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IN VITRO ANTIOXIDANT ACTIVITY OF ALCOHOLIC EXTRACT OF PODS OF PROSOPIS CINERARIA (L.) DRUCE

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ABSTRACT

The aim of present study was to estimate the *in vitro* antioxidant activity of alcoholic extract of pods of *Prosopis cineraria* (L.) Druce (*Fabaceae*) evaluated by using DPPH radical scavenging activity and hydrogen peroxide radical scavenging activity assay. The antioxidant activity is compared with ascorbic acid as standard. The IC_{50} values of *Prosopis cineraria* and ascorbic acid were found 34.59 µg/ml and 14.33 µg/ml respectively for the DPPH radical scavenging activity while 64.90 µg/ml and 135.78 µg/ml respectively for hydrogen peroxide radical scavenging activity. Thus, alcoholic extract of *Prosopis cineraria* pods possess potent antioxidant activity in hydrogen peroxide model and may be useful for preparation of neutraceuticals as potent antioxidant to treat various human diseases.

KEYWORDS: *Prosopis cineraria*, Pods, Antioxidant, Free radical scavenging, DPPH.

INTRODUCTION

Natural antioxidants present in the plants scavenge harmful free radicals from our body. Recently, natural plants have received much attention as sources of biological active substances including antioxidants. Numerous studies have been carried out on some plants, vegetables and fruits because they are rich sources of antioxidants, such as vitamin A, vitamin C, Vitamin E, carotenoids, polyphenolic compounds and flavonoids. Antioxidants may be defined as radical scavengers which protect the human body against free radicals that may cause pathological conditions such as ischemia, anaemia, asthma, arthritis, inflammation, neurodegenertion, parkinson's diseases, cancer, senility, mongolism, ageing process and diabetes mellitus.[1,3] Free radicals which have one or more unpaired electrons are

produced during normal and pathological cell metabolism. Reactive oxygen species (ROS) react easily with free radicals to become radicals themselves. ROS are various forms of activated oxygen which include free radicals as well as non-free radical species.[2,8]

***Prosopis cineraria*(L.) Druce** (family: *Fabaceae*, subfamily: Mimosaceae) commonly known as "Khejri" in Rajasthan. It is the State tree of Rajasthan, India.[4] Khejri is the golden tree of Indian deserts, plays a vital role in preserving the ecosystem of arid and semi-arid areas. It is the symbol of socio-economic development of the arid regions. Since all the parts of the tree are useful, it is called kalp taru. It is also known as the 'king of desert', and the 'wonder tree'.[4],[5],[6],[7] It is commonly found in dry and arid regions of north-western India, southern India, Pakistan, Afghanistan, Iran and Arabia.[8]

The conservation of khejri trees is a religious tenet of Rajasthan's Bishnoi community. The Government of India has recently instituted the 'Amria Devi Bishnoi National Award for Wildlife Conservation' in the memory of Amrita Devi Bishnoi, who in 1731 sacrificed her life to protect the khejri trees in Khejarali village near Jodhpur. 363 other people were also killed defending the trees.[9]

It is prickly tree or shrub. It is evergreen or nearly so. New leaves appear before or simultaneously with the fall of the old leaves in summer. The small, yellow flowers appear from March to May after the new flush of leaves. The pods are formed soon thereafter and grow rapidly in size. The pods ripen from June to August. Growth of new foliage, flowering and fruiting occurs during the driest months March-June when other plants become leafless and dormant.[10],[11]

Prosopis species have been used in indigenous system of medicine as folk remedy for various ailments like leprosy, dysentery, bronchitis, asthma, leucoderma, piles, and muscular tremors and wandering of the mind. It is also known to possess anthelmintic, antibacterial, antifungal, antiviral, anticancer and several other pharmacological properties. The smoke of the leaves is considered good for eye troubles. Leaf paste of *P. cineraria* is applied on boils and blisters, including mouth ulcers in livestock and leaf infusion on open sores on the skin.[8]

Khejari is most important top feed species providing nutritious and highly palatable green as well as dry fodder, which is readily eaten by camels, cattle, sheep and goats, constituting a major feed requirement of desert livestock. The leaves are of high nutritive value, locally it is

called "Loong". Feeding of the leaves during winter when no other green fodder is generally available in rain-fed areas is thus profitable. The pods are a sweetish pulp and are also used as fodder for livestock. Khejari Pods are locally called "sangar" or "sangri". The dried pods locally called "Kho-Kha" are eaten. Dried pods also form rich animal feed, which is liked by all livestock. Green pods also form rich animal feed. Khejari produces a brown shining gum just like Arbic Gum which is obtained duringthe months of April to June. Khejari wood is reported to contain high calorific value and provide high quality fuel wood. The lopped branches are good as fencing material.[4],[5],[6],[7]

Figure 1: Pods of Prosopis cineraria (L.) Druce (Fabaceae).

MATERIALS & METHODS

Plant material collection & authentication

The plant was collected around the Jaipur, Rajasthan and authenticity of plant was confirmed from "Herbarium Department of Botany, University of Rajasthan, Jaipur. The herbarium No RUBL 20956 of the same was preserved in Herbarium Department of Botany, University of Rajasthan, Jaipur for further reference. The pods were dried in shade at room temperature and the dried pods were powdered coarsely and passed through sieve no. 40, and stored in a well closed container.

Chemicals

DPPH (1,1-Diphenyl-2-picryl hydrazyl) was purchased from Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA). Ascorbic acid was obtained from Merck Ltd., Mumbai. methanol, hydrogen peroxide (H_2O_2), potassium dihydrogen phosphate, potassium hydroxide, phosphate buffer saline (PBS, pH 7.4) were analytical grade.

Extraction of plant seeds

The powdered Material was subjected to hot continuous extraction in a soxhlet extractor, successively with different known solvents in increasing order of polarity *viz* petroleum ether

(60-80^{o} C), benzene, chloroform, alcohol. Finally, the powdered material was macerated with water for 24 hrs to obtain aqueous extract. Each time before extracting with next solvent, the powdered material was dried in hot air oven below 50^{0}C. Each extract was then concentrated by distilling off the solvent by evaporation to a water bath.[12,13] All the extracts were stored in refrigerator for qualitative analysis.

DPPH radical scavenging activity

The antioxidant activity of the alcoholic extract was determined on the basis of their scavenging activity of the stable 1, 1-diphenyl-2-picryl hydrazyl (DPPH) free radical. 01 ml of various concentrations of the extracts in methanol was added to 4 ml of a 4 mg/100 ml (0.004% w/v) methanol solution of DPPH. After 30 minutes the absorbance of the preparations were taken at 517 nm by a UV spectrophotometer (Systronics, UV-2203, India) which was compared with the corresponding % inhibition of standard concentrations (10-100 µg/ml). Extract concentration providing 50% inhibition (IC_{50}) was calculated using the graph by plotting inhibition percentage against extract concentration.[2,11-13] Ascorbic acid (AA) was used as positive controls and all tests were carried on triplicates. The free radical scavenging activity (FRSA) was calculated by using the following equation:

$$\text{DPPH radical scavenging activity (\%)} = \frac{[\text{Abs (control)} - \text{Abs (sample)}]}{\text{Abs (control}} \times 100$$

Where, Abs (control): Absorbance of DPPH radical + methanol; Abs ((sample): Absorbance of DPPH radical + extract/standard; IC_{50} value is the concentration of the sample required to scavenge 50% DPPH free radical.

Hydrogen peroxide-scavenging activity

A solution of hydrogen peroxide (20 mM) was prepared in phosphate buffer saline (PBS at pH 7.4). Various concentrations of the extract or standard in methanol (1 ml) were added to 2 ml of hydrogen peroxide solutions in PBS. After 10 min, the absorbance was measured at 230 nm against a blank solution that contained extracts in PBS without hydrogen peroxide.[11] The percentage scavenging of hydrogen peroxide and standard compounds was calculated using the following formula:

$$\%\ \text{scavenged [Hydrogen peroxide]} = \frac{(A_0 - A_1)}{A_0} \times 100$$

Where, A_0 was the absorbance of the control; A_1 was the absorbance in the presence of the sample and standards.

RESULTS AND DISCUSSION

DPPH free radical scavenging activity

The reduction capacity of DPPH radical which is induced by antioxidant was determined by the increasing in its percentage inhibition. When concentrations of ascorbic acid was increased in the methanolic solution of DPPH radical, the absorbance of the solution decreased at 517 nm and DPPH free radical scavenging effect increased respectively and its colour was changed violet to yellow; as methanolic solution of DPPH has violet colour. The IC_{50} = 14.33 µg/ml in ascorbic acid was determined from regression equation of calibration curve (y = 0.2494x +46.437, R^2 = 0.9958); means 14.33 µg/ml of the ascorbic acid was able to scavenge 50% of free radicals of the methanolic solution of DPPH (Table 1 & Fig 3).

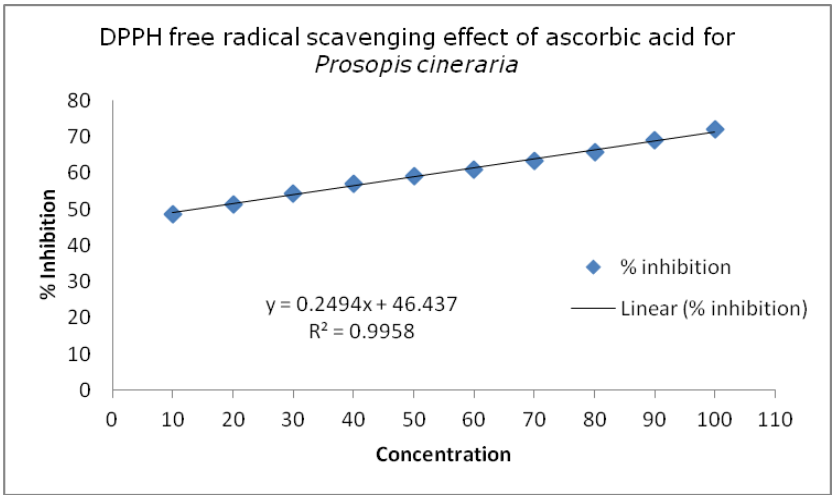

Figure 2: DPPH radical scavenging activity of standard IC_{50} = 14.33 µg/ml

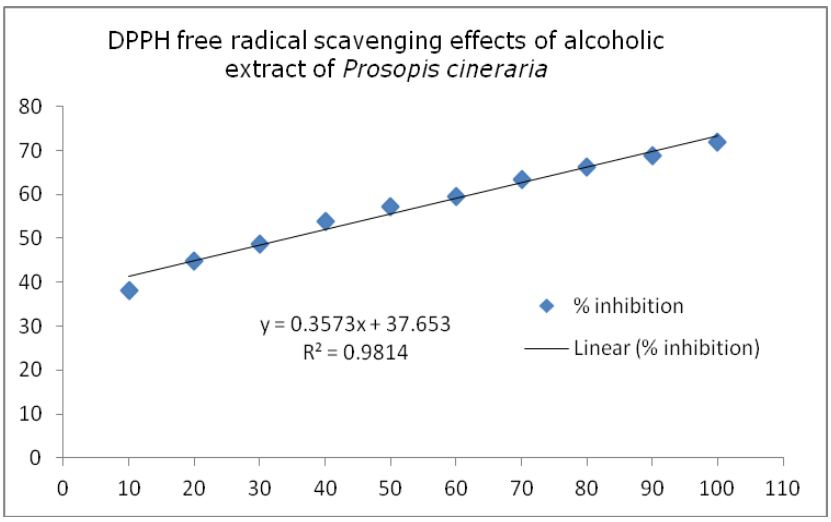

Figure 3: DPPH free radical scavenging effects of alcoholic extract of *Prosopis cineraria* IC_{50} = 34.59 µg/ml.

The scavenging effect increased with the increasing concentrations of test compound. The IC_{50} value for alcoholic pods extract was 34.59 µg/ml which was comparatively higher than the IC_{50} (14.33 µg/ml**)** of ascorbic acid (Table 2 & Fig 2). These results indicated that alcoholic extract of powdered pods of *Prosopis cineraria* produced antioxidant activity.

Hydrogen peroxide free radical scavenging activity

The radical scavenging activity of *Prosopis cineraria* extract increased with increasing in concentrations (Table 1 & Fig 5). The IC_{50} value for alcoholic extract of pods was 64.90 µg/ml which was comparatively lower than the IC_{50} **(**135.78 µg/ml) of standard (Table 2 & Fig 4). These results indicated that alcoholic extract of pods of *Prosopis cineraria* exhibited the effective antioxidant activity.

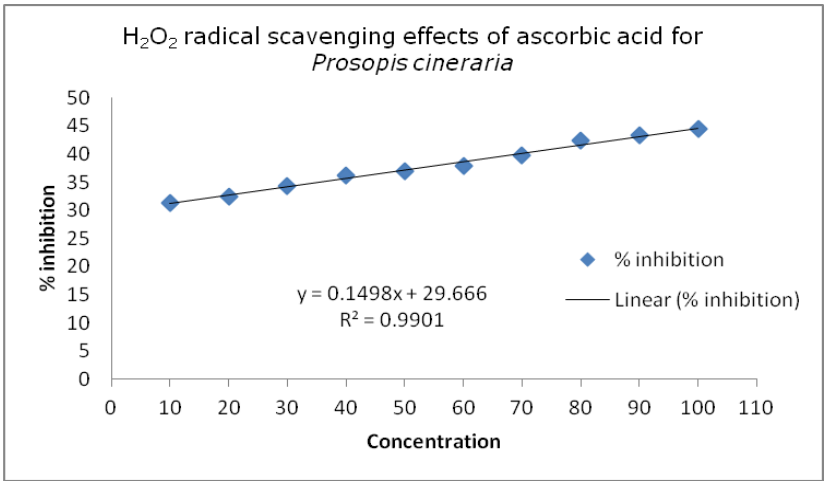

Figure 4: Hydrogen peroxide radical scavenging activity of standard IC_{50} = 135.78 µg/ml

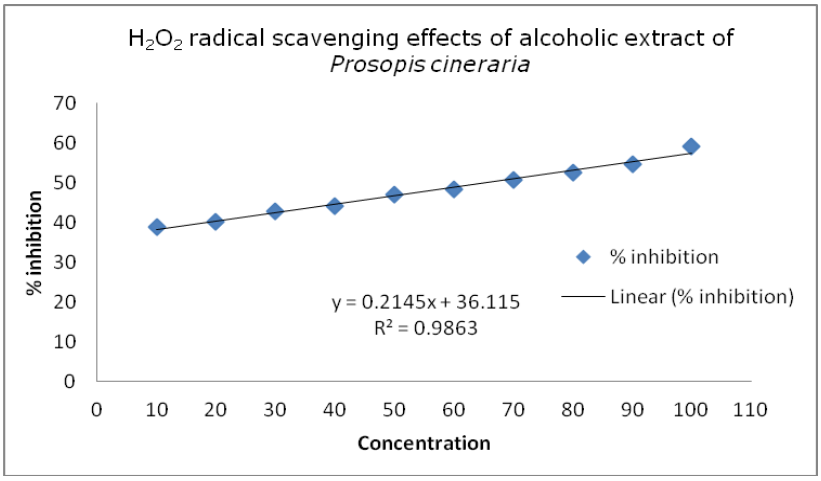

Figure 5: Hydrogen peroxide radical scavenging activity alcoholic extract of pods of *Prosopis cineraria* IC_{50} = 64.90µg/ml

Table 1: Free radical scavenging activity of alcoholic extract of *Prosopis cineraria* and ascorbic acid.

Concentration (µg/ml)	DPPH (% Inhibition alcoholic extract)	DPPH (% Inhibition ascorbic acid)	Hydrogen peroxide (% Inhibition alcoholic extract)	Hydrogen peroxide (% Inhibition ascorbic acid)
10	37.2628	49.0435	38.9536	31.3652
20	44.1034	51.7182	40.2631	32.3641
30	47.9354	54.7552	42.8042	34.2762
40	53.2334	54.023	44.2603	36.1834
50	56.6541	57.4152	47.1945	36.8729
60	58.9343	61.2144	48.3432	37.9474
70	62.7345	63.4955	50.6541	39.7842
80	65.7734	66.1589	52.5765	42.4674
90	68.4435	69.2025	54.8125	43.3024
100	71.4873	72.2441	59.2781	44.4571

Table 2: IC_{50} value (µg/ml) of alcoholic extract of *Prosopis cineraria* pods and ascorbic acid.

Compounds	DPPH	Hydrogen peroxide
Alcoholic extract	34.59	64.90
Ascorbic acid	14.33	135.78

CONCLUSION

On the basis of the results obtained in the present study, it is concluded that the alcoholic extract of pods of *Prosopis cineraria* (L.) Druce possesses the significant antioxidant activity. These finding suggest that this plant is a potential source of natural antioxidant. Further studies are warranted for the isolation and characterization of antioxidant components and also *in vivo* studies are needed for understanding their mechanism of action as an antioxidant better.

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