



In vitro antioxidant potential of stem bark of *Prosopis cineraria* Linn.

Velmurugan V*, G. Arunachalam¹ and V. Ravichandiran²

*Department of Pharmaceutical Chemistry, SRM College of Pharmacy, SRM University, Kattankulathur, Tamilnadu, India -603 203

¹PGP College of Pharmaceutical Sciences and Research Institute, Namakkal. TN, India.

²Vels University (VISTAS), Pallavaram-600117. Tamilnadu, India.

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ABSTRACT

The present study was to design and evaluation of antioxidants activity of methanolic extract of *Prosopis cineraria* (Linn.). Anti-oxidants can neutralize the side effects of free radicals by scavenging or chain breaking (like vitamin A, C, -carotene, etc.) or some other mechanism of action. These antioxidants must be constantly replenished since they are 'used up' in the process of neutralizing free radicals. The activity of methanolic extract of stem bark of *Prosopis Cineraria* Linn. (MPCL) was compared with ascorbic acid as standard 10.52 µg/ml ($y = 0.4992x + 101.25, 0.9921$) and result is 193.54 µg/ml. Data were compared by student t – test and t- value found to be 112.08 which is considered extremely significant. The results of this study show that the MPCL can be used as easily accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical industry.

Key words: *Prosopis cineraria* cinn, Antioxidant, DPPH, Nitric oxide and methanolic extract of *Prosopis Cineraria* Linn (MPCL).

INTRODUCTION

Among the various medicinal and culinary herbs some endemic species are of particular interest because they may be used for the production of raw materials or preparations containing phytochemical with significant antioxidant capacities and health benefits. Antioxidant compounds in food play an important role as a health-protecting factor. Scientific evidence suggests that antioxidants reduce risk for chronic diseases including cancer and heart disease. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. Some compounds, such as gallates, have strong antioxidant activity, while others, such as the monophenols are weak antioxidants.

The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate

degenerative disease. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxy and thus inhibit the oxidative mechanisms that lead to degenerative diseases. The preservative effect of many plant spices and herbs suggests the presence of antioxidative and antimicrobial constituents in their tissues.

Many medicinal plants contain large amounts of antioxidants other than vitamin C, vitamin E, and carotenoids. Antioxidants are compounds that inhibit or setback the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. Exogenous chemical and endogenous metabolic processes in the human body or in food system might produce highly reactive free radicals, especially oxygen derived radicals, which are capable of oxidizing bio molecules, resulting in cell death or tissue damage.

Oxidative damage plays a significantly pathological role in human disease. Free radicals lead to cellular necrosis, which is implicated in some membrane pathophysiological conditions including atherosclerosis, rheumatoid arthritis as well as toxicity of many xenobiotics, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, ageing, inflammatory response syndrome, respiratory diseases, liver diseases, cancer and AIDS. Many herbal plants contain antioxidant compounds and these compounds protect cells against the damaging effects of reactive oxygen species (ROS), such as singlet oxygen, superoxide, peroxy radicals, hydroxyl radicals and peroxy nitrite.

*Corresponding author.

Velmurugan V

Department of Pharmaceutical Chemistry
SRM College of Pharmacy, SRM University
Kattankulathur, Tamilnadu, India -603 203

MATERIALS AND METHODS

Plant material collection

The stem bark of Prosopis Cineraria (Linn.) were collected from Cuddalore district of Tamilnadu in the month of October 2008 and was authenticated by Dr. M. Raghuram, Asst. Professor, Acharya Nagarjuna University, Guntur (AP). The bark was air dried to a constant weight and made into coarse powder.

Preparation of the extracts

About 450 g of the coarse powder was extracted with methanol by Continuous hot percolation method (Soxhlet apparatus). The marc was then macerated with water to get aqueous extract. The methanol extracts were then evaporated under reduced pressure and they were stored in refrigerator till use.

Preliminary phyto-chemical screening

The methanolic extract was taken for various qualitative chemical tests to determine the presence of various phyto constituents like alkaloids, glycosides, carbohydrates, phenolics and tannins, phytosterols, fixed oils, protein and amino acids, flavanoids, saponins, gums and mucilage using reported method.

In- Vitro Antioxidant Assay

Nitric oxide radical scavenging assay

Nitric oxide scavenging assay was measured by spectrophotometric method. The methanolic extract screened for Nitric oxide (NO) radical scavenging activity (Dhenge RM. et. 2008). 1 ml sodium nitropruside (10 mm) in 0.5 M phosphate buffer (pH 7.4) was mixed with 3.0 ml of the different concentrations (25 – 100 µg/ml) of the sample dissolved in methanol and incubated at 25°C for 15 min. Above samples were reacted with Griess reagent (1% sulphanilamide in 5% H3PO4 and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride in water).

The absorbance of the chromophore formed during the diazotization of nitrate with sulphanilamide and subsequent coupling with N-(1-naphthyl) ethylenediamine was read at 546 nm. The same reaction mixture without extract of plant but with equivalent amount of 0.5 M phosphate buffer served as control. Ascorbic acid was used as standard.

$$\% \text{ Inhibition} = (\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Sample}}) \times 100 / \text{Abs}_{\text{Control}}$$

Where, Abs_{Control} is absorbance of control at time = 0 and Abs_{Sample} is absorbance of test sample. The IC₅₀ Value for extracts was also calculated. The results are mentioned in table.1.

DPPH radical scavenging assay

The free radical scavenging activity by different plant extracts was done according to the method reported by (Gyamfi et al, 2002). Fifty micro liters of the plant extract in methanol, yielding 100µg/ml respectively in each reaction was mixed with 1ml of 0.1Mm DPPH in methanol solution and 450µl of 50mM Tris-HCl buffer (pH 7.4). Metha-

nol (50µl) only was used as control of experiment. After 30 min of incubation at room temperature the reduction of the DPPH free radical was measured reading the absorbance at 517nm. L-Ascorbic acid and BHT used as controls. All determination was performed in triplicate. The percentage of DPPH radical scavenging ability of the sample was calculated by using following formula:

$$\% \text{ Inhibition} = (\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Sample}}) \times 100 / \text{Abs}_{\text{Control}}$$

Where Abs_{Control} is absorbance of control at time = 0 and Abs_{Sample} is absorbance of test sample. The IC₅₀ Value for extracts was also calculated. The results are mentioned in table-2

RESULTS:

Table: 1-Nitric Oxide Radical Scavenging Activity- Blank-0.224

S.No	Conc	STD	%Inhibition	Test	%Inhibition
1	25	0.127	43.30±0.2682	0.208	7.14±0.123
2	50	0.126	43.75±0.088	0.198	11.60±0.435
3	100	0.119	46.87±0.345	0.168	25.00±0.896
4	200	0.088	60.71±0.584	0.142	36.60±0.548
5	400	0.087	61.16±0.391	0.136	39.28±0.842
6	600	0.085	62.05±0.567	0.130	41.96±0.222
7	800	0.084	62.50±0.077	0.117	47.76±0.264
8	1000	0.048	78.50±0.598	0.091	59.37 ±0.212

The results were expressed as mean±SD of three independent values

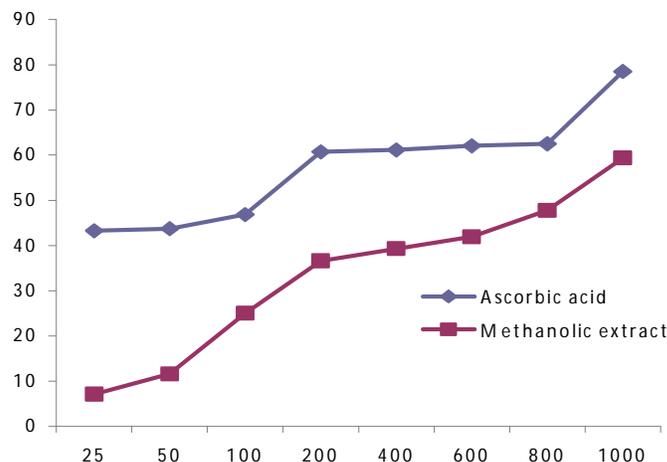


Figure:1-Nitric Oxide Radical Scavenging Activity

Table:2-DPPH Radical Scavenging Activity - Blank-0.456

S.No	Conc	STD	%Inhibition	Test	%Inhibition
1	25	0.232	49.12±0.213	0.258	12.54±0.312
2	50	0.185	59.42±0.326	0.222	24.74±0.678
3	100	0.160	64.91±0.578	0.184	37.62±0.875
4	200	0.147	67.76±0.589	0.176	40.33±0.278
5	400	0.140	69.29±0.038	0.185	59.63±0.324
6	600	0.137	69.95±0.194	0.165	63.82±0.123
7	800	0.128	71.92±0.443	0.135	70.39±0.952
8	1000	0.094	79.38±0.395	0.090	80.20±0.284

The results were expressed as mean±SD of three independent values.

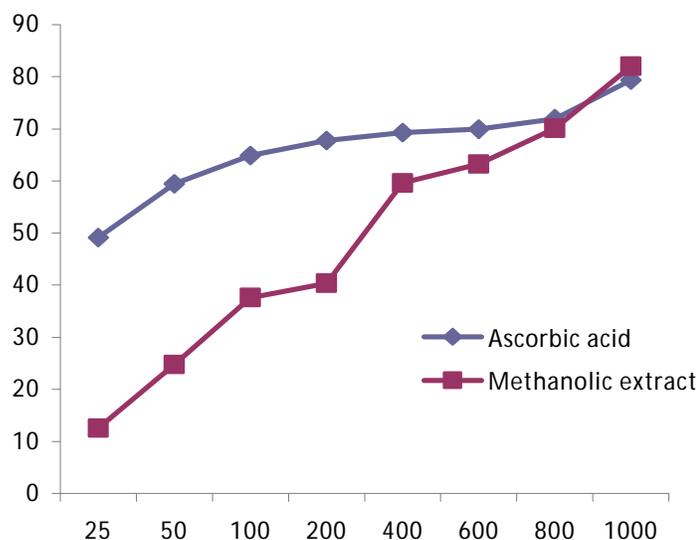


Figure-2 -DPPH Radical Scavenging Activity

DISCUSSION

Since ancient times, plants have been a veritable source of drugs. However, man tends to ignore the importance of herbal medicine. Recently, much attention has been directed towards extracts biologically active compounds isolated from popular species. Different medicine from traditional medicinal plants has been tested to identify the source of the therapeutic effects. It was found that 70% methanolic extract of *Prosopis Cineraria* (Linn.) extract contained terpenoids, saponins, phenolic compounds and tannins, proteins and amino acids, carbohydrates, mucilage and flavonoids. Free radicals are produced under certain environmental conditions and during normal cellular function in the body. These molecules are missing in an electron giving them an electric charge. So that the activity of methanolic extract of stems barks of *Prosopis Cineraria* (Linn.) was compared with ascorbic acid as standard $10.52 \mu\text{g/ml}$ ($y = 0.4992x + 101.25, 0.9921$) and result is $193.54 \mu\text{g/ml}$. Data were compared by student t-test and t-value found to be 112.08 which is considered extremely significant anti-oxidant activity.

CONCLUSION

The methanolic extract of stem bark of *Prosopis Cineraria* (Linn.) showed antioxidant activity by inhibiting DPPH and nitric oxide scavenging. In addition, the MPCL found to contain a noticeable

amount of total phenols, which play a major role in controlling antioxidants. The results of this study show that the MPCL can be used as easily accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical industry. However, the components responsible for the antioxidant activity of MPCL are currently unclear. Therefore, further works should be needed on the isolation and identification of the antioxidant components in MPCL.

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