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Assessment of Antioxidative Potential of Prosopis Cineraria Pods

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Abstract
The aim of the current study was to evaluate the antioxidative potential of *P. cineraria* pods extract (PCE) known to be rich in phenolics and flavonoids. Since the P. cineraria plant has long been regarded as a plant rich in nutraceuticals and used as food. The trees provide fuel, fodder, food, little timbers, medicines, gum and tannins. It has been used since ages by humans as a traditional therapeutic food to boost immunity to infectious pathogens. The antioxidant activity was determined by 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity. The results of DPPH assay in ethanolic extract of PCE showed that the scavenging of the free radicals was 28.74%, 57.67% and 94.12% at 0.05, 0.1, and 0.2 mg/ml respectively. The IC_{50} of ascorbic acid calculated was 0.092 mg/ml in the DPPH assay. *P. cineraria* had the highest total phenolic content 372.25 mg/gm estimated in terms of Gallic acid equivalent. The total flavonoid content in the extract was 152.79 mg/gm. This study indicated that PCE exhibited the highest antioxidant contents of flavonoids and phenols which can be potentially used as a readily accessible source of natural antioxidants by human beings.

Keywords: P. cineraria, phenolics, flavonoids, DPPH assay.

Introduction

Oxidative stress plays a pivotal role in the pathogenesis of several metabolic, chronic disorders or cancers (Aminjan *et al.,* 2019). The variety of endogenous and exogenous antioxidants provide protection against oxidative damage and chronic ailments (Cadet *et al.,* 2012). The increasing exposure of humans to radiation is a major cause of oxidative stress which has lended urgent need to look into preferably plant products to look into new antioxidant compounds which can protect against oxidative stress as they are cost effective and with hardly any side effects.

To find new novel pharmaceutical antioxidative agents, plants are helpful and valuable resources.
The polyphenolic compounds are major ingredients from the natural sources which are reported to have significant antioxidant potential (Carocho and Ferreira., 2013). In fruits, vegetables, nuts, seeds, leaves, roots, and barks primarily natural antioxidants viz., flavonoids, phenolic compounds, carotenoids etc are present (Asif., 2015). *P. cineraria* member of Fabaceae family, an indigenous plant comprises 44 species mentioned in traditional medicine to cure various ailments. It is distributed mainly in arid and semi-arid regions with less rainfall area all over the world (Cangiano *et al.,* 2020), whereas in India it is found in Haryana, Rajasthan, Punjab, Uttar Pradesh, Gujarat and Tamilnadu. It is commonly known as Khejri (Hindi and Sanskrit), Janti/Loong tree (Rajasthani), Jand (Punjabi), Sami (Gujarat), Sumri (Tamil) and Jammi (Telugu) and in Sind it is known as Kandi.

Due to the presence of various primary and secondary metabolites *P. cineraria* is reported to have various pharmacological activities including analgesic and antipyretic, antihyperglycemic, antioxidant, anti-hypercholesterolemic, antitumor, nootropic activity, anticonvulsant effects etc. (Kulshreshtha *et al.,* 2019).

The present study was undertaken to assess the antioxidant properties. This scientific data obtained may serve as a reference for future research, preparation of various herbal formulations, analytical analysis and preparing monographs about this plant.

MATERIALS AND METHODS

Plant material

Fruit of *P. cineraria* was collected in the month of July from Dausa district, Rajasthan, India. The plant was identified and has been deposited as a voucher specimen (RUBL 211666) for future reference in the Department of Botany, University of Rajasthan, Jaipur, India. The work was carried out in the Department of Zoology, University of Rajasthan, Jaipur.

Preparation of the extract

The fresh pods of *P.cineraria* were collected from Dausa district (Rajasthan, India) in summer season were washed with distilled water and dried under shade, mechanically grinded to get coarse powder and then passed through number 40 sieve meshes. Ethanolic (50%) extract of *P. cineraria* was then prepared by refluxing for 48 hrs. (16hrs×3) at 50^0C. The extract obtained was vacuum evaporated in a rota evaporator so as to get in powder form which was then dissolved in double distilled water. For oral administration fixed weight/volume of the extract to solvent after complete dissolution of the extract was used.

Estimation of bioactive compounds

The ethanolic extracts of *P. cineraria* fruits were screened for the presence of bioactive compounds using standard methods.

1.Total phenolics

The total phenolic content were determined by using the Folin Ciocalteu assay. In a test tube 1.0 ml aliquot of diluted extract (extract-water, 1:40 (v/v), two replicates) was mixed with 1.0 ml of 1 N of Folin-Ciocalteu's reagent. This mixture was allowed to stand for a 2 to 5 min followed by the addition of 2.0 ml of 20% Na_2CO_3. This mixture was incubated at room temperature for 10 min and then centrifuged for 8 min. The absorbance of the supernatant was read at 730 nm on a Perkin-Elmer Lambda 12 UV/VIS Spectrophotometer (Norwalk, CT). The total phenolic content was expressed as Gallic acid equivalents (GAE) in milligrams per gram dry material (Fig.1).

2. Flavonoids

Flavonoid content was measured by the aluminum chloride colorimetric assay. To the sample 0.5 ml of 2% $AlCl_3$ ethanolic solution was added. Extract samples were evaluated at a final concentration of 1mg/ml. Absorbance was measured at 420 nm (Fig2). The total flavonoid content was calculated as quercetin (mg/g) using the following equation based on the calibration curve as follows:

$$y = 14.549x + 0.0584, R2 = 0.9981$$

where x was the absorbance and y was the quercetin equivalent (mg/g).

3. DPPH scavenging assay

For DPPH scavenging assay, the ethanolic sample stock solutions (1.0 mg/ml) were diluted to final concentrations of 250, 125, 50, 25, 10 and 5 g/ml. To the different sample solutions of 2.5 ml concentrations 1 ml of a 0.3 mM DPPH ethanol solution was added and allowed to react at room temperature. The absorbance was read after 30 min at 518 nm and converted into the percentage antioxidant activity (AA) by using the following formula given below:

$$AA\% = 100 - \left[\frac{\{(Abs_{sample} - Abs_{blank})x100\}}{Abs_{control}}\right]$$

The blank consisted of 1.0 ml ethanol and 2.5 ml of plant extract solution. For negative control DPPH solution (1.0 ml; 0.3 mM) and ethanol (2.5 ml) was used. The positive controls were those using the standard solutions. The EC_{50} values were calculated by linear regression of plots where the abscissa represented the concentration of tested plant extracts and the ordinate the average percent of antioxidant activity from three separate tests.

Statistical analysis

The experiments were performed in triplicate and the data were statistically analyzed as mean ± SE. All graphs were plotted using MS Excel ®software 2010.

Results

Phenolic compounds are the most widely distributed secondary metabolites in the plant kingdom. Phenolic compounds are potent chain breaking antioxidants due to the presence of hydroxyl groups.

In the present study the total phenolic content was found to be 372.25 mg/gm in terms of Gallic acid equivalent. Being a Fabaceae family, *P. cineraria* fruits showed significant levels of phenols which may play a critical role as a source of phytomedicine. The polyphenolic compounds are known to possess antioxidant activity.

The phenolic compounds have redox properties which are thought to be responsible for various activities such as neutralizing free radicals, quenching of triplet and singlet oxygen, decomposing peroxides etc.

The antioxidant activity shown by flavonoids depends on the structure and position of OH groups.

Total flavonoid content of *P. cineraria* fruit were measured by using standard quercetin equivalent; it was found to be 152.79 mg/gm.

DPPH radical scavenging assay is related to inhibition of lipid peroxidation. To neutralize free radicals antioxidants transfer electron or hydrogen atoms to DPPH. This method revealed that the scavenging of the free radicals was 28.74, 57.67 and 94.12% at 0.05, 0.1, and 0.2 mg/ml respectively for the ethanolic extract of *P. cineraria* fruit. The IC_{50} of ascorbic acid was 0.092 mg/ml in the DPPH assay (Table 1). From the results it can be concluded that the ethanolic extract possesses hydrogen donating capability and thus the ability to scavenge free radicals.

DISCUSSION

It is to be noted that flavonoids belong to phenolic compounds, widely present in higher plants. These have been recognized as inhibitors of xanthine oxidase, lipoxygenase and cyclooxygenase (Metodiewa *et al.,* 1997; Hayashi *et al.,* 1988; Walker *et al.,* 2000). Thus, it plays an important role in maintaining homeostasis between intracellular oxidative stress and antioxidants.

Flavonoids can prevent damage caused by free radicals by directly scavenging the ROS species. Due to the high reactivity of the hydroxyl group of the flavonoids, free radicals are made inactive. Therefore, presence of various classes of flavonoids in the ethanolic extract of *P. cineraria* fruits indicates strong radio-protective ability.

In the present study, high phenolic content was observed which indicated strong anti-oxidative properties. Previous studies have noted that phenolic compounds can regulate oxidative insult by transfer of single electron, sequential loss of proton, metal chelation, and last but most important through hydrogen atom transfer (Zeb, 2020). Likewise, flavonoids are the major constituents of polyphenols present in plant or plant based products (Chaves *et al.,* 2020). In the present study, fruit of *P. cineraria* was examined for concentration of flavonoids, result showed >150 mg/g of the ethanolic extract which is higher than concentration of flavonoids in black tea (17.4 mg/g) or green tea (20.8 mg/g) (de Maat *et al.,* 2000). Such a high concentration of flavonoid is ideal for extensive oxidative insult. It was further confirmed by DPPH assay, where at 0.2 mg/ml concentration of *P. cineraria* fruit extract could scavenge 94% of free radicals. Result of this study was in accordance with Poonar and Gehlot (2000), authors of this study reported close to 80% scavenging ability of *P. cineraria* fruit. They also reported alteration in level of anti-oxidative potential as the pod develops into larger fruit. These studies confirm that *P. cineraria* contains bioactive compounds that have strong anti-oxidative properties.

Conclusion

Sangri, one of the most important ingredients of Panchkuta, was analyzed in terms of antioxidant potential, so they could be recommended to be included in the diet looking into their nutritional value and possible role as radioprotector due to the presence of various antioxidant anomalies. There are substantial anecdotal reports indicating that the consumption of sangri could ameliorate a wide range of illnesses. *In-vitro* bioactivity results of the extract also support such health beneficial claims. Further clinical investigations are needed for the potential products present in *P. cineraria* for preparations of pharmaceutical drugs.

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Fig. 1: Total Phenol.

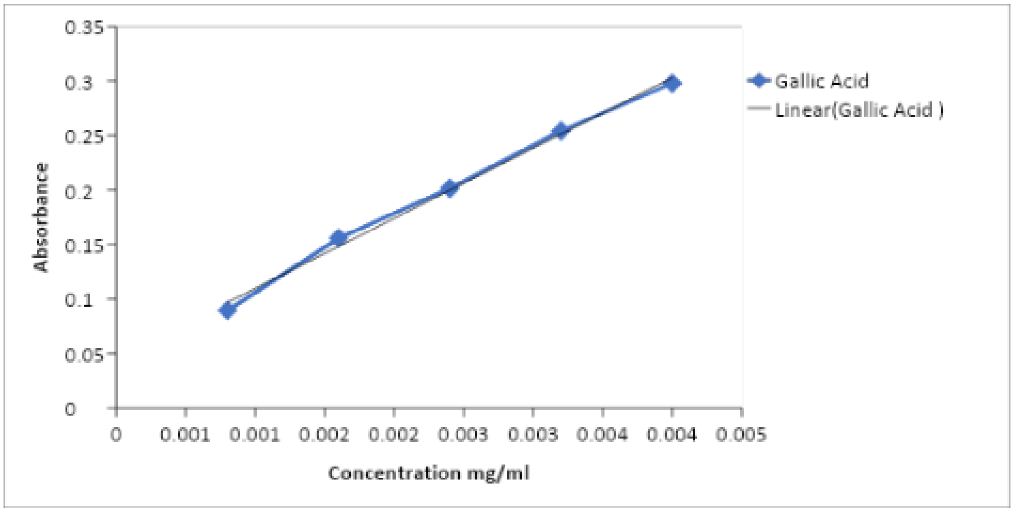

Fig. 2: Flavonoid.

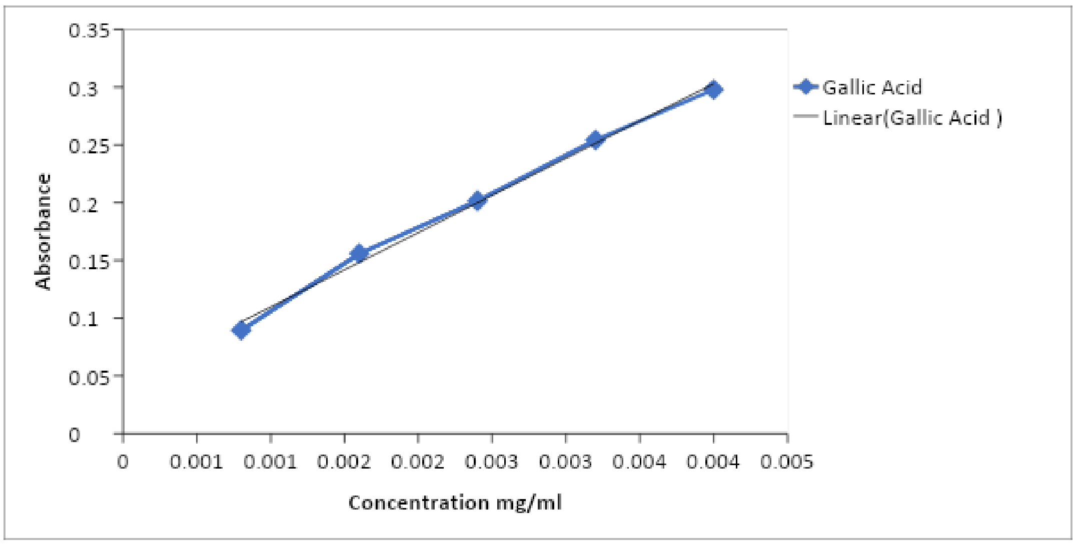

Table. 1: DPPH Assay.

CONC.mg/ml	% INHIBITION	IC 50 mg/ml
0.05	28.74	
0.1	57.67	0.092
0.2	94.12	