Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2015; 7(2); 311-314

ISSN: 0975-4873

Research Article

Free Radical Scavenging Activity of *Urginea indica*, *Alhagi maurorum*, *Crinum asiaticum* and *Prosopis cineraria*

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Available Online" 15th March, 2015

ABSTRACT
The free radical scavenging activity of methanol, chloroform and ethyl acetate extracts of Urginea indica, Alhagi maurorum, Crinum asiaticum and Prosopis cineraria were evaluated using 1,1-diphenyl-2-picryl- hydrazyl (DPPH) free radical. Ascorbic acid used as standard antioxidant. The results showed that the methanolic and ethyl acetate extract of Alhagi maurorum and chloroform extract of Urginea indica showed significant antioxidant activity and their radical scavenging activity approached the activity of standard.

Keywords: Urginea indica, Alhagi maurorum, Crinum asiaticum, Prosopis cineraria, DPPH, antioxidant activity.

INTRODUCTION

During oxidative stress reactive oxygen species (ROS), such as superoxide anion (O_2^-), hydroxyl ($HO^\cdot$), and peroxyl ($HOO^\cdot$, $ROO^\cdot$) radicals are produce which play important roles in de- generative or pathological processes, such as aging[1], cancer, coronary heart disease, Alzheimer's disease[2], neurodegenerative disorders, atherosclerosis, diabetes, and inflammation[3]. These ROS are formed by a electron reduction process of molecular oxygen (O_2). ROS can easily initiate the lipid peroxidation, causing damage of cell membrane of phospholipids and lipoproteins by propagating a chain reaction cycle[4]. Thus antioxidant defence system has evolved with aerobic metabolism to counteract oxidative damage from ROS. Most living species have efficient defence system to protect themselves against oxidative stress induced by ROS[5].

Natural antioxidants are the secondary metabolites of plants[6] includes carotenoids, flavonoids, cinnamic acids, benzoic acids, ascorbic acid, tocopherols etc. Several classes of antioxidants are present in various dietary supplements have been suggested for the health benefits. Consumption of these products leds to the reduction of various inflammatory and oxidative stress biomarkers[7,8,9]. The antioxidant effect of these compounds is connected with their chemical structure and their capability to delocalize electrons over the aromatic ring. When these compounds react with a free radical the captured electron is delocalized and stabilized by the resonance effect of the aromatic nucleus preventing free radical chain reactions. This is often called radical scavenging, but polyphenolic compounds inhibit oxidation by various mechanisms, mainly depending on the source material and possible presence of synergists or antagonists. Literature survey indicates that there is an inverse relationship between the dietary intake of antioxidant rich food and the incidence of human diseases[10,11]. Hence search for new synthetic and natural antioxidants is essentially importent. Although initial research on antioxidants was mostly on isolated pure compounds, recent focus is more on natural formulations[12,13].It has been found that compounds in their natural formulations are more active than their isolated forms[14]. In view of this, specific parts of *Urginea indica*, *Alhagi maurorum*, *Crinum asiaticum* and *Prosopis cineraria* were selected in the present study and their extracts were screened for antioxidant activity.

MATERIALS AND METHODS

Chemicals

1,1-Diphenyl-2-picryl-hydrazyl (DPPH) was obtained from Sigma Aldrich Co., St. Louis, USA. All other chemicals used were of analytical grade.

Preparation of crude plant extract

The plants selected in this study were collected locally and verified by Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India. Plant materials consisting of bulb of *Urginea indica*, aerial parts of *Alhagi maurorum*, bulb of *Crinum asiaticum* and stem bark of *Prosopis cineraria* were collected and dried. Shade dried plant material (100 gm) was powdered and extracted with chloroform, ethyl acetate and methanol separately. Each extraction step was completed in 48 hours. The extracts were filtered hot and solvent was removed under reduced pressure. The concentrated extracts were dried under vaccuo.

Two dimensional thin layer chromatography: 2 mg of each one of the twelve extract samples and one hesperidine,used as standard, were dissolved into 3 ml of methanol and after this they were spotted onto cellulose plates (20 × 20 cm^2 ,

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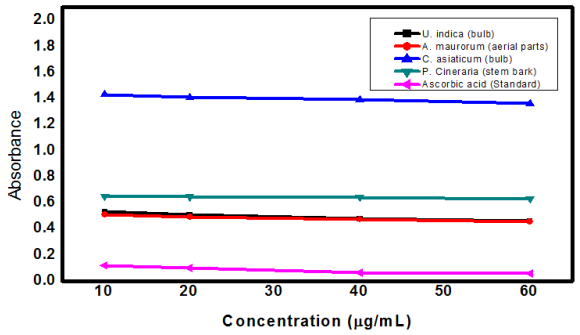

Fig. 1: DPPH free radical scavenging activity of standard ascorbic acid and methanolic plant extracts

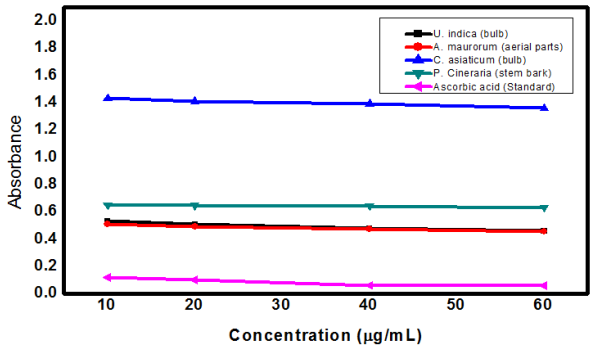

Fig. 2: DPPH free radical scavenging activity of standard ascorbic acid and ethyl acetate plant extracts

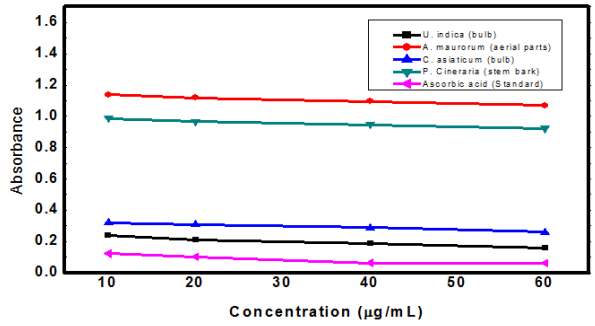

Fig. 3: DPPH free radical scavenging activity of standard ascorbic acid and chloroform plant extracts

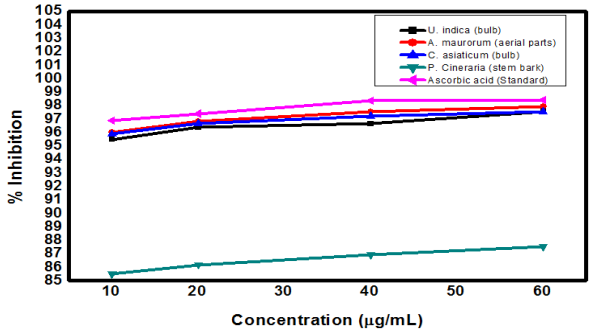

Fig.4: Comparative Antioxidant activity of selected plant methanolic extracts

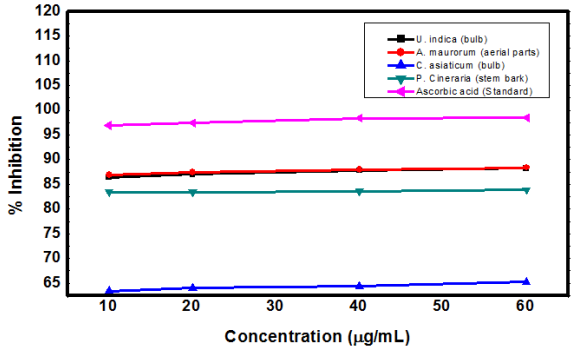

Fig.5: Comparative Antioxidant activity of selected plant ethyl acetate extracts

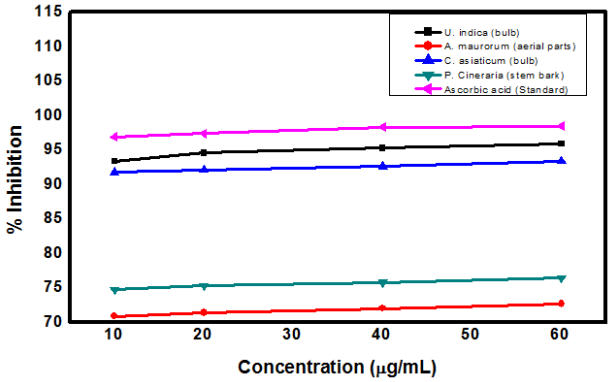

Fig. 6: Comparative Antioxidant activity of selected plant chloroform extracts

0.1 mm thick). The plates were developed in ethyl acetate: acetic acid: water (4:1:2) system in one direction and after drying, they were developed in the second direction using 15% acetic acid[15]. Spots were observed under uv light with and without the presence of ammonia fumes.

Free radicals scavenging activity: DPPH assay was carried out according to the method Naik et al[16]. A solution (2.5 ml) of $2X10^{-3}$ µg/ml of 1,1-diphenyl-2-picrylhdrazyl (DPPH) in methanol was mixed with equal volume of extract/test compound/ascorbic acid (standard) solution in methanol and kept in dark for 30 min. The absorbance at 517 nm was monitored at different concentration (10, 20, 40 and 60 µg/ml) at different time interval (minute) using UV-Vis spectrophotometer (Pharmaspec-1700 Shimadzu) at nm. Blank was also carried out to determine the absorbance of DPPH, before interacting with the extract. The percentage of inhibition of extract was calculated using the following eqavation.

$$\text{DPPH scavenging activity (\%)} = \frac{AB - AA}{AB} \times 100$$

Where AB = absorbance of DPPH in MeOH;
AA = Sample=absorbance of DPPH+ sample extract or standard in MeOH

RESULTS AND DISCUSSION

Two dimensional thin layer chromatography: TLC plates examined under UV with and without the presence of ammonia, showed that yellow, orange, violet and deep purple spots. According to mabry et al. (1970),deep purple or violet spots turning to yellow in the presence of NH_3 fumes indicate the presence of flavones with 5-OH and 4'-OH or 3-OH substituted flavonols and 5-OH and 4'-OH and some 5-OH flavanones and 4'- OH chalcones lacking B-ring hydroxyl groups, deep purple colour spot not changing in the presence of NH_3 fumes indicate the presence of 5-OH flavones or flavonols in the molecule in

Table 1 : Antioxidant activity of the selected plants by free radical scavenging activity

Plant Species	Test Extract	Conc.	Absorbance (µg/ml)	%inhibition	IC_{50} (µg/ml)
U. indica (bulb)	MeOH	10 µg/ml	0.176	95.50	22.61
		20 µg/ml	0.142	96.37	
		40 µg/ml	0.130	96.97	
		60 µg/ml	0.095	97.57	
	Ethyl Acetate	10 µg/ml	0.532	86.40	24.98
		20 µg/ml	0.509	86.99	
		40 µg/ml	0.479	87.75	
		60 µg/ml	0.460	88.24	
	Chloroform	10 µg/ml	0.239	93.38	23.00
		20 µg/ml	0.211	94.60	
		40 µg/ml	0.185	95.27	
		60 µg/ml	0.160	95.91	
A. Maurorum (aerial parts)	MeOH	10 µg/ml	0.155	96.03	22.48
		20 µg/ml	0.123	96.85	
		40 µg/ml	0.096	97.54	
		60 µg/ml	0.080	97.95	
	Ethyl Acetate	10 µg/ml	0.511	86.94	24.91
		20 µg/ml	0.490	87.47	
		40 µg/ml	0.471	87.96	
		60 µg/ml	0.455	88.37	
	Chloroform	10 µg/ml	1.139	70.89	30.39
		20 µg/ml	1.119	71.40	
		40 µg/ml	1.095	72.01	
		60 µg/ml	1.070	72.65	
C. asiaticum (bulb)	MeOH	10 µg/ml	0.161	95.88	22.55
		20 µg/ml	0.130	96.67	
		40 µg/ml	0.108	97.23	
		60 µg/ml	0.096	97.54	
	Ethyl Acetate	10 µg/ml	1.432	63.40	33.91
		20 µg/ml	1.409	63.99	
		40 µg/ml	1.392	64.42	
		60 µg/ml	1.361	65.21	
	Chloroform	10 µg/ml	0.322	91.77	23.61
		20 µg/ml	0.308	92.12	
		40 µg/ml	0.288	92.63	
		60 µg/ml	0.259	93.38	
P. Cineraria (stem bark)	MeOH	10 µg/ml	0.567	85.50	25.20
		20 µg/ml	0.540	86.19	
		40 µg/ml	0.511	86.94	
		60 µg/ml	0.487	87.55	
	Ethyl Acetate	10 µg/ml	0.649	83.41	26.20
		20 µg/ml	0.647	83.46	
		40 µg/ml	0.643	83.56	
		60 µg/ml	0.632	83.84	
	Chloroform	10 µg/ml	0.987	74.77	28.87
		20 µg/ml	0.966	75.31	
		40 µg/ml	0.947	75.79	
		60 µg/ml	0.923	76.41	
Ascorbic acid (Standard)		10 µg/ml	0.121	96.90	
		20 µg/ml	0.101	97.41	22.33
		40 µg/ml	0.064	98.36	
		60 µg/ml	0.061	98.94	
Blank		-	3.913		

which 4'-OH is absent or substituted. In most of the extracts examined by TLC and UV light, deep purple or violet colour appeared which indicate the presence of flavanones, flavones and flavonols. These compounds are

in the form of diglycosides as demonstrated by the R_f values in the two dimensional TLC using hesperidin as a standard.

Free radicals scavenging activity: Free radical scavenging activity expressed in terms of IC_{50}. Among the methanolic extracts of *Urginea indica*, *Alhagi maurorum*, *Crinum asiaticum* and *Prosopis cineraria*, the extract of *Alhagi maurorum* shows significant free radical scavenging activity (see Table 1, Fig. 1 and Fig. 4). In this case, the low absorption value at 10 µg/ml indicated its effectively even at a very low concentration. Also, IC_{50} was found to be equivalent to that of the reference compound, ascorbic acid (22.33 µg/ml) thus, indicating the high antioxidant potency the extract of aerial parts of *A. mauroum* (IC_{50} = 22.48 µg/ml).

Among the chloroform extracts of *Urginea indica*, *Alhagi maurorum*, *Crinum asiaticum* and *Prosopis cineraria*, the extract of *Urginea indica* shows significant free radical scavenging activity (see Table 1,Fig. 3 and Fig.6). In this case, the low absorption value at 10 µg/ml indicated its effectively even at a very low concentration. Also, IC_{50} was found to be equivalent to ascorbic acid (22.33 µg/ml) thus, indicating the high antioxidant potency the extract of *Urginea indica* (IC_{50} = 23.00 µg/ml).

The ethyl acetate extracts of *Urginea indica*, *Alhagi maurorum*, *Crinum asiaticum* and *Prosopis cineraria*, the extract of *Alhagi maurorum* shows best free radical scavenging activity (see Table 1,Fig. 2 and Fig.5). In this case, the low absorption value at 10 µg/ml indicated its effectively even at a very low concentration. Also, IC_{50} was found to be equivalent to ascorbic acid (22.33 µg/ml) thus, indicating the high antioxidant potency the extract of *Alhagi maurorum* (IC_{50} = 24.91 µg/ml).

CONCLUSION

Methanol, chloroform and ethyl acetate extracts of *Urginea indica*, *Alhagi maurorum*, *Crinum asiaticum* and *Prosopis cineraria* were examined by DPPH for their free radical scavenging activity. Results showed that the methanolic and ethyl acetate extract of *Alhagi maurorum* and chloroform extract of *Urginea indica* have significant radical scavenging activity approaching the activity of ascorbic acid as standard examined by the same tests.

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