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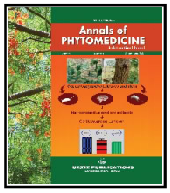

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Effect of extraction solvents on antioxidant potential of *Prosopis cineraria* (L.) leaves

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Abstract

The purpose of this study was to investigate the bioactivity profile, and to investigate the effect of three extraction solvents (acetone, methanol, aqueous) on the antioxidant activity of *Prosopis cineraria* (L.) leaf extract. *P. cineraria* leaves were characterized by their chemical composition and mineral profile. Higher phenolic content, total flavonoids and antioxidant activity could be obtained using acetone as solvent, followed by methanol and aqueous solution.The test plant material contained significant amounts of total phenolic content (6.84 ± 0.18 mg GAE/g) and total flavonoids (3.56 ± 0.78 mg CE/g) in the acetone extract. The DPPH free radical scavenger activity of *P. cineraria* leaf extract increased with increasing levels of highly fluctuating concentrations. The acetone extract showed the best DPPH free radical scavenger activity at the IC_{50} value (205.15 mg/ml) and also the highest total antioxidant capacity (4.98 ± 0.89 mg AAE/g).

1. Introduction

Ayurveda plays an important role, especially in India, known as the world's oldest medical system. Therefore, medicinal plants have been known for thousands of years as a rich source of therapeutic agents that improve the body's ability to stay healthy and fight disease (Srinivasulu *et al*., 2017; Ali, 2020; Singh and Singh, 2021; Nehra *et al*., 2022). As secondary metabolites, various chemicals exist in various compositions and provide therapeutic properties for medicinal plants (Karthikeyan *et al*., 2009; Mehrotra, 2021). Currently, the focus is mainly on natural sources of antioxidants, rather than on possible synthetic antioxidants, as they have harmful effects on human health (Maizura *et al*., 2011; Manju and Pushpa, 2020; Devi *et al*., 2021; Nehra *et al*., 2021).

P.cineraria is a medium-sized evergreen prickly tree commonly called Khejri. It belongs to the family Leguminosae and subfamily Mimosoideae. In India, it is especially cultivated in the drylands of Haryana, Rajasthan, Punjab, Gujarat, western Uttar Pradesh and the Deccan. *P.cineraria* is known as the Thar Desert BoonTree, commonly known as Jandi or Khejri (Diagne *et al*., 1992). It is known to be 'Kalpvirkash' because every part of the tree is useful (Panwar *et al*., 2014). Leaf paste applied to small mouth ulcers and drips are used on open wounds of the skin. Because, the leaves of this plant play a large role, commonly known as runes, its smoke is thought to be suitable for a variety of eye problems, and the leaf's paste is used to treat sorethroats and blisters. Leaf paste applied to bovineoral ulcers and used for open wounds of drip skin (Chopra *et al*., 1958; Nadkarni, 1954; Jewers *et al*., 1974). A mixture of fresh leaves and lemon juice is used for indigestion (Ghazanfar and Al-Al-Sabahi, 1993).

Extraction takes an important step to help recover the desired medicinal bioactive ingredient from the plant by removing unnecessary components using the solvent using a selective solvent (Dhanani *et al*., 2017; Singirikonda *et al*., 2021; Devi *et al*., 2021). Therefore, the main purpose of this study is to evaluate the antioxidant capacity of powerful antioxidant compounds, especially by investigating the most effective solvents for extracting phenols and flavonoids from *P. cineraria* leaves.

2. Materials and Methods

2.1 Plant material and chemicals

Leaf samples of *P. cineraria* were procured from Research farm, Department of Forestry at CCS Haryana Agricultural University in Hisar. The voucher number is BSI/AZRC/ I.12015/Tech./2015-16-(PI. Id.)/790, Dated 01/03/2016. The proposed studies were conducted in Chemistry, CCS HAU, Hisar. Before bringing the plant material and processing it, the material was stored in the shade at room temperature. Similarly, all chemicals and standards were purchased from Sigma Aldrich and Merck.

2.2 Proximate composition

Proximity analysis of *P. cineraria* leaves were performed on water content, crude fiber (Maynard, 1970), ash content and crude protein according to the standard method described in AOAC (Association of Official Analytical Chemists) (Horwitz *et al*., 1970).

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2.3 Mineral and chemical analysis

Minerals were estimated by the methods of Jackson (1973) and Ruig *et al*. (1986). The difference between the amount of total sugar and the content of reducing sugar was used to compute the content of non-reducing sugar. The tannin concentration as a catechin equivalent was calculated using the Burns Vanillin HCl method (Burns, 1971). The alkaloid content was estimated using the method of Harborne (1973), and the starch was estimated using the method of Sadasivam and Manickam (1996).

2.4 Preparation of extract of *P. cineraria* leaves

With a thimble filter paper of Whatman No. 1, a powder sample of *P. cineraria* leaves were obtained and placed in a typical Soxhlet device fitted in a 500 ml round-bottom flask. About 300 ml of solvent (acetone, methanol and distilled water) was added to upto 1.5 siphons. Each solvent was used for extraction at boiling temperature. The siphon mechanism occurs after the chamber is completely filled with solvent and contains some of the dissolved phytochemicals. At the three extraction steps, the volume of each filtered solvent was recorded. Total sugars, reducing and non-reducing sugars were measured using aqueous extracts, whereas total phenol, total flavonoids, and DPPH free radical scavenging activity were measured using acetone, methanol, and aqueous extracts.

2.5 Phytochemical content

2.5.1 Determination of total phenolics

Each extract (0.2 ml) was diluted with the appropriate solvent of each extract was diluted with the respective solvent. Also, 1 ml of 1 mol/l Folin Ciocalteu reagent and 2 ml of Na_2CO_3 (20%, w/v) were added and mixed and distilled water was used to make a volumeof 10 ml. After standing for 8 min, the mixture was centrifuged at 6000 rpm for 10 min. The absorbance of the supernatant was measured on blanks prepared with a 730 nm, UV-Visible double-beam spectrophotometer (Shimadzu Corporation, UV 1900). Blanks were prepared in the same way, but instead of extracts, each solvent was included. Since the standard analysis of total phenol is performed from the standard curve of the extract by the Folin Ciocalteu method (Singleton and Rossi, 1965) and displayed in milligrams of gallic acid equivalent per gram (mg GAE/g).

2.5.2 Determination of total flavonoids

For total flavonoids estimation, each concentration of the standard solution was taken in approximately 1 ml rather than 4 ml re-distilled water, 0.3 ml of 5% $NaNO_2$ was added, and after 5 min, 0.3 ml of 10% $AlCl_3$ was mixed. Soon, 2 ml of 1 M NaOH was added and again distilled water was used to bring the volume to 10 ml. After the solution was thoroughly mixed. The absorbance of the solution was measured on a UV-visible double beam spectrophoto-meter (Shimadzu Corporation, UV 1900) prepared with a blank of 510 nm. Similarly, blanks were prepared, but instead of extracts, each solvent was included. The amount of total flavonoids present in the standard curve of the extract was calculated using catechins. Standard analysis of total flavonoids was done by aluminum chloride colorimetric assay (Ribarova and Atanassova, 2005), expressed in milligrams (mg CE/g) of catechin equivalents per gram.

2.6 Evaluation of DPPH free radical scavenging activity

The dry mass of each solvent extract of *P. cineraria* leaves were noted in aqueous, methanol and acetone solvent and further method of Hatano *et al*. (1988) was used to assess DPPH-free radical scavenging activity.

2.7 Total antioxidant capacity

Estimate the total antioxidant capacity extract of *P. cineraria* leaves by the modified phosphorus molybdenum method (Prieto *et al*., 1999). Put, 0.3 ml each extract into a glass tube, add 3 ml phosphorus molybdenum reagent, mix the solution well and put the lid on. Incubate them for 90 min at 95°C. The contents of the vial were then cooled, and the absorbance of the created blank was measured in a UV-VIS double beam spectrophotometer at 695 nm (Shimadzu Corporation, UV 1900). Calculated as aqueous extracts from the total antioxidant capacity calibration curve and expressed as mg AAE/g.

2.8 Statistical analysis

For statistical analysis, each sample is taken in triplicate and results are expressed as mean ± standard error (S.E.). In online statistical analysis, one-way analysis of variance (ANOVA) and two-way analysis of variance (ANOVA) were used to assess significant differences between the means of the samples (OPSTAT). Regression analysis in Microsoft Excel was used to calculate the IC_{50} values of DPPH free radical scavenging activity. The Karl Pearson method in Microsoft Excel was used to calculate correlations between total phenolics, total flavonoids, and DPPH free radical trapping IC_{50} values. All additional calculations were completed in Microsoft Excel 2016.

3. Results

3.1 Composition profiling, mineral and chemical analysis

P. cineraria leaves are composed of water content (6.78 ± 0.07%), crude fiber content (15.14 ± 0.12%), ash content (9.97 ± 0.32%) and crude protein content (22.98 ± 0.36%) and estimated as percent w/w. The content of minerals (Fe, Mn, Zn,Cu), and the data are presented in Table 1. Mineral content of *P. cineraria* leaves, Fe (325.22 ± 0.65 ppm), Mn (45.7 ± 0.62 ppm), Zn (44.18 ± 0.17 ppm) and Cu (13.18 ± 0.64 ppm), tannin content (19.48 ± 0.77 mg CE/g), alkaloid content (3.51 ± 0.23% w/w), starch content (16.07 ± 0.69 mg / g) and total sugars content (0.77 ± 0.34 mg/g,total non-reducing sugar content (0.03 ± 0.27 mg/g)with total reducing sugar content (0.74 ± 0.34 mg/g) were all found in leaves analyzed by chemical analysis.

3.2 Effects of extracting solvent on phytochemical parameters

For *P. cineraria* leaves, total phenolic content (TPC) and total flavonoid content (TFC) were estimated using three different solvent systems. In various solvent extracts, acetone extract of *P. cineraria* leaves gave the best TPC (6.84 ± 0.18 mg GAE/g), followed by methanol extract (2.28 ± 0.34 mg GAE/g) and aqueous extract (0.71 ± 0.79 mg GAE/g). TFC was determined to be catechin equivalent (CE). Similarly, *P. cineraria* leaves also contained high TFCs in acetone extract (3.56 ± 0.78 mg CE/g), methanol extract (1.69 ± 0.47 mg CE/g) and aqueous extract (0.35 ± 0.79 mg CE/g). Table 2 shows the DPPH scavenging activity of *P. cineraria* leaves affected by the extraction solvent. The DPPH scavenging ability of the sample

extracts was reported as aper centage of DPPH scavenging. In leaf aqueous, methanol, and acetone extracts, the proportion of DPPH free radical scavenging activity is steadily increasing when the concentration of the extract is increased. The maximum DPPH free radical scavenging activity of the leaf is indicated by acetone extract, followed by methanol and aqueous.

Table 1: Proximate composition, mineral and chemical analysis of *P. cineraria* leaves

Proximate composition (% w/w)			
Moisture content	**Crude fibre content**	**Ash content**	**Crude protein content**
6.78 ± 0.07	15.14 ± 0.12	9.97 ± 0.32	22.98 ± 0.36
Mineral analysis (ppm)			
Fe	**Mn**	**Zn**	**Cu**
325.22 ± 0.65	45.7 ± 0.62	44.18 ± 0.17	13.18 ± 0.64
Chemical analysis			
Tannin content (mg CE/g)	**Alkaloid content (% w/w)**	**Starch (mg/g)**	**Total sugars (mg/g)**
19.48 ± 0.77	3.51 ± 0.23	16.07 ± 0.69	0.77 ± 0.34

Table 2: DPPH free radical scavenging activity (%) and IC_{50}-value (µg/ml) of different extracts of *P. cineraria* leaves

Extracts	Free radical scavenging activity of DPPH at various concentrations (µg/ml)							IC_{50} (µg/ml)
	1000	500	250	100	50	25	10	
Acetone	90.72	85.70	61.48	37.27	23.21	11.69	6.27	205.15
Methanol	85.78	74.36	55.89	27.22	13.00	6.93	4.74	234.76
Aqueous	77.82	69.17	44.36	24.25	11.65	5.64	3.38	282.28

The IC_{50} value of the acetone extract was the lowest at 205.15 µg / ml, followed by the methanol extract at 234.76 µg/ml and the aqueous extract at 282.28 µg/ml. This resulted in the extract made with acetone having the highest DPPH free radical scavenging activity, followed by the methanol and aqueous extract of leaves.The phytochemical components present in the extract contributed to the major antioxidant activity. High phenol content acts as an antioxidant from most plant materials. Total antioxidant capacity is calculated from acetone, methanol, and aqueous extracts from standard curves and is expressed in mg AAE/g. Total antioxidant capacity was highest with acetone extract (4.98 ± 0.89 mg AAE/g), followed by methanol extract (1.97 ± 0.07 mg AAE/g) and aqueous extract (0.57 ± 0.08 mg AAE/g) as shown in Figure 1.

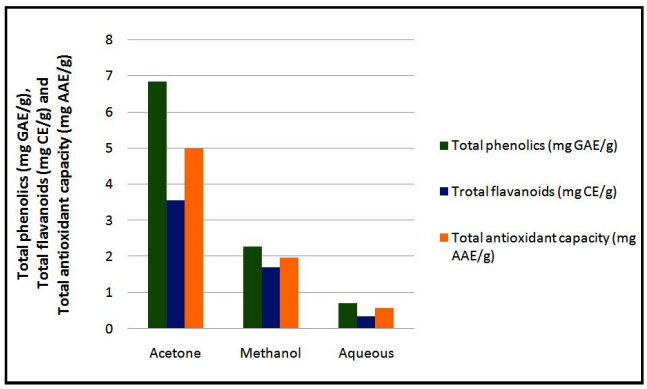

Figure 1: Effect of extraction solvents on total phenolics, flavonoids and total antioxidant capacity of *P. cineraria* leaves.

The correlation was evaluated to understand the relationship between total phenol, total flavonoids and the IC_{50} value of DPPH free radical scavenging activity. We obtained a correlation between total phenol and the IC_{50} value of DPPH-free radical scavenging activity. Also, between the IC_{50} values of total flavonoids and DPPH free radical scavenging activity. All of these correlation analyzes were performed at the 1% significance level. There was a very significant correlation between total phenolics and DPPH-free radical scavenging activity IC_{50} values (r = 0.918) in leaves. The relationship between total flavonoids and the IC_{50} value of DPPH free radical scavenging activity from the leaves has a negative correlation and is very important (r = 0.928). A correlation between total phenolics and total flavonoids was also observed in leaves. The results showed that the correlation between them was positive and highly significant in leaves (r = 0.999).

4. Discussion

Recent research has revealed that free radicals, also known as reactive oxygen species (ROS), are the primary cause of most physiological impairments, tissue damage, pathological processes, and diseases affecting humans (Tawaha *et al*., 2007; Sathishkumar *et al*., 2009). Antioxidants are increasingly acknowledged in medical and biological sciences as beneficial to health and illness (Oliveira *et al*., 2009). The main reason for the antioxidant action is due to the phenolic compounds' redox characteristic, which aids in the decomposition of peroxides and helps them adsorb and neutralise free radicals (Ito *et al*., 1983). Traditionally, the raw and decoction forms of this plant's leaves have been used.

The moisture content (6.78%), crude fibre content (15.14%), ash content (9.97%), and crude protein content (22.98%) of *P. cineraria* leaves were measured in the current study. Other scientists calculated the moisture content of *P. cineraria* leaves as well. The moisture content of leaves was found to be 3.893 % by Pathak and Kumar (2017). As a result, our data is very close to what other

studies have estimated. As a result, the information gathered is consistent. The crude fibre content of leaves was 16.67 %, according to Hussain *et al.*, (2017). The crude fibre content in leaves was estimated to be 20.1% by Afifi and Al-rub (2018). As a result, the data obtained for crude fibre content in leaves is comparable to that obtained by other researchers. As a result, the determined data is in agreement. Leaf ash concentration was calculated to be 6.15 per cent by Bhandari *et al.* (1979). According to Afifi and Al-rub (2018), leaves contain roughly 12.2 % ash. As a result of the varied locales, the ash content in leaves varies. Ranjhan *et al.* (1964) calculated the crude protein content of leaves to be 15.3 %. According to Pal and Chanchal (2017), the crude protein content of leaves is high (14-15%). According to Afifi and Al-rub (2018), the crude protein concentration in leaves is approximately 12.2%. As a result of the various regions and the availability of various conditions in that particular place. All of this occurred as a result of changes in the outcomes of other researchers' data.

In the current study, the Fe, Mn and Cu concentrations in leaves were 325.22, 45.70, and 13.18 ppm, respectively. In leaves, the tannin concentration (19.48 mg CE/g), alkaloid content (3.51%), and starch content (16.07%) were calculated. Bohra (1980) found that the tannin level in leaves was 11.6%. The tannin content of leaves was estimated to be 5.751% by Ehsen *et al.* (2016). Tannin concentration in leaves was determined to be between 8 and 10% by Afifi and Al-rub (2018). Pathak and Kumar (2017) calculated the alkaloid content of *P. cineraria* leaves to be 43.3793 mg/ml in stem bark and 75.381 mg/ml in leaves. Hussain *et al.* (2017) looked at the alkaloid content of *P. cineraria* leaves, which was 9.4%. As a result, the information obtained is consistent with that of other researchers.

Total phenolics concentration in acetone, methanol, and aqueous extracts was 6.84, 2.28, and 0.71 mg GAE/g in leaves in the current study. Choudhary *et al.* (2011) calculated that the total phenolic content of the 80 per cent methanol extract of *P. cineraria* stem bark was 6.95 mg GAE/g DW. The phenol content of *P. cineraria* leaves was 0.331 %as investigated by Ehsen *et al.* (2016). The total phenol concentration in crude extracts of *P. cineraria* leaves was 548 ± 1.45 mg/100 g in aqueous extract and 654 ± 2.54 mg/100 g in methanol extract, according to Mohan *et al.* (2017). As a result, the findings are consistent with those of other researchers. Total flavonoids concentration in acetone, methanol, and aqueous extracts was 3.56, 1.69 and 0.35 mg GAE/g in leaves in the current study. Kapoor and Bansal (2013) calculated the flavonoid content of *P. cineraria* leaves grown in Rajasthan's Nagaur region, finding total flavonoid content of 5.68 mg/g dry weight in Alaye, 6.13 mg/g dry weight in Khinvsar, and 6.21 mg/g dry weight in Merta. Ehsen *et al.* (2016) looked at the flavonoid content of *P. cineraria* leaves and found that it was 1.113% per cent. According to Mohan *et al.* (2017), the total flavonoid concentration in crude extracts of *P. cineraria* leaves was 184.03 ± 1.87 in aqueous extract and 231±0.43 in methanol extract. As a result, the findings are consistent with those of other researchers.

According to current research, the proportion of DPPH free radical scavenging activity increases as the concentration of extract increases. At 1000 µg/ml, aqueous extract has the highest DPPH free radical scavenging activity (77.82%), followed by 69.17 to 3.38 % at 500 to 10 µg/ml. Similarly, the highest percentage of DPPH free radical scavenging activity in methanol extract is 85.78 % at 1000 µg/ml, followed by 74.36 to 4.74% at 500 to 10 µg/ml. The greatest percentage in acetone extract is 90.72% at 1000 µg/ml and ranges from 85.70 to 6.27% at 500 to 10 µg/ml. This is due to the fact that acetone extract has a greater ability to donate hydrogen than methanol and aqueous extract, resulting in a colour change from purple to blue. Other studies looked at the DPPH free radical scavenging capacity of different solvent extracts in leaves. *In vitro* antioxidant activity of DPPH ranged from 20.1 to 108.1 % in hydro-ethanolic leaf extract at concentrations of 200 to 1000 µg/ml, according to Sharma *et al.* (2014). The IC_{50} was at 503 µg/ml. The antioxidant activity of *P. cineraria* leaves was examined by Mohan *et al.* (2017), who found that it ranged from 10.43 to 89.36 % in methanol extract and 9.54 to 80.86 % in aqueous extract at concentrations of 31.25 to 1000 µg/ml. As a result, the information gathered appears to be consistent with that of other researchers.

5. Conclusion

Current research findings are of great importance in the fields of nutritional supplements and pharmaceuticals. The present study uses the *P. cineraria* minerals Fe, Mn, Zn, Cu leaves to decompose these plant parts to enrich the nutrients in the soil, and to serve as a fertilizer to help improve the growth of other crops. As a result of this investigation, it was found that acetone solvent extract of *P. cineraria* leaf exhibited better antioxidant activity and high phenolic and flavonoid content. However, further research is needed to identify the individual components that form the antioxidant system and develop applications for the food and pharmaceutical industries.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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