was measured at 415 nm versus reagent blank containing water instead of the sample. Rutin was used as a standard compound for the quantification of total flavonoids and the standard curve was drawn.A similar procedure was conducted for all extracts. All determinations were carried out in triplicates. Total flavonoid content was expressed interms of rutin equivalent per milligrams (µg RE/mg) of extracts.

2.6. DPPH radical scavenging activity

The free radical scavenging activity of various fractions of CA flowers was determined in vitro by
performing DPPH scavenging assay. The quantitative determination of radical scavenging activity was done . Ascorbic acid was used as standard. 100 µg/ml of AA was prepared by dissolving 10 mg in 100 ml methanol. 2mL of each frcation and standard at various concentrations (100, 50, 25, 12.5, 6.25, 3.125, 1.625, and 0.812 µg/mL) were added to 3 ml of 0.004% freshly prepared DPPH solution. Then the reaction mixture was kept at room temperature under dark condition for 30 min and the absorbance was measured at 515 nm (Systronic UV-Visible Spectrophotometer-1203). Methanol was used as a blank, the methanol and DPPH solution as a baseline control (ADPPH). All determinations were carried out in triplicates. The degree of decolorization of DPPH from purple to yellow indicated the scavenging efficiency of the fractions. The percent inhibition was calculated as:
%Inhibition = A Blank- A Sample × 100/ A Blank
Where A Blank is the absorbance of the control reaction and A sample is the absorbance of sample.

Data analysis

All determinations were carried out in triplicates and data were presented as mean ± SD. A p-value

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less than 0.05 were considered statistically significant.

III. RESULTS

3.1. Total phenolics and flavonoids content

The total phenolic content (TPC) was determined by using Folin-Ciocalteu reagent and it is reported as micrograms per miligrams (µg/mg) of gallic acid equivalent (GAE) by reference to gallic acid standard curve (Y= 0.0101X+0.0165 and r2= 0.9902). **Figure 1** shows all the fractions contained a considerable amount phenolic content and it was found that CAEA fraction had the highest TPC (120.6 ± 0.55) µg of GAE/mg followed by CAWA (95.19±0.00), CAME (60.58±0.961) and CAPE (50.96±0.00) µg/mg of gallic acid equivalent respectively. The total flavonoid content (TFC) is expressed as µg/mg of rutin equivalents (RE) by reference to rutin standard curve (Y= 0.005X+0.028 and r2= 0.9933). It was found that the CAEA fraction had the highest TFC (84.38± 3.125) µg of RE/mg followed by CAWA (79.16±1.804), CAME (41.67±1.804) and CAPE (14.48±1.005) µg/mg of rutin equivalent respectively.

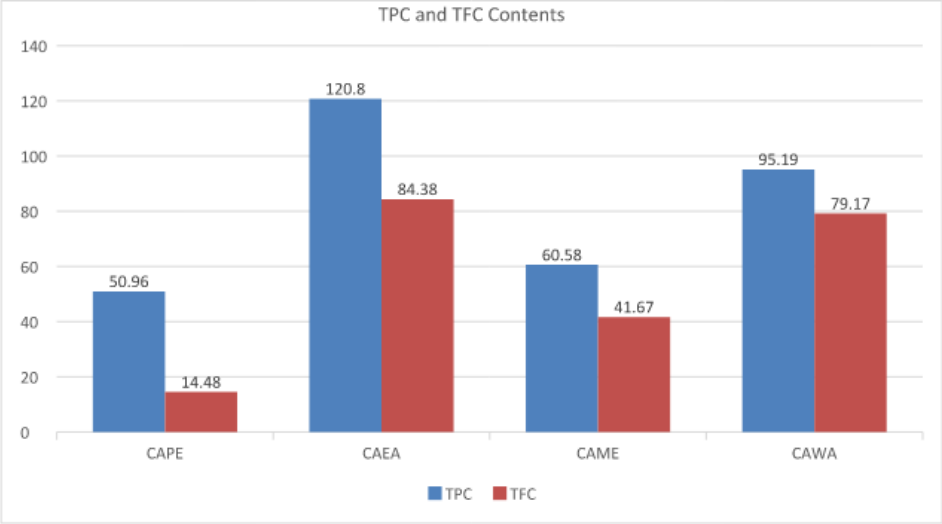

Fig. 1. Total phenolic and flavonoid content of various fractions of Cassia auriculata

3.2. DPPH SCAVENGING ASSAY

ntioxidant potential is based on scavenging ability of DPPH radical by fractions and it was measured with the standard antioxidant Ascorbic acid (AA). Figure 2 shows that the % inhibition of standard and CA fractions in the order of CAEA > CAME > CAWA > CAPE.

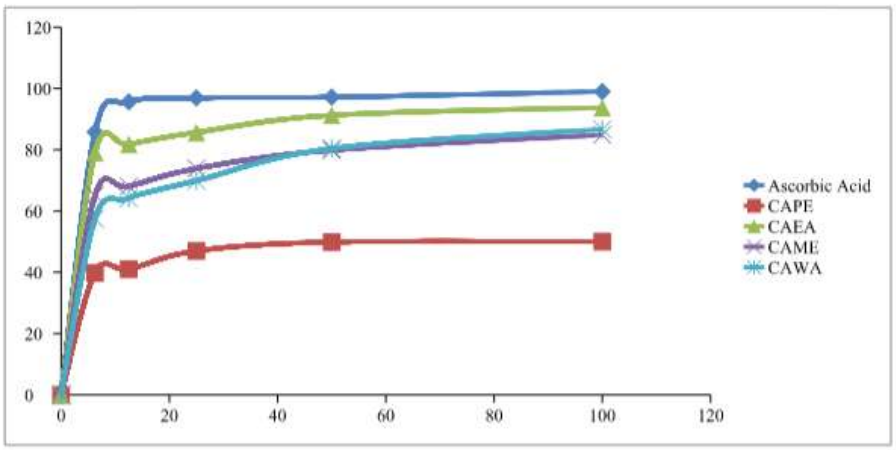

Fig. 2. DPPH radical scavenging activity of Cassia auriculata

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IV. DISCUSSION:

Since free radical generation is a necessary component of normal cellular function, our bodies are always producing excess free radicals. These free radicals can come from endogenous or exogenous sources, and they are the cause of many degenerative diseases, including diabetes, cancer, atherosclerosis, ageing, heart disease, and neurodegenerative illnesses like Alzheimer's disease. .. As far as we are aware, a vast array of strong antioxidants exist that can stop the spread of free radicals. Hence in this study we evaluate a potent antioxidant by performing in vitro antioxidant assays. DPPH is nitrogen centered free radical which shows strong absorbance at 517 nm. Since DPPH radical involves a hydrogen atom transfer process . it gets reduced either by accepting an electron or by abstraction of hydrogen atom from antioxidant compound The Scavenging capability of DPPH radical is related to the inhibition of lipid peroxidation and it was determined by decrease in intensity of violet colour. Hence when more antioxidants occur in the fractions, the more DPPH reduction will occur and it relates to high scavenging capability of respective fractions. In this assay, the good antioxidant activity of CAEA and CAME fractions on DPPH radical may be attributed to a direct role in trapping free radicals by donating hydrogen atom. In the present study among all fractions tested, the ethyl acetate, methanol and aqueous fraction showed significantly higher percent inhibition and considerably correlated with phenolic and moderately with flavonoid content. Hence scavenging ability of ethyl acetate and aqueous fractions may due to the presence of a greater number of phenolic compounds rather than flavonoids. The plant phenolic compounds and flavonoids have been reported to show strong antioxidant activity in biological systems, acting as oxygen radical and free radicals' scavengers due to ability of benzene rings to transfer electrons (Brown,1995). Present study revealed that relatively highest phenolic and flavonoid content are found in ethyl acetate and aqueous fractions and moderate content was found in methanol fraction, while lowest contents was found in pet ether fractions. This results may arise due to higher solubility of phenolic and flavonoid compounds in ethyl acetate and hydro alcohol pet ether and thus it suggests the efficient antioxidant property of Cassia auriculata herb.

V. CONCLUSION

All the extracts obtained from the Cassia auriculata studied here showed phenolics, flavonoids and antioxidant capacity, although with different efficiencies. The results showed that Cassia auriculate is good source of phytochemicals including phenolic compounds and offer opportunities for development of value-added products, nutraceutical and food applications to enhance health benefits.

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CONFLICT OF INTEREST:
There are no conflicts to declare.

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