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In vitro antioxidant potential of *Prosopis cineraria* leaves

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ABSTRACT

Damage to cells caused by free radicals is believed to play a central role in the aging process and in disease progression. Many aromatic, medicinal and spice plants contain compounds that possess confirmed strong antioxidative components. The medicinal value of plants have assumed an important dimension in the past few decades owing largely due to the discovery as a rich source of antioxidants that combat oxidative stress through their redox active secondary metabolites and the rising concerns about the side effects of synthetic drugs. These factors have inspired the widespread screening of plants for possible antioxidant properties. Scientific interests in medicinal plants are emerging as plants are invaluable sources of new drugs and plant based antioxidants are preferred to the synthetic ones because of safety concerns. To evaluate antioxidant activity of different solvent fractions obtained from the leaves of Prosopis cineraria. Scavenging ability of the extracts for radicals like DPPH, ABTS, hydroxyl, superoxide, nitric oxide and hydrogen peroxide were performed to determine the potential of the extracts. All six fractions showed to have scavenging activity. The ethyl acetate and methanolic extracts showed to have maximum scavenging activity

Keywords: *Prosopis cineraria,* free radicals, antioxidant, medicinal plants, radical scavenging.

INTRODUCTION

Plant derived bioactive compounds are attractive candidates for drug development [1]. Free radicals are formed continuously as normal by-products of oxygen metabolism during mitochondrial oxidative phosphorylation which will be scavenged by antioxidants present in the system. *Prosopis cineraria* (Fabaceae) is a small to moderate sized tree found in the regions of Arabia and various parts of India such as Rajasthan, Gujarat, Haryana, Uttar Pradesh and Tamilnadu. The bark is prescribed for scorpion stings [2]. *Prosopis cineraria* flower is pounded mixed with sugar and used during pregnancy as safeguard against miscarriage [3] .The smoke of the leaves is good for eye troubles [4] .The pod is considered astringent in Punjab. Bark of the tree is used in the treatment of asthma, bronchitis, dysentery, leucoderma, leprosy, muscle

tremors and piles [5, 6] .The plant is recommended for the treatment of snakebite. However, to date no research on antioxidant properties of the *Prosopis cineraria* have been scientifically documented. In this study we report the antioxidant activities of various extracts of *Prosopis cineraria* leaf.

MATERIALS AND METHODS

Extract preparation

The leaves of *Prosopis cineraria* were collected fresh, rinsed with tap water and blotted dry using a filter paper and used for extract preparation. The components present in the leaves were extracted using solvents of increasing polarity, from non polar to polar (Petroleum ether, Benzene, Chloroform, Ethyl Acetate, Methanol and Aqueous Extract) using Soxhlet apparatus. The extract was evaporated and the residue obtained was dissolved in Dimethyl sulfoxide (20mg/20 µl) and further used for various *in vitro* assays.

Figure1: *Prosopis cineraria* leaves

Chemicals

All chemicals and solvents used were of analytical grade.

Diphenyl picryl Hydrazyl (DPPH) radical scavenging activity

The radical scavenging and antioxidant potential of the plant extracts were determined by the ability of plant extracts to scavenge the stable free radical DPPH and convert it into Diphenyl picryl hydrazine. The degree of decolorization from purple to yellow color was measured spectrophotometrically at 517 nm [7]. 500µl of 0.3mM DPPH prepared in methanol was mixed with 2.5 ml of extracts. The reaction mixture was mixed well and left in dark at room temperature for 30 min. The absorbance was measured spectrophotometrically at 517 nm. The scavenging ability of the plant extract was calculated using this equation;

$$\text{DPPH Scavenging activity (\%)} = [(\text{Abs control} - \text{Abs sample})] / (\text{Abs control})] \times 100$$

where Abs control is the absorbance of DPPH + methanol; Abs sample is the absorbance of DPPH radical + sample (i.e. extract).

Azino bis ethyl bezthiozoline sulphonic acid (ABTS) scavenging activity

The percent inhibition of ABTS radical by plant extracts were determined by the ability of plant extracts to scavenge the cationic free radical ABTS. The extent of decolorization was measured at 745nm [8]. The working solution was prepared by mixing 7 mM ABTS solution and 2.45 mM potassium persulphate solution and allowed to react for 12 h at room temperature in the dark. The percent of scavenging inhibition capacity of $ABTS^+$ of the extract was calculated from the following equation:

$$\% \text{ inhibition} = [(\text{Abs control} - \text{Abs sample})] / (\text{Abs control})] \times 100$$

Hydrogen peroxide scavenging activity

The ability of the plant extracts to scavenge Hydrogen peroxide radical was determined by measuring the decrease in absorbance at 230nm spectrophotometrically [9]. Plant extracts were mixed with 3.0 ml of 40 mM H_2O_2 solution prepared in phosphate buffer (0.1 M pH 7.4) and incubated for 10 min. The absorbance of the solution was taken at 230 nm against blank solution containing the plant extract without H_2O_2.

$$\text{Scavenging activity (\%)} = [(\text{Abs control} - \text{Abs sample})] / (\text{Abs control})] \times 100$$

Hydroxyl scavenging activity

The extent of hydroxyl radical scavenging activity by plant extracts were measured calorimetrically by studying the reaction between Dexyribose and the plant extracts for hydroxyl radical generation with Fe3+/Ascorbatre/EDTA/H_2O_2 system [10]. The assay quantifies the 2-deoxyribose degradation product, by its condensation with TBA.

$$\text{Scavenging activity (\%)} = [A0 - A1] / A0 \times 100$$

Where A0 was the absorbance of the control and A1 was the absorbance in the presence of the samples.

Inhibition of Nitric oxide generation

The extent of nitric oxide generation was studied using Griess reagent method [11]. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generate nitric oxide which interacts with oxygen to produce nitrite ions determined by the use of Griess reagents. 300µl of 100 mM sodium nitroprusside dissolved in 0.5 ml phosphate buffer saline (pH 7.2) was mixed with plant extract .The mixture was incubated at 25°C. After 150 min, 0.5 ml of incubation solution was withdrawn and mixed with 0.5 ml of Griess reagent. The mixture was incubated at room temperature for 30 min. The absorbance was measured at 540 nm. The amount of nitric oxide radical was calculated following this equation

$$\% \text{ inhibition of NO} = \text{Abs control} / \text{Abs sample} \times 100$$

Statistical analysis used: All the parameters studied were analyzed statistically using Sigma Stat statistical package (Version 3.1). One way ANOVA with $P<0.05$ was considered significant and, one way ANOVA followed by post-hoc Fischer analysis was done to test the levels of statistical significance.

RESULTS

The percent scavenging activity of the different extracts of *Prosopis cineraria* leaves are shown in the Fig 2. From the results it is evident that all the six extracts of leaves could scavenge DPPH, ABTS and hydrogen peroxide radicals. Comparatively, the petroleum ether extract of leaves was less effective than the other extracts.

Figure 2: DPPH, ABTS, H_2O_2 SCAVENGING ACTIVITY

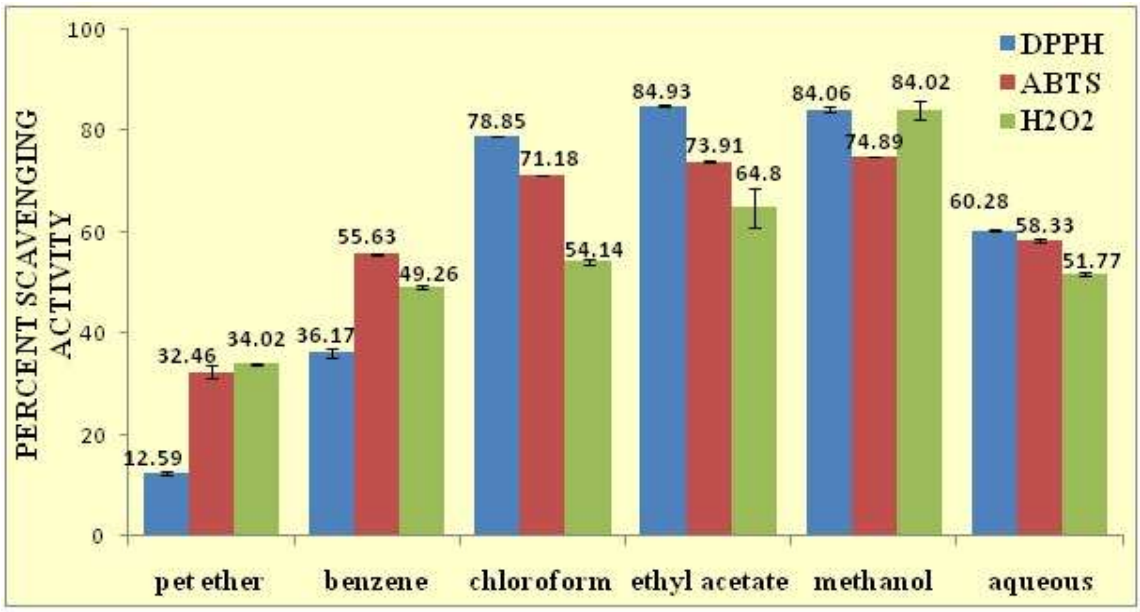


Figure 3: PERCENT INHIBITION OF NITRIC OXIDE GENERATION

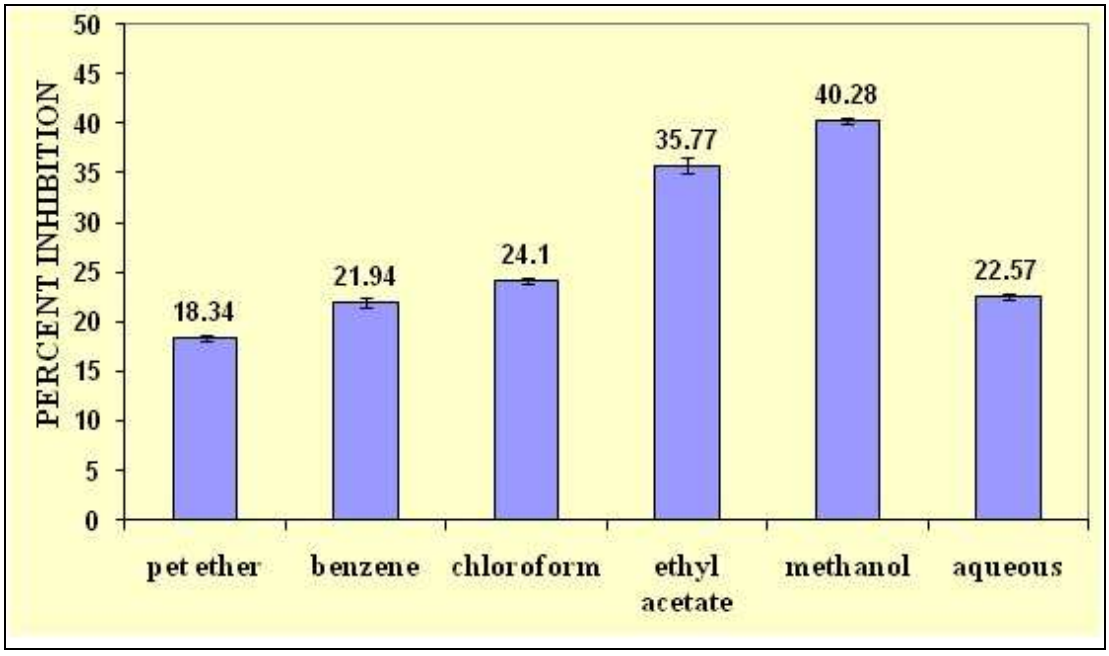
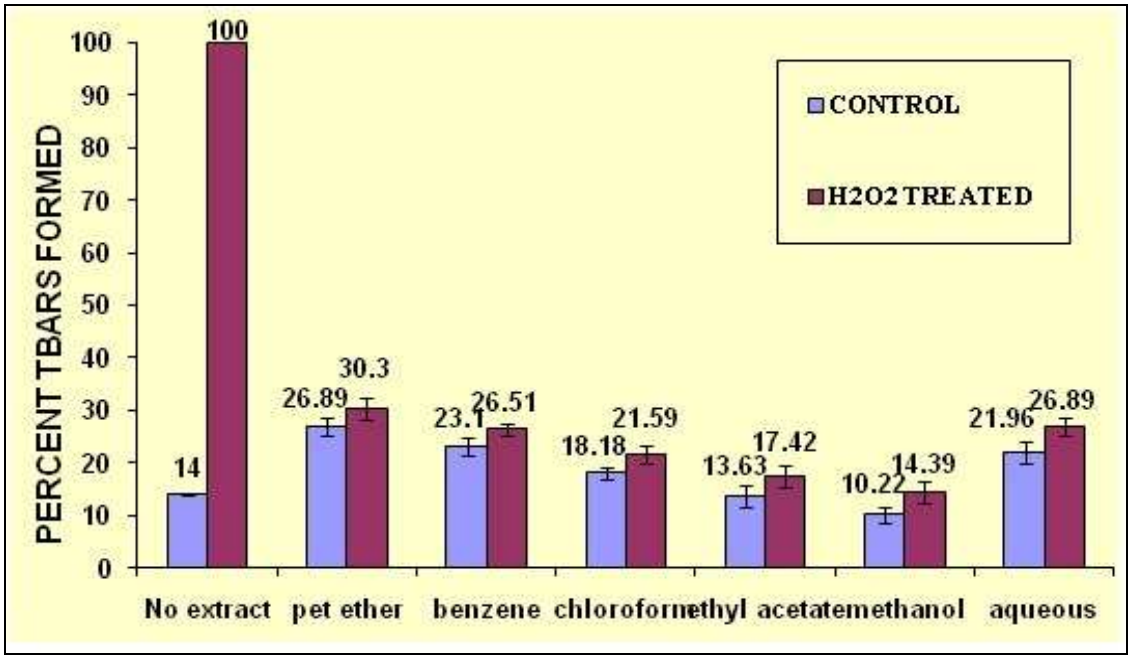


Figure 4: HYDROXYL RADICAL SCAVENGING ACTIVITY

The extent of inhibition of nitric oxide generation *in vitro* in the presence of the extracts of leaves of *Prosopis cineraria* were determined and presented in Fig 3. All the extracts of leaves

showed inhibitory activity. The methanolic extract of leaves showed maximum activity. The effects of *Prosopis cineraria* on H_2O_2- induced damage to deoxyribose was quantified as the amount of TBARS formed, and the results are represented in Fig 4. H_2O_2 exposure resulted in a steep increase in TBARS formation. Co treatment with plant extracts showed decrease in TBARS formation.



The values of the positive control (H_2O_2 treated) group were fixed as 100% damage and the per cent damage in the other groups were calculated relative to this.

DISCUSSION

The present study was aimed to assess the antioxidant activity of *Prosopis cineraria* leaf extracts. Antioxidants are compounds that prevent the oxidation of essential biological macromolecules by inhibiting the propagation of the oxidizing chain reaction. Keeping in mind the adverse effects of synthetic antioxidants, researchers have channeled their interest in isolating natural antioxidants [12] which are very effective to control the oxidative stress and hence prevent the initiation of disease propagation. The result of DPPH scavenging activity assay in this study indicates that the plant was potently active. This suggests that the plant extract contain compounds that are capable of donating hydrogen to a free radical in order to remove odd electron which is responsible for radical's reactivity. Among six extracts ethyl acetate and methanolic extracts show maximum scavenging activity followed by chloroform and aqueous extracts. However, this study provides a definitive report about the free radical scavenging capacity of *Prosopis cineraria*, since the antioxidant activity of a drug may depend on the free radical scavenging activity [13]. The aqueous, methanolic and ethanolic extracts of *Melissa officinalis*, *Matricaria recuttia* and *Cymbopogan citrates* were found to possess DPPH scavenging activity [14]. The DPPH scavenging ability of *Prosopis cineraria* leaves implicate the strong medicinal properties of the plant. Another screening method for antioxidant activity is the ABTS radical cation decolourization assay. This assay is widely used to assess the antioxidant capacity. In the present investigation, this method showed results quite similar to those obtained in the DPPH reaction. Of the successively extracted *Aphanamixis polystachya* bark with hexane, ethyl acetate, methanol and water, the methanolic extract possessed potent

ABTS scavenging activity [15]. The acetone, ethanol, methanol and water extracts from seed and calyx parts of persimmon (*Diospyros kaki* cv. Fuyu) fruit had relatively strong ABTS radical scavenging activity, exhibiting high antioxidant capacity. The highest ABTS activity was detected in the ethanol extract [16].With the support of the above studies, the ability of *Prosopis cineraria* leaf extracts in effectively scavenging ABTS radicals reveals the strong radical scavenging potential of the leaves. Hydrogen peroxide is a normal cellular metabolite that is continuously generated and maintained at low concentration. The ability of *Prosopis cineraria* leaf extracts to scavenge hydrogen peroxide in an in *vitro* system was carried out in the present study and the results revealed that the ethyl acetate and methanolic extracts exhibited a strong scavenging effect against hydrogen peroxide. The petroleum ether fraction of *Coccinia grandis* showed strong H_2O_2 scavenging activity followed by chloroform and ethyl acetate fractions [17]. From the results of our study, it is clear that *Prosopis cineraria* leaf extracts can effectively scavenge H_2O_2, which shows the strong antioxidant activity of the leaves. Hydroxyl radical is the most reactive among the ROS. The effect of *Prosopis cineraria* leaf extracts on hydroxyl radical-induced damage to deoxy ribose was analysed. The results showed that all the six extracts exhibited strong protection against hydroxyl radical, with the methanolic extract having maximum activity than the other five. The methanol extract of *Lagerstroema speciosa* L. showed higher hydroxyl radical scavenging activity when compared to the ethyl acetate, ethanol and water extracts [18]. The ethanol extract of the leaves of *Stachytarpheta angustifoliav* [19] efficiently inhibited hydroxyl radicals. From the results obtained, it is evident that *Prosopis cineraria* leaf extracts possess very good hydroxyl radical scavenging activity. The available literatures are supportive to our results. The leaf extracts of *Prosopis cineraria*, were analysed for their effect on the inhibition of NO generation. The results revealed that the methanolic extract and ethyl acetate extract exhibited maximum inhibition. The various extracts (methanol, hexane and ethyl acetate) of the shoots of *Anacardium occidentale* were measured for their antioxidant activities, the methanolic extract exhibited the maximum scavenging of superoxide anion and nitric oxide radicals [20]. Among the four fractions of *Geranium sibiricum*, the ethyl acetate fraction showed the highest nitric oxide scavenging activity [21]. With these literatures, it is clear that *Prosopis cineraria* leaf extracts exhibited good nitric oxide scavenging activity, which reiterated the strong antioxidant potential of the leaves.

Thus, the present study found that *Prosopis cineraria* leaves have effective *in vitro* antioxidant and radical scavenging activity.

CONCLUSION

Methanolic and ethyl acetate extract of *Prosopis cineraria* leaves showed to have maximum scavenging activity followed by chloroform and aqueous extract. On comparing the two extracts, methanolic extract of leaves were found to possess maximum scavenging activity indicating that leaves possesses good antioxidant activity. This is indicative of the fact that the active components get extracted in the methanolic extract and are responsible for their radical scavenging ability.

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