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Research Article

# Antioxidant Activity of Metanolic Extracts of Stem barks of *Parkinsonia aculeata* Linn

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**Abstract:** Antioxidents are important in protective against various diseases like hypertensive, diabetes, cardiovascular disease and cancer. In present study of antioxidant activity of metanolic extracts of stem and stembark of *Parkinsonia aculeate* L. was carried out using standard in vitro models. The antioxidant activity was evaluated in vitro by using three different assays viz., Nitric oxide Scavenging activity, DPPH Scavenging activity and Hydrogen peroxide Scavenging activity. The result of the study depends of concentration of the metanolic extracts of the stem bark of *Parkinsonia aculeata* L. This study will provide suitable opportunities for further investigation to verify these activities in vivo.

**Keywords:** Antioxident activity, DPPH, Nitirc Oxide, Hydrogen peroxide.

## INTRODUCTION

*Parkinsonia aculeate* L. belongs to the family Leguminoceae and commonly known as "Jerusalem thorn". It is hand species valued as an ornamental tree, found in dry regions of America, India (Specially in Rajasthan), Pakistan and Italy. Decoction of the seed is used potential sources for low cost protein[1] and free amino acids[2]. It also shows trypsin inhibitor activity and helps in the prevention of diabeties[3] . Extracts of stem bark of this plant were screened for spermatogenic effects[4] and antibiotic activities[5] .

Many antioxidants compounds obtained from plant sources have been identified as free radical or active oxygen Scanvergers[6]. In recent time, therefore interest has increased significantly in finding naturally occurring antioxidants for use food or medicinal substances to replace the synthetic antioxidant[7]. Plant constituents like flavonoid and phenolic compounds are broadly distributed and have been reported to extract multiple biological effects including antioxidant, anti-inflammatory or anticarcinogenic etc[8].

The aim of the present investigation is to evaluate in vitro antioxidant and free radical Scavenging activity of the *Parkinsonia aculeate* L. Stembark extracts.

## EXPERIMENTAL

Aluminum chloride, trichloroacetic acid, Sulphanilic acid, Nitroblue tetrazolium chloride (NBT), Phenazine methosulphate (PMS) and nicotinamide adenine dinucleotide (NADH) were purchased from Sisco Research Lab, India. Ferrozine and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) Potassium ferricyanide, ferric chloride sodium carbonate, naphthyl ethylene diamine dihydrochloride (NEDD) were purchased from Ronkem fine chemicals Ltd. India. And used L- ascorbic acid, gallic acid and quercetin from Department Lab. All the regents were of analytical grade and used without further purification.

**Plant materials and extraction procedures:** *Parkinsonia aculeate* L. stembark collected during January 2011 from College Campus and was authenticated by Departemnt of Botany, University of Rajasthan, Jaipur. About 500g. of powered stembark of the plant was first defatted wit petroleum ether and extracted with methanol for 72 Hr. the extract was concentrated under reduced pressure using rotary evaporator. Methanolic extract of the stembark directly used for the determination of antioxidant activities.

**1.Hydrogen peroxide scavenging activity**: Hydrogen scavenging activity was determined by the method described by Jayaprakasha *et al*[9]. A solution of hydrogen peroxide (20mM) was prepared in phosphate–buffer saline (PBS at $P^H$ 7.4). Different concentrations of the extract and standard compound in methanol (1 ml) added to 2 ml of hydrogen peroxide solution in PBS. After 20 min. the absorbance was measured at 230 nm. All the data presented are average of triplicate analysis.

**2. DPPH scavenging activity:** The free radical scavenging activity was determined by the DPPH assay described by Blios[10]. DPPH method is one of the most simple and inexpensive method to measure antioxidant activity of plants and involves the use of the free radical (DPPH). It is a stable free radical and is often used to evaluate the antioxidant activity of several plants. Antioxidants on interaction with DPPH, either transfer electron or hydrogen atom to DPPH and thus neutralizing its free radical character. The odd electron in the DPPH free radical gives a strong absorption at 517 nm and its purple in colour. 0.1 mM solution of DPPH in methanol was prepared and 5 ml of this solution added to 1 ml of sample solution in methanol at different concentrations. After 1 hr. the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity.

**3. Nitric oxide scavenging activity:** Nitric oxide scavenging activity of methanolic extract was carried out based on method described by Marcocci with slight modifications[11]. The reaction mixture (10 ml) containing sodium nitropruside (5 ml, 10 mM), phosphate buffer saline (1 ml) and the extracts, the compound and standard solution (1 ml) were incubated at 25°C for 3 hr. after incubation, 1 ml of the reaction mixture was removed and 1 ml of sulphanilic acid regent (0.33% in 20% glacial acetic acid) was mixed and allowed to stand for 10 min. for completion of diazotization reaction and then 1 ml of 0.1% solution of naphthyl ethylene diamine dihydrochloride (NEDD) was added, mixed and allowded to stand for another 0.5 hr. in diffused light. The absorbance was measured at 540 nm against corresponding blank solution.

**Statistical analysis:** All values are expressed as means ± SD. Statistical analysis was performed by one-way ANOVA. Differences were considered significant at $p < 0.05$. The values for $IC_{50}$ were estimated graphically by linear regression analysis.

## RESULTS AND DISCUSSION

**Hydrogen peroxide scavenging activity:** Hydrogen peroxide scavenging activity of extract is compared with ascorbic acid, quercetin and gallic acid. Result has been summarized in **Table -1**. the ability of

extract to scavenge hydrogen peroxide is compared with standard compounds that are ascorbic acid, quercetin and gallic acid. $IC_{50}$ values of extract, ascorbic acid, quercetin and gallic acid were found to be 316±0.494, 198.61±1.631, 38.34 ± 2.138 and 50.49 ± 0.167 µg $mL^{-1}$, respectively. These results showed that extract was effective in scavenging hydrogen peroxide. Hydrogen peroxide scavenging activity of extract and standard compounds exhibited the following order: quercetin > gallic acid > ascorbic acid > extract.

**DPPH scavenging activity:** The extract showed maximum hydrogen donating ability in the presence of DPPH free radical at high concentration. As shown in **Table -2,** the extract showed a strong hydrogen donating capacity and can powerfully scavenge DPPH radical. The extract showed antioxidant activity with $IC_{50}$ value of 21.86 ± 0.625 µg $mL^{-1}$ . However, the known antioxidants such as ascorbic acid and quercetin exhibited $IC_{50}$ values of 16.81 ± 0.549 and 8.02 ± 0.246 µg $mL^{-1}$, respectively. The loss of the DPPH radical based on the absorbance at 517 nm wavelength can be monitored by decreased optical density[12]. The result obtained in this investigation reveals that the DPPH radical scavenging ability of methanolic extract of stembark may be attributed to the hydrogen donating ability.

**Nitric oxide scavenging activity:** Nitric oxide (free radical) has an important role in various types of inflammatory and other physiological conditions.[13] The procedure is based on the principle that sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. In presence of extract, production of nitrite ions was reduced due to scavenging of nitric oxide, which interacts with oxygen to produce nitrite ions. $IC_{50}$ value of extract was found to be 671.20 ± 11.82 µg $mL^{-1}$; however gallic acid exhibited 79.31 ± 0.546% inhibition at a concentration of 20 µg $mL^{-1}$ as show in Table-3, which indicates that the methanolic extract of stembark possess the nitric oxide scavenging activity.

**Table- 1**

| **Hydrogen Peroxide Scavenging Activity Of Extract And Standard** | | | | |
|---|---|---|---|---|
| Concentration (µg $mL^{-1}$) | Percentage inhibition (mean ± SD) | | | |
| | Extract | Ascorbic acid | Quercetin | Gallic acid |
| 20 | * | * | 37.36 ± 2.137 | 36.00±1.808 |
| 40 | * | * | 49.17 ± 2.213 | 40.82 ± 1.212 |
| 60 | * | * | 68.79 ± 2.100 | 58.09 ± 1.715 |
| 80 | * | * | 83.22 ± 0.674 | 68.94 ± 1.625 |
| 100 | 8.0 ± 1.563 | 39.29 ± 0.828 | 93.94 ± 0.652 | 83.95 ± 0.968 |
| 200 | 18.12 ± 1.381 | 53.55 ± 1.136 | * | * |
| 300 | 46.00 ± 1.406 | 66.18 ± 1.585 | * | * |
| 400 | 72.23 ± 1.474 | 78.22 ± 1.815 | * | * |
| 500 | 90.06 ± 0.663 | 96.92 ± 0.906 | * | * |
| $IC_{50}$ Values in µg $mL^{-1}$ (mean ± SD) | 316.64 ± 0.494 | 198.61 ± 1.634 | 38.34 ± 2.138 | 50.49 ± 0.167 |

**Table- 2**

| **Dpph Scavenging Activity of Extract And Standard** | | | |
|---|---|---|---|
| Concentration ($\mu g\ mL^{-1}$) | Percentage inhibition (mean ± SD) | | |
| | Extract | Ascorbic acid | Quercetin |
| 5 | * | 32.28 ± 2.348 | 38.21 ± 0.214 |
| 10 | 38.46 ± 0.945 | 42.71 ± 1.128 | 58.73 ± 0.183 |
| 20 | 48.17 ± 1.430 | 62.29 ± 0.986 | 83.60 ± 1.006 |
| 30 | 58.66 ± 0.650 | 88.62 ± 1.089 | 90.74 ± 0.355 |
| 40 | 70.39 ± 0.934 | 93.55 ± 0.416 | * |
| 50 | 80.97 ± 2.135 | * | * |

**Where * not determined.**

**Table- 3**

| **Nitric Oxide Scavenging Activity Of Extract Andstandard** | |
|---|---|
| Concentration ($\mu g\ mL^{-1}$) | Percentage inhibition (mean ± SD) |
| 200 | 16.61 ± 3.036 |
| 400 | 38.76 ± 1.099 |
| 600 | 46.50 ± 1.929 |
| 800 | 58.55 ± 1.679 |
| 1000 | 78.05 ± 1.638 |
| Gallic acid (20 $\mu g\ mL^{-1}$) | |

**Where * not determined.**

## CONCLUSION

The results obtained in the present study reveals that the methanolic extract of the stembark of *Parkinsonia aculeata* L. can successfully scavenge various reactive oxygen species / free radicals under *in vitro* conditions. The presence of phenolics and flavonoids further suggest the possibility of its antioxidant activity. Further purification of the extract is required for the identification of active principles which may be valuable in a number of free radical mediated disease processes.

## REFERENCES

1. D. Prakash, A Niranjan, S.K. Tiwari and P. Pushpangandan, *Int. J. Food Sci. Nutri.*,2001, **52**, 337..
2. S. Jaiswal, A. Batra, S. Verma and M.M. Bokodia, *J. Sci. Ult.*,1984. **50**, 24..
3. A.C.R. Leite T.G. Arayo, B.M. Carvalho, N.H. Silva and M.B.S. Maia, *J. Ethnopharmacol.*, 2007,**111**, 547.
4. R.S. Gupta, H. Rehwani, V. Khusalami, K. Tanwar and .Y.C. Joshi, *J. Pharm. Biol.*, 2007,**45**, 1
5. M. Rani, S. Kumar and S.B. Kalidhar, *Indian J. Pharm. Sci.*,1999, **61**, 315.

6. W. Zheng and S.Y. Wang., *J. Agric. Food Chem.*, 2001, **49**, 5165.
7. N. Ito, S. Fukushima, A. Hasegawa, M. Shibata and T. Ogisa, *J. Nat. Cancer Inst.* 1983,**70**, 348
8. A.L. Miller, *Alt. Med. Rev.*,1996, **1**, 103.
9. G.K. Jayaprakash, L.J. Rao and K.K. Sakasiah, *Bioorg. Med. Chem.*, 2004,**12**, 5141.
10. M.S. Bilos, *Nature*, 1958,**26**, 1199.
11. L. Marcocci, L. Pocker, M.T. Dory-Lafaix, A. Sakaki and M. Gardes Albert, *Methods Enzymol.*, 1994,**234**, 462.
12. N. Singh and P.S. Rajini, *Food Chem.,2004,* **85**, 611.
13. G.R. Schinella, H.A. Tournier, J.M. Prieto, M. Morduyovich and J.L. Riso, *Life Sci.,2002,* **70**, 1023.

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