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Effect of Extraction Solvents on Antioxidant Activity of Khejri (*Prosopis cineraria* L.) Stem Bark

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ABSTRACT

Studying the physiological effects of the three extraction solvents (water, methanol, and acetone) on the antioxidant activity of Khejri stem bark extract was the goal of this research (*Prosopis cineraria* L.). Khejri stem bark is characterized by their chemical and mineral composition. Higher phenol content, total flavonoid and antioxidant activity could be obtained using acetone as a solvent, followed by methanol and aqueous solution. The tested plant material contained a significant amount of total phenols (2.31 ± 0.13 mg GAE/g) and total flavonoids (0.88 ± 0.14 mg EC/g) in the acetone extract. With an increase in dynamically variable concentration levels, Khejri stem bark extract's ability to scavenge DPPH free radicals rises. The total antioxidant capacity of the acetone extract was the greatest (1.180.89 mg AAE/g), and the acetone extract demonstrated the strongest free radical scavenging activity of DPPH and IC_{50} value was 179.44 mg/ml.

Keywords Ayurveda, Therapeutic agents, Phytonutrients, *Prosopis cineraria,* Extracts oxidative inhibitor.

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INTRODUCTION

Worldwide, medicinal plants have been valued and known for thousands of years as abundant remedies for disease and disease prevention. In the form of chemically active substances, the medicinal value of these plants has a definite physiological effect on the human body (Fitzgerald *et al.* 2020). Phytochemicals were found to be present in all parts of the plant, but the essential ingredients of Ayurvedic rasayans and various types of Unani medicines were found to be present primarily in the stem and roots (Maheboob *et al.* 2018). Medicinal plants have great therapeutic potential due to their high antioxidant content (Devi *et al.* 2023, Nehra *et al.* 2022).

Prosopis cineraria Linn. (Fabaceae/Leguminosae) commonly known as Khejri is a medium-sized evergreen prickly tree (Meghwar and Dhanker 2022). In India, it is especially cultivated in the drylands of

Haryana, Rajasthan, Punjab, Gujarat, western Uttar Pradesh and the Deccan. Because every part of the tree helps in some way, therefore, it is also known as the "King of the Desert" because it is used for fodder, food, fuel, fiber and forest in the Indian Thar Desert (Panwar *et al.* 2014). During the severe famine of Rajputana in 1868-69, many lives were saved using husks as food, and also the cakes were made from husk flour. In nature, the bark has a dry, pungent, bitter, and sharp taste. It treats leprosy, dysentery, bronchitis, asthma, leukoderma, piles and tremors of the muscles and wandering of the mind. It is also used for tanning leather and has abortive and laxative properties. Meanwhile, it is primarily prescribed for the treatment of snake bites and scorpion stings and also plant is the excellent source of potash (Joshi *et al.* 2022).

Extraction uses selective solvents to perform significant steps, as will help you recover the physiologically active medicinal ingredient desired from plants and to help relieve non-desired with the help of solvents (Dhanani *et al.* 2017, Aggarwal *et al.* 2022). As different bark and leaf extracts with different solvents exhibited different biological activities. The activities of bronchodilators and vasodilators are shown by the crude methanol extract from the intermediate body bark by overloading Ca^{2+} channels. On the other hand, its water and methanol extract will show modest antibacterial activity compared to ciprofloxacin.

New chemicals are of great interest because they have been isolated from medicinal plants and are known to be an important source of new drugs due to their therapeutic effects (Devi *et al.* 2023). As sources of alternative medicine, drugs derived from native plants and herbal remedies have great potential and are used to treat health-related illnesses (Moond *et al.* 2023). Among the trees, the legume family tree is very interesting because it has antioxidant power against oxidative stress. This tree contains bioactive substances and is a good source of phytochemicals for promoting our health. Various chemicals and phytochemicals, including minerals, alkaloids, crude protein, crude fiber, total sugar, total phenols, and flavonoids, are the main constituents of various parts of the tree.

Now, in the age of health and illness, antioxidants are now recognized in medical biology (Goel *et al.* 2022). Oxidation of essential biological macromolecules is avoided by using antioxidant compounds that suppress the oxidation chain reaction (Pisoschi and Pop 2015). Oxidative stress induces the imbalanced production of antioxidants and the formation of reactive oxygen species, which play a role in the defense of our body, and promotes various problems such as aging and cancer (Singh *et al.* 2021). Because it causes cardiovascular disease, Parkinson's or Alzheimer's disease, degenerative diseases and inflammation (Forman and Zhang 2021). Currently, we are focusing on the natural resources of antioxidants rather than the synthetic antioxidants available such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and tert-butyl hydroquinone (TBHQ), as they have a detrimental effect on human health (Xu *et al.* 2021). The present study was aimed to determine the photochemical and its antioxidant potential (DPPH and total antioxidant capacity) in stem-bark of the plant, *Prosopis cineraria* L.

MATERIALS AND METHODS

Plant material and chemicals

Samples of stem bark of Khejri (*Prosopis cineraria* L.) were collected from Department of Forestry of CCS Haryana Agricultural University in Hisar. The proposed study was carried out in laboratory of chemistry Department in Campus of CCS HAU, Hisar. The raw materials are kept at room temperature in the shadow before to carry out in process for research work. Likewise, all chemicals and standards were purchased from Sigma Aldrich and Merck.

Proximate composition

According to the standard procedure outlined in the AOAC (Association of Official Analytical Chemists), proximity analysis in Khejri stem bark was done for estimation of moisture, crude fiber, ash, and crude protein content (Moond *et al.* 2023).

Mineral and chemical analysis

The minerals were estimated using the method of

Jackson and Ruig (Jackson 1973, Ruig *et al.* 1986). The unreduced sugar content was calculated as the difference between the total sugar content and the reducing sugar content. The tannin content was determined in stem-bark by Burns method or vanillin HCl [Vanillin-HCl reagent: Prepared by mixing equal volumes of reagent A and B, A=HCl in methanol (8%) and B=Vanillin in methanol (4%)] and catechin used as a standard (Burns 1971). The alkaloid content was estimated by the method of Harborne (Harborne 1973) and the starch was estimated by the method of Sadasivam (Sadasivam 1996).

Preparation of extract of Khejri stem bark

A sample of Khejri stem bark powder was taken using thimble Whatman No. 1 filter paper and placed in a conventional soxhlet apparatus fitted in a 500 ml round bottom flask. Approximately 300 ml of solvent (water, methanol and acetone) was added to a maximum of 1.5 siphons and extraction was done in each solvent at their boiling temperature of the solvent. The siphon mechanism occurs after the cavity is completely filled with solvent and contains some soluble photochemical.

When extraction was completed then volume of filtrate was recorded. Water-based extracts were used to measure total sugars, reducing and non-reducing sugar while the free radical scavenging activities of total phenols, total flavonoids and DPPH were assessed using extracts in water, methanol and acetone.

Determination of total phenolics

Each extract (0.2 ml) was diluted with the corresponding solvent. Further, 1 ml of 1 mol / L Folin Ciocalteu reagent and 2 ml of Na_2CO_3 (20%, w / v) were added and mixed well and distilled water was used to obtain a volume of 10 ml. The mixture was left to stand for 8 min and centrifuged at 6000 rpm for 10 min. Similarly, blanks were prepared, but instead of extracts, every solvent was included. The absorbance of the sample was measured on UV-VIS DBS (double beam spectrophotometer) Shimadzu Corporation, UV 1900 at wavelength 730 nm against a blank prepared.

Since the standard analysis of total phenols was performed from the standard curve of the extract using the Folin Ciocalteu method (Moond *et al.* 2023) and is shown in milligrams of gallic acid equivalent per gram (mg GAE/g).

Determination of total flavonoids

The total flavonoid content was determined by aluminium chloride colorimetric analysis (Goel *et al.* 2022) and to estimate the total number of flavonoids, each concentration of the standard solution was taken at approximately 1 ml, instead of 4 ml of re-distilled water, 0.3 ml of 5% $NaNO_2$ was added, and after 5 min, 0.3 ml $AlCl_3$ 10% is Mix. Immediately add 2 ml of 1 M NaOH and add distilled water to a volume of 10 ml. After the solution was well mixed, the absorbance of the solution was measured on a dual UV spectrophotometer (Shimadzu Corporation, UV 1900) prepared with a 510 nm blank. Likewise, blanks were prepared, but instead of extraction, all solvents were included. The amount of total flavonoids present in the standard curve of the extract was calculated using catechin and expressed in milligrams (mg CE/g) of catechin equivalent per gram.

Evaluation of DPPH free radical scavenging activity

The dry mass of each solvent for extracting Khejri stem bark was recorded in water, methanol and acetone solvents and further standard method was used to evaluate the DPPH free radical scavenging activity (Devi *et al.* 2023).

Total antioxidant capacity

The total antioxidant capacity from stem bark extract was estimated using the modified molybdenum method (Prieto *et al.* 1999). Place 0.3 ml of each extract in a glass tube, add 3 ml of phosphorus molybdenum reagent, mix the solution and close the stopper. Incubate them for 90 min at 95 °C. The contents of the vial were then cooled and the absorbance measured at 695 nm on the blank prepared in a double beam UV-VIS spectrometer (Shimadzu Corporation, UV 1900). Calculated as an aqueous extract from the standard curve of total antioxidant capacity and expressed in mg AAE / g.

Statistical analysis

For statistical analysis, each sample was taken in triplicate and the results were expressed as the mean ± standard error (SE). One-way analysis of variance and two-way analysis of variance (ANOVA) were performed to assess the significant difference between sample means in online statistical analysis (OPSTAT). The IC_{50} value of DPPH free radical scavenging activity was calculated by regression analysis in Microsoft Excel. Correlations between total phenols, total flavonoids, and DPPH free radical trap IC_{50} values were calculated using the Karl Pearson method in Microsoft Excel. All other measurements were performed in Microsoft Excel 2016.

RESULTS AND DISCUSSION

Composition profiling, mineral and chemical analysis

Khejri bark consists of moisture content (7.70 ± 0.05%), crude fiber content (22.54 ± 0.02%), ash content (16.50 ± 0.36%) and crude protein content (14.67 ± 0.34%). The mineral content (Fe, Mn, Zn, Cu), and the data are shown in Table 1. Mineral content in stem bark of Khejri is, Fe (384.52 ± 0.75 ppm), Mn (53, 13 ± 0.42 ppm), Zn (13.62 ± 0.27 ppm) and Cu (19.48 ± 0.54 ppm). The stem bark was analyzed by chemical analysis for tannin content (1.55 ± 0.07 mg EC / g), alkaloid content (0.78 ± 0.13%), starch content (5. 98 ± 0.19 mg / g) and the content of total sugars (0.55 ± 0.24 mg / g, total content of reducing sugars (0.47 ± 0.27 mg / g) and total content of non-reducing sugars (0.08 ± 0.14 mg /g).

Table 1. Proximate composition, mineral and chemical analysis of Khejri stem bark.

Proximate composition (%)			
Moisture content	Crude fiber content	Ash content	Crude protein content
7.70± 0.05	22.54± 0.02	16.50± 0.36	14.67± 0.34
Mineral analysis (ppm)			
Fe	Mn	Zn	Cu
384.52± 0.75	53.13± 0.42	13.62± 0.27	19.48± 0.54
Chemical analysis			
Tannin content (mg CE/g)	Alkaloid content (%)	Starch (mg/g)	Total sugars (mg/g)
1.55± 0.07	0.78± 0.13	5.98± 0.19	0.55± 0.24

Effects of extracting solvent on phytochemical parameters

For Khejri stem bark, the total phenol content (TPC) and the total flavonoid content (TFC) were estimated using three different solvent systems. Among the different solvent extracts, the acetone extract of Khejri stem bark gave the best TPC (2.31 ± 0.13 mg GAE/g), followed by the methanol extract (1.55 ± 0.14 mg GAE/g) and the aqueous extract (0.32 ± 0.09 mg EAG/g). TFCs were identified as catechin equivalents (CEs). Likewise, Khejri stem bark also contained high TFCs in acetone extract (0.88 ± 0.14 mg EC / g), methanol extract (0.23 ± 0.07 mg EC/g) and the aqueous extract (0.12 ± 0.03 mg EC / g). Table 2 shows that the DPPH scavenging activity of Khejri stem bark was affected by the extraction solvent. The DPPH scavenging capacity of sample extracts was reported as a percentage of DPPH collection. In the leaf extracts, methanol and acetone, the percentage of DPPH free radical scavenging activity increased steadily with increasing concentration of the extract. Maximum DPPH free radical scavenging activity from stem bark was indicated by acetone extraction, followed by methanol and aqueous. The total phenols, flavonoids and antioxidant activity was studied in *Avicennia officinalis* and reported that and total phenolics in various solvent fractions are in the order as: The TPC values decrease in the order: Ethyl acetate > acetone > chloroform > ethanol > methanol > dichloromethane and flavonoids content in the order as: Acetone > methanol > ethyl acetate > ethanol >

Table 2. DPPH free radical scavenging activity (%) of different extracts of Khejri stem bark.

	DPPH free radical scavenging activity at different concentration (µg/mL)					
Extracts	500	250	100	50	25	10
Aqueous	73.38	42.14	23.66	10.72	5.73	0.92
Methanol	82.37	51.90	26.38	12.55	6.91	2.26
Acetone	89.75	60.62	32.06	18.45	11.86	4.10

Table 3. IC_{50} (µg/mL) values among different solvent (aqueous, methanol and acetone) extracts of Khejri stem bark.

	Stem bark extracts		
	Aqueous	Methanol	Acetone
Quadratic regression equation	$y = -0.0001x^2 + 0.2044x+0.7507$ $R^2 = 0.9942$	$y = -0.0002x^2 + 0.2566x+0.5431$ $R^2 = 0.9987$	$y = -0.0002x^2 + 0.2948x+3.5407$ $R^2 = 0.9979$
IC_{50} (µg/mL)	279.04	236.24	179.44

chloroform > dichloromethane and the percentage of scavenging effect on the DPPH radical was increased monotonically with increasing the concentration of *Avicennia officinalis* extracts from 50 to 350 µg/ml. Among the investigated extracts, *Avicennia officinalis* extracts using acetone exhibited maximum DPPH radical scavenging activity (Nguyen *et al*. 2022).

DPPH free radical scavenging activity (%) of different extracts of Khejri stem bark was mentioned in Table 2. The IC_{50} value of the acetone extract was the lowest at 179.44 µg/mL, followed by the methanol extract at 236.24 µg/mL, and the water extract at 279.04 µg/mL (Table 3). This resulted in the acetone extract having the highest DPPH free radical scavenging activity, followed by aqueous methanol and leaf extracts. The phytochemicals present in the extract contribute to the main antioxidant activity. High content of phenol acts as an antioxidant in most plant materials. The total antioxidant capacity is calculated from the acetone, methanol and water extracts of the standard curve, expressed in mg AAE/g. The total antioxidant capacity of the acetone extract was the highest (1.18 ± 0.89 mg AAE/g), followed by the methanolic extract (1.07 ± 0.03 mg AAE/g) and the water extract (0.27 ± 0.05 mg AAE/g).

The correlation was evaluated to understand the relationship between the IC_{50} values of total phenols, total flavonoids, and DPPH free radical scavenging activity. We obtained the correlation between total phenolics and the IC_{50} value of DPPH free radical scavenging activity. In addition, the IC_{50} value of total flavonoids is between the free radical scavenging activity of DPPH. All these correlation analyses were performed at the 1% significance level. There is a very significant correlation between total phenols

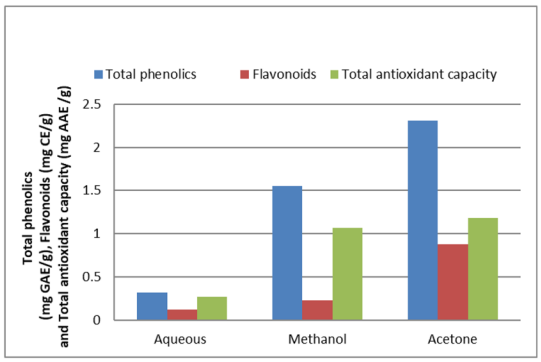

Fig. 1. Effect of extraction solvents on total phenolics, flavonoids and total antioxidant capacity of Khejri stem bark.

and the IC_{50} value of DPPH free radical scavenging activity (r=0.916) in stem bark. Total flavonoids are negatively correlated with the IC_{50} value of the DPPH free radical scavenging activity of stem bark, which is very important (r=0.905). A correlation was also observed between the total phenolics in the stem bark and the total flavonoids. The results showed that the correlation between them was positively correlated in stem bark and very significant (r = 0.989) and the effect of different solvents on total phenol, flavonoids and total antioxidant capacity is shown in Fig.1.

CONCLUSION

The current research results are of great significance in the field of nutritional supplements and medicines. This research uses the bark of the mineral stem Khejri Fe, Mn, Zn, Cu to break down these plant parts to enrich soil nutrients and as a fertilizer to help promote the growth of other crops. The results of this study found that acetone solvent extract of Khejri stem bark showed better antioxidant activity and high content of phenols and flavonoids. However, more research is needed to determine the various components that make up the antioxidant system and to develop applications in the food and pharmaceutical industries.

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