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Nutraceutical Properties of *Prosopis cineraria* (L.) Druce Pods: A Component of "Panchkuta"

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Prosopis cineraria (L.) Druce is a tree endemic to hot deserts of India, belonging to the family Leguminosae. Pods locally called "Sangri" are considered as dry fruit of desert and are one of the main ingredients of quintessential Rajasthani dish - The Panchkuta. In the present work, we have attempted to study different characteristics of the pods, to understand its health benefits. Sangri pods were studied for various phytochemicals like alkaloids, saponins, tannins, flavanoids, flavanols and total phenolics. As this plant is found in water stress (or deficient area) so antioxidant potential of pods has also been investigated. Acetone and methanol extracts of Sangri pods are the most potent bioactive extracts. Nutritional analysis shows it as a good source of proteins and minerals like calcium, sodium and potassium. This study demonstrates that *P. cineraria* pods may be employed as nutraceutical food with rich nutrition, disease prevention and health promoting effects.

Keyword: Endemic Plant, Phytochemical Analysis, Antioxidant Activity, ICP-OES.

1. Introduction

Nutritional and medicinal properties of plants are known since a long time, however, their disease preventing and health promoting aspects are realized in recent times. Plant foods such as vegetables and fruits play a vital role in health of human beings providing carbohydrates, fats, proteins, vitamins and minerals[1]. Various health agencies have established dietary recommendations for prevention of chronic diseases, cancer and atherosclerosis[2,3]. The primary compounds that are thought to provide the protection afforded by fruit and vegetables are the antioxidants[4]. Regular intake of natural antioxidants commonly present in fruits and vegetables have gained importance among consumers and scientist as studies indicate their consumption coupled with lowering risk of cardiovascular disease and cancer[5,6].

Vitamins, phenolics, and carotenoids are three major groups of natural antioxidants possessing defensive property present in fruits and vegetables[7]. The nutraceutical value and the antioxidant activity of wild, semi-cultivated or neglected vegetables is regarded worldwide as an important area of the nutritional and phytotherapic research[8-12].

P. cineraria grows in dry and arid regions of Arabia and in regions of India mainly Rajasthan, Haryana, Punjab, Gujarat, Western Uttar Pradesh and drier parts of Deccan. The tree is locally known as Jandi or Khejri (India), Jand (Pakistan) and Ghaf (Arabic). It is the State tree of Rajasthan, India[13]. *P. cineraria* are small to medium size tree evergreen and thorny. It is also

known as “wonder tree” and “king of desert” as all the parts of tree are useful[14]. The importance of the medicinal value of this tree has been highlighted in our ancient literature[15]. The bark of the tree has abortifacient and laxative properties and is also used as a remedy for rheumatism in the central provinces.

The leaves are of high nutritive value and locally called "Loong". Leaf paste of *P. cineraria* is applied on boils and blisters, including mouth ulcers in livestock[16]. The smoke of the leaves is considered good for eye troubles[17]. Leaf extracts of *P. cineraria* have been reported to show antibacterial, antihyperglycemic, antihyperlipidemic and antioxidative activities[18]. Pods are brown to chocolate in colour on ripening, each containing several seeds embedded in sweet dry yellow pulp. *P. cineraria* pods are locally called "sangar" or "sangri". The pod is considered astringent in Punjab. Sangri pods are known to prevent protein and mineral deficiency[14]. Cooked pods of Khejri are used as a functional food in Rajasthan, for the amelioration of numerous illnesses[19,14]. Recently, Nair *et al.*[20] has reported LPO, COX-1 and -2 enzyme inhibitory activities of Sangri pods.

Since, Sangri pods have not been much explored for their phytochemicals and bioactivities, present study was aimed to investigate its phytochemicals and antioxidant activity to be used as a potential source of nutraceutical. Sangri pods have been recently qualitatively screened for phytochemicals[21].

2. Material and Methods

2.1 Plant Material

P. cineraria (L) Druce dried fruits or Sangri were collected from Delhi, India and were identified at NISCAIR under reference no. NISCAIR/RHMD/Consult/-2011-12/1702/02.

The partially crushed samples were used to study various parameters.

2.2 Reagents

Folin-Ciocalteu’s Phenol reagent (SRL), Gallic acid (HiMedia), Dimethyl Sulfoxide (SRL), Aluminium Chloride (Fisher), Sodium Hydroxide (SRL), Ascorbic Acid (SRL), 2,2'-Azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (Sigma), Trolox (Aldrich), TPTZ (Fluka), Ammonia Solution (SRL), Ferrozine (SRL), Ferrous Chloride (Thomas Baker), Petroleum Ether (Loba Chemie), Methanol (Thomas Baker), Dichloro methane (Fisher), Ethanol, Ethyl acetate, Acetone, ICP Multielement Standard (Qualigens), Distilled Water.

2.3 Extraction

Partially crushed samples were extracted with Petroleum ether (PE), Dichloromethane (DCM), Ethyl acetate (EtOAc), Methanol (MeOH) and Acetone (Ac). The extracts were dried under vaccum and stored at 4°C.

2.4 Nutritional Analysis

Macrokjeldhal method was used for estimation of crude protein content[22]. Crushed samples of dried fruits were put in an oven at 105°C for 24 h. Difference in weight determines the moisture content[23]. The ash content was analyzed by AOAC method Ref. 942.05. The fat content of the samples was determined by using PE as a solvent. Total carbohydrate[22] and Energy calorific value[22] were also calculated. Crude fiber was analyzed by AOSC 1975 method[24]. Mineral content was analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES).

2.5 Phytochemical Analysis

2.5.1 Crude Alkaloids Determination: 2.5 g of the sample was weighed into a 250 ml beaker and 100 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed[25].

2.5.2 Saponins Determination
The method of Obadoni and Ochuko[26] was used to determine saponins. 5 g of samples powder was put into a conical flask and 50 ml of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue reextracted with another 50 ml of 20% ethanol. The combined extracts were reduced to 10 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 15 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a waterbath. After evaporation the samples were dried in the oven to a constant weight and the saponin content was calculated as percentage.

2.5.3 Tannin Determination
5 g sample was mixed with 50 ml distilled water on a shaker for 1 hr. 5 ml of filtrate was pipette out into a tube and mixed with 0.1 M $FeCl_3$ (3 ml) in 0.1 N HCl and 0.008 M potassium ferrocyanide. Absorbance was measured at 605 nm within 10 min. Tannic acid (100 ppm) was used as standard[27].

2.5.4 Total Phenolics Determination (TPH)
Folin Ciocalteu reagent method was used to determined total phenols[28]. An aliquot (100 µl) of extract was mixed with 250 µl of Folin–Ciocalteu reagent and allowed to stand at room temperature for 5 min. Sodium bicarbonate (20%, 1.5 ml) was added to the mixture and incubated at room temperature for 120 min. The absorbance was measured at 765 nm using a spectrophotometer. A standard curve was plotted using different concentrations of gallic acid and the amount of total phenolics was calculated as gallic acid equivalents in mg/ g of dried extract.

2.5.5 Total flavonoids determination
Total flavonoid content was determined using aluminium chloride ($AlCl_3$) method, using Rutin as a standard[29].The sample extract (250 µl) was added to 4.5 ml distilled water followed by 5% $NaNO_2$ (0.03 ml). After 5 min at 25°C, $AlCl_3$ (0.03 ml, 10%) was added. After further 5 min, the reaction mixture was treated with 2 ml of 1 M NaOH. Finally, the reaction mixture was diluted to 10 ml with water and the absorbance was measured at 510 nm. The results were expressed as rutin equivalents (RE) in mg/g of dried extract.

2.5.6 Total flavonol determination
Total flavonols of extracts were estimated as mg RE /g extract, from the rutin calibration curve[30]. The reaction mixture was prepared by mixing 0.5 ml of extract solutions with 0.5 ml (20 g/l) $AlCl_3$ and 1.5 ml (50 g/l) of CH_3COONa. The absorbance of reaction mixture was read at 440 nm after incubation at 20°C.

2.6 Antioxidant Activity
2.6.1 DPPH radical scavenging activity
The scavenging effect on α,α-diphenyl-β-picrylhydrazyl (DPPH) free radical was measured by the method of Shimada *et al.*[31] with some modification. DPPH solution (1 ml) was mixed with 0.1 ml of extract (at a concentration range of 0.5-1 mg/ml). The mixture was shaken and left for 30 min at room temperature. The absorbance of the resulting solution was measured at 517 nm using a spectrophotometer. For blank, 0.1 ml DMSO was used instead of the sample. Ascorbic acid and Trolox were used as standards. The concentration of extracts required to scavenge 50% of DPPH radicals, called inhibitory concentration (IC_{50}) was also calculated.

2.6.2 ABTS assay
ABTS radical-scavenging activity of the hydrophilic fractions was determined by a procedure reported by Re *et al.*[32]. The ABTS solution was prepared by mixing 8 mM of ABTS salt with 3 mM of potassium persulfate in 25 ml of distilled water. The solution was held at room temperature in the dark for 16 h before use. The ABTS solution was diluted with ethanol in order to obtain an absorbance between 0.8 and 0.9 at

734 nm. Fresh ABTS solution was prepared for each analysis. Antioxidant or standard solutions, 10 µl were mixed with 990 µl of diluted ABTS solution and incubated for 10 min. The absorbance at 734 nm was read. Ethanol was used as a blank. Trolox and ascorbic acid were used as standards. The concentration of extracts required to scavenge 50% of ABTS radicals, called inhibitory concentration (IC_{50}) was also calculated.

2.6.3 FRAP assay

FRAP solution (900 µl) was mixed with certain concentration of the plant extract (100 µl) and incubated at 37˚C for 4 min. The absorbance of the reaction mixture was measured at 593 nm. Trolox was used for standard curve[33].

2.6.4 Statistical analysis

All data were reported as the mean ± SD of three measurements.

3. Results and Discussion

3.1 Nutritional Analysis

In the present study, potential benefits were shown by nutritional attributes of Sangri pods (Table 1). Moisture content and dry matter analysis reporting during nutritional analysis is very important because it directly affects the nutritional content of Sangri pods. The moisture content was quite low (8.55±0.341%) which may be advantageous in view of the sample's shelf-life. Sangri pods were found to be rich in carbohydrates (51.01±1.179%). There was an appreciable amount of protein (28.42±0.56%) making it as a good source of protein, while fibre content are also good. There is evidence that the dietary fibre has a number of beneficial effects related to its indigestibility in the small intestine[34]. Sangri pods has very low amount of fat (2.30±0.328) which makes it ideal diet for overweight people.

Table 1: Proximate analysis of Sangri pods (%)

Species	Ash	Moisture	Crude fat	Total protein	Total carbohydrate	Crude Fiber
Sangri	9.712±1.034	8.55±0.341	2.30±0.328	28.42±0.56	51.01±1.179	5.00±2.541

All the values are mean of triplicate determinations

Sangri pods were found to be rich in P, Mg, Zn, Se, Ca and Fe by ICP-OES analysis (Table 2). Relatively high Zn contents are relevant in nutritional aspects, as Zn supplementation in diabetes mellitus proved to have an antioxidant effect[35]. Iron is used against anaemia, tuberculosis and disorder of growth[36]. Ca and P are important minerals as they are bound together in the bone, teeth and ligament of the body. Every organ in the body, especially the heart, muscles, and kidneys, needs the mineral Mg. It also contributes to the makeup of teeth and bones.

Table 2: Mineral content of Sangri pods (ppm)

Analyte	Conc. (ppm)
Sb	0.613
As	4.7460
Be	0.0081
Cd	0.0777
Ca	22.859
Cr	0.530
Co	0.0294

Cu	4.143
Fe	21.80
Pb	0.780
Li	0.291
Mg	185.9
Mo	0.1732
Ni	0.681
P	890.2
Se	29.40
Sr	N.D.
Tl	7.116
Sn	4.270
Ti	0.404
V	7.453
Zn	98.573

All the values are mean of triplicate determinations

3.2 Phytochemical Analysis

The phytochemical content of Sangri pods was analyzed and was found to be very promising. The values of tannins, saponins and crude alkaloids were determined on dry weight basis (g/100 g) (Table 3). Moderate quantity of alkaloids and saponins was found in Sangri pods while tannin content was very high. Alkaloids are good spasmolytic and anesthetic agents while saponins helps in boost of the immune system, lowering the cholesterol levels in the blood and reducing the risk of getting intestinal cancer. Tannins are known to produce anthelmintic activities as they bind to free proteins in the gastrointestinal tracts of host animal[37,38].

Table 3: Phytochemical analysis of Sangri pods (g/100g)

Species	**Crude alkaloid**	**Tannins**	**Saponins**
Sangri	2.076± 0.045	41.4±1.34	6.94±0.702

All the values are mean of triplicate determinations.

Various reports have shown that phenolic compounds contribute to quality and nutritional value in terms of modifying color, taste, aroma, and flavor and also in providing health beneficial effects. Therefore, total phenolic, flavonoid and flavonol content of Sangri pod extracts were also estimated (Table 4). Total phenolic content of extracts were in following order: MeOH > Acetone > PE > DCM > EtOAc. Thus, methanol is the best solvent for obtaining high yield of phenolics in Sangri pods. Phenolics provide plant defense mechanisms to neutralize reactive oxygen species (ROS) in order to survive and prevent molecular damage and damage by microorganisms, insects, and herbivores[39].

Table 4: Polyphenolic compounds of Sangri pods

Polyphenolic compounds	**PE extract**	**DCM extract**	**EtOAC extract**	**Acetone extract**	**MeOH extract**
Total Phenolic[a]	61.071±0.43	47.975±1.65	42.73±1.27	62.73±0.67	65.59±0.63
Total Flavonoid[b]	82±1.01	556.33±0.52	595.33±0.76	872.67±1.54	504.3±1.76
Total Flavanol[c]	104.76±0.81	213.33±1.32	47.85±0.87	127.61±077	ND

All the values are mean of triplicate determinations and expressed in mg/ g; a- Gallic acid equivalent;b- Rutin equivalent; c-Rutin equivalent

Flavonoids have a wide range of biological activities, such as cell-proliferation-inhibiting, apoptosis-inducing, enzyme-inhibiting, antibacterial, and antioxidant effects[40-42]. The flavonoid content of the extracts was in order: Acetone > EtOAc > DCM > MeOH >PE, with Ac extract having very high amount of flavonoid content. DCM extract was found to be rich in flavanols, followed by Acetone extract. However, MeOH extract did not show any presence of flavanols.

3.3 Antioxidant activity

The DPPH method is an easy, rapid, stable and sensitive way to determine the antioxidant activity of a specific compound or plant extracts[43]. In this assay, DPPH free radical accepts hydrogen and gets reduced by an antioxidant. Acetone extract showed highest DPPH scavenging activity followed by EtOAc and DCM extracts of Sangri pods. However, MeOH and PE extracts showed negligible activity. High scavenging activity of Acetone extract can be explained on account of its high flavonoid content. Similarly, EtOAc and DCM extracts also had good amount of flavonoids. Thus, flavonoids contribute to the DPPH scavenging activity of Sangri pods extracts.

Trolox equivalent antioxidant capacity (TEAC) of the Sangri pods extracts was evaluated as percent inhibition of ABTS free radicals. ABTS radical is a blue chromophore produced by the reaction between ABTS and potassium persulfate. The antioxidant activity as determined by ABTS assay was found to be highest in MeOH extract and least in case of DCM extract.

In FRAP assay, reduction of the ferric-tripyridyltriazine to the ferrous complex forms an intense blue colour which can be measured at a wavelength of 593 nm. The intensity of the colour is related to the amount of antioxidant reductants in the samples. FRAP activity is maximum in case of DCM followed by MeOH extract and least in case of PE extract.

4. Conclusion

“Sangri”, one of the most important ingredients of Panchkuta was analyzed in terms of nutritional, phytochemicals and antioxidant potential for their use as functional foods and nutraceutical to provide health benefits. There are substantial anecdotal reports indicating that the consumption of Sangri pods could ameliorate a wide range of illnesses. Our in vitro bioactivity results of the extracts from pods also support such health-beneficial claims. Thus, there is enormous scope for future research and further pharmacological investigation on *P. cineraria*.

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