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Antioxidant Activity of Some Medicinally Important Arid Zone Plants

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Abstract : Arid zone of Rajasthan has its own importance and specific characteristic with respect to endemic and a large number of plants of economic importance and medicinal use. Dichloromethane and methanolic extracts of twelve arid zone plants (*Aerva tomentosa* Forsk., *Gisekia pharnaceioides* L., *Heliotropium marifolium* Retz., *Lepidagathis trinervis* Nees., *Mimosa hamata* Willd., *Mollugo nudicaulis* Lam., *Polycarpea corymbosa* Lam., *Portulaca pilosa* L., *Sericostoma pauciflorum* Stocks. ex Wight., *Trianthema decandra* L., *Tribulus terrestris* L. and *Verbesina encelioides* (Cav.) Benth. & Hook. fil ex Gray), used in Indian phytotherapy for the treatment of inflammation, jaundice, urinary disorders and other kidney problems were screened *in vitro* for antioxidant activity by DPPH assay. All the methanolic extracts of the selected plant species exhibited appreciable activity as compared to the dichloromethane extracts, among these *A. tomentosa*, *H. marifolium*, *M. nudicaulis*, *P. corymbosa* and *M. hamata* exhibited higher antioxidant activity with 6.5 µg/ml RC_{50} value.

Key words : Arid zone plants, antioxidant activity, DPPH, % inhibition.

Introduction

Currently there has been an increased interest globally to identify antioxidant compounds that are pharmacologically potent and have low or no side effects for use in preventive medicine and food industry. The widespread use of traditional herbs and medicinal plants has been traced to the occurrence of natural products with medicinal properties. As plants produce significant amount of antioxidants to prevent the oxidative stress caused by photons and oxygen, they represent a potential source of new compounds with antioxidant activity (Aquil *et al.,* 2006). The role of plants in disease prevention and cure have been attributed, in part, to antioxidant properties of their constituents- liposoluble vitamin A and E, the water-soluble vitamin C and a wide range of amphipathic molecules, broadly termed phenolic compounds. The antioxidant activity of these compounds is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, singlet oxygen quenchers, and metal chelators (Rice-Evans *et al.,* 1997; Morel *et al.,* 1994; Ivanova *et al.,* 2005). Despite many studies on medicinal plants resources (Katewa *et al.,* 2001; Katewa *et al.,* 2003; Jain *et al.*, 2004, 2005), a large number of these plants and associated indigenous uses still require proper documentation and need to explore the usefulness of many of them for modern therapy. Arid zone of Rajasthan constitutes an apt example where medicinal plants are widely used in everyday life as part of folk medicinal remedies. However, a little is known about the antioxidant potential of arid zone plants (Aquil *et al.,* 2006). The aim of the present study is

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to establish the antioxidant capacity of some of the most popular arid zone medicinal plants, which are widely used in traditional system of medicine.

Materials and Methods

Plant materials

All plant materials were collected from different areas of Rajasthan, identified by one of the author Prof. S. C. Jain, their voucher specimens were prepared for authentication and are on deposit at the Herbarium, Department of Botany, University of Rajasthan, Jaipur, India.

Preparation of extracts

100 g different plant materials (whole plant, leaves and flowers) of selected plants were air-dried, powdered and successively extracted with dichloromethane and methanol (250 ml each) overnight at room temperature seperately. The resultant extracts were filtered and the plant residues were re-extracted (3X) and the combined extracts were evaporated to dryness at 35ºC *in vacuo*, and used for the antioxidant activity.

Antioxidant potentialities

2, 2- Diphenyl-1- picrylhydrazyl (DPPH) and quercetin were obtained from Hi media, India. The method used by Fogliano et al. (1999) was adopted with suitable modifications to our particular circumstances. Methanolic solution of DPPH (20 mg/ 10 ml) was used.

For qualitative assay, different extracts and quercetin as standard (20 mg) were dissolved in 1 ml methanol, out of which 1 il was applied on TLC plates (Silica G; 20x20 cm). Later, these plates were sprayed with DPPH (20 mg/10 ml) and exposed to daylight until discoloring of the background (6 hr). The resulting yellow colour on the plates was determined as active antioxidant constituents. This method was also used for positive and negative controls. For quantitative assay, each of the extract (8 mg) was dissolved separately in 10 ml of methanol and various concentrations (80, 60, 40, 20 and 10 µg) were prepared. Each 2.5 ml of test extract was mixed with DPPH (20 mg/ 10 ml) and allowed 30 minutes for any reaction to occur. The absorbance of the colour developed was measured at 517 nm by UV spectrophotometer (Varian type Cary PCB 150 Water Peltier System with Standard Cuvettes). The negative control and standard quercetin as positive control was subjected to the same procedure. Three replicates were used and the average absorption was noted for each concentration. Data was processed using EXCEL and concentration, that cause 50% reduction in absorbance (RC_{50}), was calculated. Percent inhibition of DPPH was calculated by following equation (Lee *et al.,* 1998):

$$\% \text{ Inhibition} = 1 - (A_1/A_2) \times 100$$

where, A_1 is the absorbance of the test sample and A_2 as the absorbance of control reaction.

Results and Discussion

The ethnobotanical data of twelve medicinally important arid zone plant species which includes botanical name, family, common name, part used, key ailments, mode of administration, key constituents, status and habit are summarized in Table 1. DPPH is a stable free radical and often used to evaluate the antioxidant activity of several natural compounds (Fogliano *et al.,* 1999; Naik *et al.,* 2003). Antioxidants on interaction with DPPH, either transfer electron or hydrogen atom to DPPH, thereby neutralizing its free radical character. DPPH shows strong absorption at 517 nm.

Extracts of *A. tomentosa, G. pharnaceioides, H. marifolium, L. trinervis, M. hamata, M. nudicaulis, P. pilosa, P. corymbosa, S. pauciflorum, T. decandra, T. terrestris* and *V. encelioides* showed

Table 1 : Ethnobotanical data of some medicinally important arid zone plants

	Botanical name	Family	Common name	Herbarium no.	Plant part	Key ailments	Mode of administration	Key constituents
1	Aerva tomentosa Forsk.	Amaranthaceae	Magbira	10	Wp	Skin infection, inflammation, abdominal warms	Plant extract for remove swelling	α-Amyrin, chrysin
2	[a]*Gisekia pharnaceioides* L.	Aizoaceae	Balu ka sag	64	Wp	Female diseases, defective semen, destroys fat, malfunctioning of sex organs	Ashes of whole plant used to cure eczema	Myristone, triacontane, tetracosanol
3	*Heliotropium marifolium* Retz.	Boraginaceae	Chota-santari	69	Wp	Emetic, snake bite, ulcer.	Plant extract for killing roundworms	Heliotrine, europine, indicine
4	Lepidagathis trinervis Nees.	Acanthaceae	-	79	Wp	Tonic, fever	Leaves applied to boils to draw out pus	-
5	Mimosa hamata Willd.	Mimosaceae	Jinjani	130	Wp	Tonic, in urinary complaints, glandular swellings, blood-purifier	Paste of leaves is applied over glandular swellings and is used	Gallic acid, mimonoside A-C, 4-ethylgallic acid
6	Mollugo nudicaulis Lam.	Ficoidaceae	-	87	Lvs, Wp	Used in athrepy and whooping cough	Pounded leaves used cold or warm as poultice over boils and inflammatory swellings; used for bites from animals and given with molasses in form of a pill in jaundice	Cyanogenic glycosides, terpenes, saponins
7	Polycarpea corymbosa Lam.	Caryophyllaceae	-	99	Lvs, Wp	As remedy for venomous bites from reptiles, jaundice	-	α -1-Barrigenol, stigmasterol, camelliagenin
8	*Portulaca grandiflora* Hook.	Portulacaceae	Bichuni	131	Lvs, St	Depurative, Hepatitis, swelling and pain in the pharynx, cirrhosis of the liver with ascites	The fresh juice of the leaves and stems is applied externally as a lotion to snake and insect bites, burns, scalds and eczema	Betalains, portulacaxanthin II, III

9	Sericostoma pauciflorum Stocks. ex Wight.	Boraginaceae	Karvas	110	Lvs	Dehydration, acidity	Roots ground up with milk and given internally considered specific in orchitis; juice of leaves dropped into the nostrils to relieve one-sided headache	α-, β- Amyrin, sericostinyl acetate, triterpenes
10	Trianthema decandra L.	Ficoidaceae	Gadabani	132	Rt, Rt bark, Lvs	Hepatitis, asthama, and suppression of the menses, inflammation of testicles	-	-
11	*Tribulus terrestris* L.	Zygophyllaceae	Gokhru	119	Ft, Lvs, Wp	Cooling, diuretic, tonic, aphrodisiac, appetite, carminative, Impotence, painful micturition, calculus affection, urinary discharges, kidney disease and gravel, kidney stone, wound, rheumatism, leucorrhoea	Juice of lvs. with *Cassia obtuse* , *Glinus lotoides* , mixed with rice water (neeragaram) consumed for reduce body heat and give cooling effect; mucilaginous water extract of the plant is taken as a remedy for impotency	Sapogenin, diosgenin, harmine, saponins, tannins
12	Verbesina encelioides (Cav) Benth. & Hook. Fil ex Gray	Asteraceae	Nakli-surajmukhi	126	Wp	Analgesic, febrifuge, emetic, insecticide, antiinflammatory, gum sores, hemorrhoid, spider-bite	Herb infusion for reduce swellings	Galegine, n-tricontane, taraxesterol, phytol

[a]Endangered plant species
Abberiviations: Wp = Whole plant, Lvs = Leaves, St = Stem, Rt = Root

Table 2 : Antioxidant activity of selected arid zone medicinal plants

Plant name	Part used	Yield (%)		RC50 (µg/ml)		% Inhibition of DPPH at µg/ml concentrations									
						DCM					MeOH				
		DCM	MeOH	DCM	MeOH	10	20	40	60	80	10	20	40	60	80
1. *A. tomentosa*	Fl	0.66	1	19.5	7	46.5	50.22	60.17	62.22	75.42	70.82	75.02	87.32	88.9	96.5
2. *G. pharnaceioides*	Wp	0.13	9.93	15	7.5	28.97	68.82	71.2	73.57	76.6	69.42	71.7	77.87	83.3	83.45
3. *H. marifolium*	Fl	1.1	3.55	8.5	6.5	56.77	60.02	65.57	68.97	93.97	72.97	76.2	91.62	92.05	92.37
4. *L. trinervis*	Wp	7.54	57.49	20	8	44.92	47.82	64.22	65.65	81.5	63.02	75.6	95.47	95.85	96.17
5. *M. hamata*	Lf	1.77	2.59	9	6.5	55.9	64.3	66.4	71.2	72.2	81.5	82.2	84.42	85.52	88.32
6. *M. nudicaulis*	Wp	3.79	0.33	15.5	6.5	45.92	53	54.07	56.35	67.1	77.97	79.72	85.5	86.42	90.82
7. *P. corymbosa*	Wp	0.46	2.62	7.5	6.5	67.67	78.62	90	92.35	95.42	83	85.37	90.32	90.92	94.4
8. *P. grandiflora*	Fl	18.63	3.18	10	8	51.1	56.12	58.12	59.42	62.05	61.6	63.45	66.6	68	80.8
9. *S. pauciflorum*	Wp	0.37	1.71	60	14.5	37.65	41.67	46.5	49.8	65.47	44	56.12	81.97	85.45	94.4
10. *T. decandra*	Wp	0.86	4.14	69	9	40.57	41.92	44.12	46.72	54.05	57.07	65.82	71.2	78.45	90.65
11. *T. terrestris*	Fl	4.4	2.9	8	7.5	60.6	63.5	66.07	67.87	80.17	67.22	71.52	73.57	76.75	82.87
12. *V. encelioides*	Fl	4.51	3.03	60	7.5	43.92	44.07	47.05	48.5	56.47	66.4	69.65	75.65	93.67	94.32

Abbreviations: Fl= Flower, Wp= Whole plant, Lvs= Leaves,
DCM= Dichloromethane, MeOH= Methanol,
RC50 = Concentration of the extract (µg/ml) at which the absorbance (at 517 nm) decreases to half of its initial value

appreciable antioxidant activity and % inhibition of DPPH where RC_{50} (µg/ml) ranged from 6.5-69 (Table 2). Dichloromethane extract of *P. corymbosa* (RC_{50} 7.5 ìg/ml) and *T. terrestris* (RC_{50} 8 ìg/ml) were highly active in antioxidant activity. It is noteworthy that the maximum activity in methanol extracts was exhibited by *H. marifolium*, *M. nudicaulis*, *P. corymbosa* and *M. hamata,* (RC_{50} 6.5 µg/ml). % Inhibition of DPPH was highest (96.50 %) in methanol extract of *A. tomentosa* that is followed by (96.17 %) methanol extract of *L. trinervis* at 80 µg/ml concentration

This ethnomedicobotanical study on the arid zone medicinal plant species has revealed the enormous diversity in the region and the popular use of these plants by the local tribals for a wide range of common ailments like round worms, fever, cough, asthma etc. A comparison of the present information, with earlier records on Indian medicinal plants uses (Aquil *et al.,* 2006; Kirtikar and Basu, 1933; Chopra *et al.,* 1956; Jain, 1991; Asolkar *et al.,* 1992) revealed the antioxidant potentials of the traditional plants selected in the study. The majority of plants listed in this paper are known to contain various active principles of therapeutic value and biological activity against a number of diseases. Many of these plants have folk medicinal claims but lack phytochemical and pharmacognostical information, *e.g., G. pharnaceioides, M. nudicaulis, S. pauciflorum* and *T. decandra* etc. of the present study which needs to be investigated for their bioactive phytochemicals and thus, constitute promising materials for future research in phytomedicine.

Many assay methods for antioxidant activity *in vitro* and *in vivo* have been developed, but only a few rapid and reliable methods applicable to antioxidant activity assay for a huge number of plant extract sample exist (Miller *et al.,* 1993; Aruma and Cupett, 1994). Total antioxidant capacity assay, such as DPPH method is most common for antioxidant activity for large-scale examination. The improved DPPH method described by Fogliano *et al.,* (1999) was successfully used in this study to systematically assess the total antioxidant capacity of the medicinal herb extracts on a large scale, being simple, fast, reliable, inexpensive, and also very adaptable to both hydrophilic and lipophilic antioxidants/systems. This efficient and effective method can be used for systematic screening of medicinal herbs and dietary plants for their relative antioxidant content. Several studies have revealed that intake of natural antioxidants is correlated with low incidence of cancer, heart diseases, diabetes, and other diseases associated with ageing (Hertog *et al.,* 1995; Cai *et al.,* 2004). Therefore, arid zone plants can be considered to be a rich source of antioxidants.

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References

Aqil F., Ahmad I. and Mehmood Z. (2006): Antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants, *Turk. J. Biol.*, **30**, 177-183.

Aruoma O.I. and Cuppett S.L. (1994): Antioxidant Methodology: *In Vivo* and *In Vitro* Concepts. AOCS Press: Illinois.

Asolkar L.V., Kakkar K.K. and Chakre O.I. (1992): Glossary of Indian Medicinal Plants with Active Principles. Council of Scientific and Industrial Research: New Delhi, India.

Cai Y., Qiong L., Mei S. and Harold C. (2004): Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer, *Life Sci.,* **74**, 2157-2184.

Chopra R.N., Nayar S.L. and Chopra I.C. (1956): Glossary of Indian Medicinal Plants. Council

of Scientific and Industrial Research: New Delhi, India.

Fogliano V., Verde V., Randzzo G. and Ritieni X. (1999): A method for measuring antioxidant activity and its application to monitoring the antioxidant capacity of wines, *J. Agric. Food Chem.,* **47**, 1035-1040.

Hertog M.G.L., Kromhout D., Aravanis C., Blackburn H., Buzina R., Fidanza F., Giampaoli S., Jansen A., Menotti A., Nedeljkovic S., Pekkarinen M., Simic B.S., Toshima H., Feskens E.J.M., Hollman P.C.H. and Katan M.B. (1995). Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study, *Arch. Internal Med*., **155**, 281-286.

Ivanova D., Gerova D., Chervenkov T., and Yankova T. (2005): Polyphenols and antioxidant capacity of Bulgarian medicinal plants. *J. Ethnopharmacol.,* **96**, 145-150.

Jain A., Katewa S.S., Choudhary B.L. and Galav P.K. (2004): Folk herbal medicines used in birth control and sexual diseases by tribals of Southern Rajasthan, India, *J. Ethnopharmacol.,* **90**, 171-177.

Jain A., Katewa S.S., Galav P.K. and Sharma P. (2005): Medicinal plant diversity of Sitamata wildlife sanctuary, Rajasthan, India, *J. Ethnopharmacol.,* **102**, 143-157.

Jain S.K. (1991): Dictionary of Indian Folk Medicine and Ethnobotany. Deep Publications: New Delhi, India.

Katewa S.S., Choudhary B.L., Jain A. and Galav P.K. (2003): Traditional uses of plant biodiversity from Aravalli hills of Rajasthan, *Indian J. Trad. Knowl.,* **2**, 27-39.

Katewa S.S., Guria B.D. and Jain A. (2001): Ethnomedicinal and obnoxious grasses of Rajasthan, India, *J. Ethnopharmacol.,* **76,** 293-297.

Kirtikar K.R. and Basu B.D. (1933): Indian Medicinal Plants. Vol. 1, Lalit Mohan Basu: Allahabad, India.

Lee S.K, Mbwambo Z.H., Chung H.S., Luyengi L., Games E.J.C. and Mehta R.G. (1998): Evaluation of the antioxidant potential of natural products, *Com. Chem. High T. Scr.,* **1**, 35-46.

Miller N.J., Rice-Evans C.A., Davies M.J., Gopinathan V. and Milner A. (1993): A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates, *Clinical Sci.,* **84**, 407-412.

Morel I., Lescoat G., Cillard P. and Cillard J. (1994): Role of flavonoids and iron chelation in antioxidant action, *Methods Enzymol.,* **234**, 437-443.

Naik G.H., Priyadarsini K.I., Satav J.G., Banavalikar R.P., Sohoni D.P., Biyani M.K. and Mohan H. (2003): Comparative antioxidant activity of individual herbal components used in Ayurvedic medicine, *Phytochem.,* **63**, 97-104.

Rice-Evans C.A., Miller N.J. and Paganga G. (1997): Antioxidant properties of phenolic compounds, *Trends Plant Sci.,* **2**, 152-159.