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Research Article

ANTIOXIDANT POTENTIAL AND FREE RADICALS SCAVENGING ACTIVITY BY POD EXTRACTS OF *ACACIA SENEGAL WILLD*

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ABSTRACT

Acacia Senegal Willd (Leguminosae), known as Arabic gum tree. Its mature pods with seeds were extracted using solvents of different polarities and explored for *in-vitro* free radical scavenging activity. Preliminary assays of both extracts of *Acacia Senegal* pod scavenges 1,1-diphenyl-2-picrylhydrazyl stable free radicals in concentration dependent manner. All studied extracts possess electron donating ability and reduce ferric ion to ferrous in a cell free system at pH-7.4. It has also been found from total antioxidant capacity as assessed by reduction of molybdate showed *Acacia Senegal* Pod-hydro alcoholic to possess when compared to standard ascorbic acid equivalents per milligrams of the extracts. Pod hydro alcoholic extracts were found more effective when compared to Pod water extract in scavenging 1,1-diphenyl-2 picrylhydrazyl, reducing ferric ion and molybdate reduction in antioxidant capacity. The significant correlations exist between extract concentrations and percentage scavenging activity of radicals in all models. Results clearly indicate that *Acacia Senegal Pod* is effective free radical scavenger and chain breaking antioxidant.

Keywords: Acacia Senegal, Arabic gum tree, Antioxidant, Free Radical Scavenging, Kher.

INTRODUCTION

Free radicals may be designated as molecular sharks that damage molecules in cell membranes, mitochondria, DNA and are very unstable, tends to rob electrons from the molecules in the immediate surroundings in order to replace their own losses[1]. Reactive oxygen species (ROS) is a collective term, which includes not only the oxygen radicals but also some non-radical derivatives of oxygen; these include hydrogen peroxide, hypochlorous acid and ozone[2]. Numerous disorders like rheumatoid arthritis, cardiovascular disorders, cystic fibrosis, metabolic disorders, neurodegenerative diseases and gastrointestinal ulcerogenesis are reported as ROS mediated[3-4]. The role played by ROS in stress induced gastric ulcer and inflammatory bowel diseases have been well established, as well as their involvement in the process of ageing [5-6]. Many synthetic antioxidant components have shown toxic and/or mutagenic effects. Hence attention has been given to naturally occurring antioxidants. Therefore, identification of effective antioxidants and free radical scavengers from plants origin is an ideal strategy for new drug development. Hence, present study was design to explore the antioxidant and free radical scavenging activity of *Acacia Senegal Willd* in a prospective way.

Acacia Senegal willd (Leguminosae) also known as Arabic gum tree. Locally it is called Kumbat in Sind[7], Rajasthan and Lasbella it is called Kher[8-9], distributed mainly in tropical and sub tropical region of southern part of West Pakistan and India in Jaipur and Jodhpur, the species grows to 2-15m tall with a flat or rounded crown[10]. *Acacia Senegal* (AS) Leaves are small, grey-green, alternate cream colored flowers occur on 2-12cm long spiks, Pods are dehiscent (open by splitting at maturity) and seeds greenish-brown[11]. The tree is highly valued for centuries for gum arabic production, which is used in food, Pharmaceuticals and other industries in the USA and Europe[12]. Gum Arabic is approved for use as food additives by the US Food and Drug Administration and in on the list of substances that are generally recognized as safe [13]. Other uses include soil fertility restoration by way of atmospheric nitrogen fixation, provision of wood for fuel, local construction and poles for fence posts[14]. Folk Medicine the Demulcent, emollient gum is used in inflammation of intestinal mucosa, and externally to cover inflamed surfaces, as burns and nodular Leprosy. Also said to be used for antitussive, astringent, cold coughs, diarrhea, dysentery, expectorant gonorrhea, sore throat and urinary track[15]. Various chemical studies on *Acacia Senegal* gum contains neutral sugars (rhamnose, arabinose and galactose), acids (glucuronic acid and 4-methoxyglucuronic acid) calcium, magnesium, potassium and sodium[16]. Phytoconstituents reported in the literature from plant AS (L.) Willd: Flavone, catechin, polyphenols, tannins, chalcones, alkaloids and flavonoids[17].

Various Pharmacological activities on *Acacia Senegal* gum exudates offers protection against cyclophosphamide induced urinary bladder cytotoxicity[18]. Scavenging of nitric oxide by gum arabic has been reported to limit the acetaminophen-induced hepatotoxicity in mice[19]. Other studies have documented the antioxidant properties of gum arabic in a variety of animal model system[20]. The bark of AS are made into a poultice to treat bedsores and wounds. The investigation was initiated to identify wound-healing plants and lesser known edible plant in Oman that have high antioxidant capacity[17]. In Present study we aimed to explore the antioxidant potential extracts of *Acacia Senegal* Pods. (hydro alcoholic and aqueous).

MATERIAL AND METHODS

Chemicals Details

1,1-diphenyl-2-picrylhydrazyl (DPPH), o-phenanthroline, ferric chloride, ascorbic acid, and ethylene diamine tetra acetic acid (EDTA) were procured from Haryana Scientific & Engg. Corp., Rohtak. Matured pods with seeds of Acacia Senegal Willd was purchased from Jodhpur local market, Rajasthan. (India), the plant material was authenticated by Dr. Ashok Kumar Sharma, M.D. (Dravyaguna Vigyan), Prof. & Head of Department Shri Baba Mastnath Ayurvedic Degree College, Asthal Bohar, Rohtak.

Extraction process

Acacia Senegal shed dried plant material (matured pods with seeds) were grinded and powdered material (100 g) was used for extraction. The hydro-alcoholic and aqueous extracts were prepared by hot continuous percolation method in a Soxhlet apparatus. Both extracts were collected separately and dried in vacuum system. Hydro-alcoholic extract with 70% ethanol (Pod-HA, yield: 12.45%) and Water extract with water (Pod-W, yield: 12.95%). Both extracts were condensed by re-distillation and dried in vacuum desiccators to obtain a final extract residue.

DPPH radical scavenging activity[21]

The free radical scavenging activity was measured in terms of hydrogen donating or radical scavenging ability by using stable free radical DPPH (100 µM) in ethanol. The equal volumes of different extracts were incubated with DPPH in graded concentrations of (10 to 500 µg/ml) for 20 min. at room temperature and by using a digital UV/VIS spectrophotometer (model 371E) absorbance was recorded at 517 nm. The experiment was

performed in triplicate. Scavenging ability was measured by decreased DPPH absorbance in test sample when compared to standard DPPH solution. Results were expressed as percentage inhibition of DPPH by comparing with blank.

Percentage scavenging of free radical by test compound:

$$\%\text{age Scavenging} = \frac{\text{Absorbance of control sample- Absorbance of test sample}}{\text{Absorbance of control sample}} \times 100$$

Reduction of ferric-ions[22]

The electron donating capability (reducing ability) was studied by ferric chloride reduction in cell free system. The reaction mixture contained 1.0 ml of phosphate buffer (pH 7.4), 100 µM ferric chloride and 0.5 ml of test compounds of different concentrations (10-500 µg/ml). After 3 min. of incubation, EDTA (100 µM) and ortho-phenanthroline (300 µM) were added, reaction was allowed for 10 min at room temperature and by using a digital UV/VIS spectrophotometer (model 371E) absorbance was recorded at 510 nm. Ascorbic acid was used as standard as equivalent to 100% reduction of ferric ions, comparative reduction of Fe^{3+} by *Acacia Senegal* extract was calculated.

Percentage reduction of Ferric ion by test compound:

$$\%\text{age Reduction} = \frac{\text{Absorbance of test sample}}{\text{Absorbance of ascorbic acid}} \times 100$$

Total antioxidant capacity[23] **[By ammonium molybdate reduction method]**

Total antioxidant capacity was measured in different concentrations of extract were mixed with 3ml of reagent solution (0.6Msulphuric acid, 28mMsodium phosphate and 4mM ammonium molybdate), after 90 minutes incubation at 95^0C for, sample cool to room temperature and using a digital UV/VIS spectrophotometer (model 371E) absorbance of molybdate (V) formed was measured at 695 nm. The antioxidant activity was expressed as the number of equivalents of ascorbic acid. Ascorbic acid was used as standard equivalent to 100% antioxidant capacity, comparative antioxidant capacity of Acacia Senegal pod extracts were calculated.

Percentage antioxidant capacity of test compound:

$$\text{Percentage antioxidant capacity} = \frac{\text{Absorbance of test sample}}{\text{Absorbance of ascorbic acid}} \times 100$$

RESULTS

DPPH radical scavenging activity

Pod extracts of *Acacia Senegal* scavenged the DPPH stable free radicals in a concentration dependent manner (10-500 µg/ml). Both extracts showed maximum scavenging activity at 500µg/ml, effect was observed with reduced scavenging activity. The pod-HA showed maximum activity (69.91%) and pod-W (53%). In both the cases effect was

observed at concentration 500 µg/ml. The experiment was performed in triplicate. The interaction of *Acacia senegal* pod-HA and pod-W. extracts with DPPH radicals presented in Tab. and Fig.1a.

Reduction of ferric ion

Reduction of ferric ion i.e. Fe^{2+}- Fe^{3+} couple is known to be involved in various free radical reactions. Both extracts of pod-HA and pod-W reduce Fe^{3+} in to Fe^{2+}at pH 7.4. In this study, reduction of Fe^{3+} into Fe^{2+} by ascorbate was taken as 100%. The pod-HA and pod-W (500 µg/ml) caused a significant reduction of Fe^{3+} to (66.75%) and (51.36%) respectively. The experiment was performed in triplicate shown in Tab. and Fig. 1b.

Total antioxidant capacity [By ammonium molybdate reduction method]

The reduction of ammonium molybdate by *Acacia Senegal* pod extracts were studied and ascorbic acid was taken as 100%. It has also been found from total antioxidant capacity as assessed by reduction of molybdate showed extract of Pod- HA (600 µg/ml) extrac showed a significant reduction of molybdate to (64.77%) and the pod-W (600 µg/ml) extract showed to(40.05%). The experiment was performed in triplicate shown in Tab. and Fig. 1c.

DISCUSSION

Free radicals have been a subject of significant interest among scientists in the past decade and their possible role in human diseases has gained importance now in days[24-25]. Antioxidants neutralize toxin and volatile free radicals that are defined as atoms or groups of atoms having an unpaired electron[6]. These also include related reactive oxygen species (ROS) that leads to free radical generation, causes the cascading chain reaction in biological system. In a normal, healthy organism or human body, the generation of pro-oxidants in the form of ROS is effectively kept in check by various levels of antioxidant defense. Antioxidants present in various dietary supplements offered their beneficial effects by neutralizing these ROS during various disease conditions. Lipids, proteins and DNA are all susceptible to attack by free radicals and cellular damage induced by oxidative stress has been implicated in the etiology of numerous diseases.

DPPH radical is widely used as a model to investigate the scavenging potential of several natural compounds such as phenolic and anthocyanins or crude extract of plants[26]. The ability of pod-HA and pod-W extracts of AS to reduce DPPH radicals, supports its free radical scavenging activity. Our study indicates the proton donating property may be responsible for free radical scavenging activity of AS Pod. Antioxidant compounds for example, sesamol, gallic acid poly-phenols reduce the Fe^{3+} to Fe^{2+} and are considered as chain breaking antioxidant for their proton donating activity[27]. AS Pod reduces ferric ion at pH 7.4 which indicates its proton donating ability and supports its free radical scavenging activity. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. It is also been found from total antioxidant capacity as assessed by reduction of molybdate showed Pod-HA to possess when compared to standard ascorbic acid. It was therefore to be expected that AS pod has potent antioxidant and radical scavenging ability. This activity is believed to be mainly due to their redox properties which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. Hence present study revealed antioxidant property of AS pod extracts.

Table 1a: Interaction of Acacia Senegal[a] pod extracts with DPPH Radical

Conc. (µg/mg)	DPPH Scavenging by pod-HA		DPPH Scavenging by pod-W	
	Absorbance	%Scavenging	Absorbance	%Scavenging
500	.204±.005	69.91	.322±.003	53
400	.260±.003	61.11	.352±.003	47.98
200	.342±.003	49.25	.386±.003	42.90
100	.403±004	40.46	.463±.006	31.50
50	.520±008	23.20	.566±.001	16.50
25	.599±002	13.03	.638±.008	5.90
10	.632±004	6.73	650±.003	4.11
Control	.678±002	00.00	.678±.002	00.00

[a] All values are mean (n = 3).
Data are expressed as Mean ± S.D.

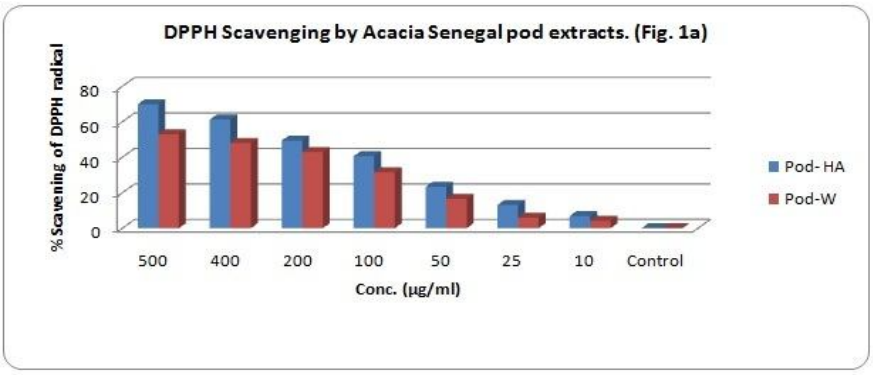

Fig. 1a : Interaction of Acacia Senegal pod extracts with DPPH Radical

Table 1b: Reduction of ferric ions by Acacia Senegal[a] pod extracts

Conc.(µg/ml)	Ferric ion reduction by pod-HA		Ferric ion reduction by pod- W	
	Absorbance	%Reduction	Absorbance	%Reduction
Ascorbic acid(300µM)	.406±.003	100	.405±.003	100
500	.271±.002	66.75	.208±.005	51.36
400	.226±.005	55.57	.190±.005	45.91
200	.208±.002	51.13	.173±.004	42.52
100	.156±.002	38.22	.122±.004	29.12
50	.088±.004	21.47	.058±.003	14.30
25	.052±.002	12.51	.021±.003	5.17
10	.018±.001	4.33	.006±.001	1.42

[a] All values are mean (n = 3).
Data are expressed as Mean ± S.D.

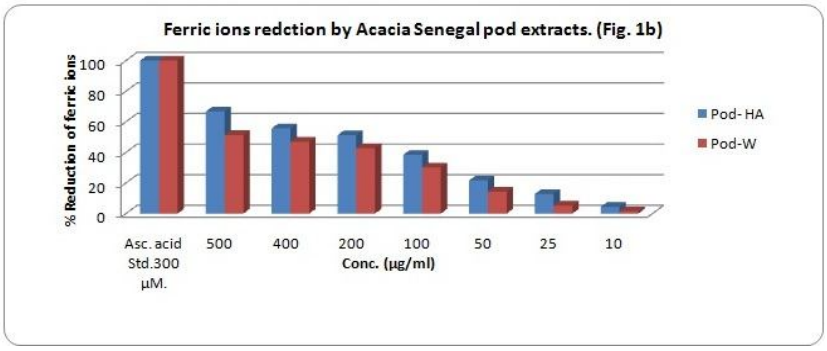

Fig. 1b: Reduction of ferric ions by Acacia Senegal pod extracts

Table 1c: Antioxidant capacity of Acacia Senegal[a] pod extracts

Conc.(µg/ml)	Antioxidant capacity of pod-HA		Antioxidant capacity of pod- W	
	Absorbance	% Antioxidant capacity	Absorbance	%Antioxidant capacity
Ascorbic acid (300µM)	.880±.006	100	.884±.004	100
600µg/.3ml	.570±.005	64.77	.354±.005	40.05
300µg/.3ml	.297±.002	33.55	.209±.005	23.44

[a] All values are mean (n = 3).
Data are expressed as Mean ± S.D.

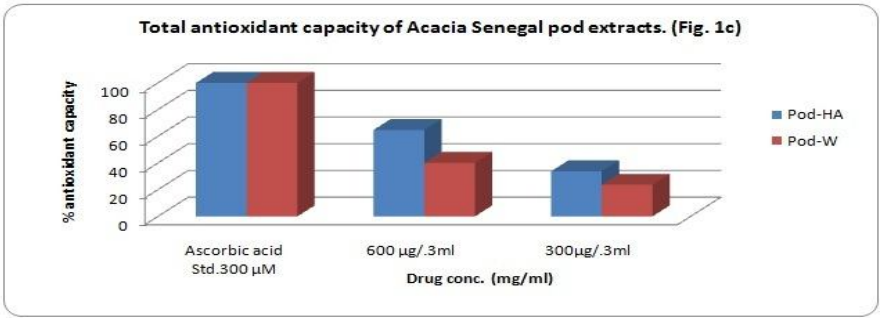

Fig. 1c: Antioxidant capacity of Acacia Senegal pod extracts

CONCLUSIONS

Present study showed that hydro alcoholic pod extract of AS is most effective antioxidant in various *in vitro* assay systems. AS has been reported to contain flavone, polyphenols, tannins, alkaloids and flavonoids[17], of these compounds, polyphenols, alkaloids and flavonoids are probably responsible for its free radical and reducing property observed in this study. As compared to previous reports, our study showed that *Acacia Senegal pod-HA* exerted a potent effect when compared to pod-W. Further studies are required to better understand these compounds and their effects on cellular function antioxidant properties of *Acacia Senegal* could be beneficial in pathological condition involving oxidative stress.

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