Available online at www.ijpsdr.com
International Journal of Pharmaceutical Sciences and Drug Research 2011; 3(1): 48-51

Research Article ISSN 0975-248X

Comparison of the Antioxidant Capacity of an Important Hepatoprotective Plants

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ABSTRACT
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 the food industry. 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. Commonly used medicinal plant extracts with standardized content of polyphenols were investigated for their antioxidant activity (AA). In the present paper ten plants (*Picrorrhiza kurroa, Tephrosia purpurea, Terminalia arjuna, Tinospora cordifolia, Glycyrrhiza glabra, Azadirachta indica, Apium graveolens, Swertia chirata, Phyllanthus amarus, and Aloe vera*) and their possible constituents responsible for its antioxidant property were compared by reducing power, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity method. The plants described contain antioxidant principles that can explain and justify their use in traditional medicine (Hepatoprotective) in the past as well as the present. These results suggest that AA determination is of interest for a comparative evaluation of in vitro antioxidant potential, but it needs to be combined with in vivo data for adequate assessment of the antioxidant capacity of medicinal plant extracts.

Keywords: Antioxidant activity; reducing powers; DPPH; Hepatoprotective.

INTRODUCTION

Nowadays, the fact of harmful effect of reactive oxygen species on human health is well-known. The capability of natural defense systems of living organisms against excess production of these species decreases when influenced with negative environmental factors or aging. [1] The traditional medicine all over the world is nowadays revalued by an extensive activity of research on different plant species and their therapeutic principles. As plants produce a lot of antioxidants to control the oxidative stress caused by sunbeams and oxygen, they can represent a source of new compounds with antioxidant activity. [2]

Reactive oxygen species (ROS) formed *in vivo*, such as superoxide anion, hydroxyl radical and hydrogen peroxide, are highly reactive and potentially damaging transient chemical species. These are continuously produced in the human body, as they are essential for energy supply, detoxification, chemical signaling and immune function. ROS are regulated by endogenous superoxide dismutase, glutathione peroxidase and catalase but due to over-production of reactive species, induced by exposure to external oxidant substances or a failure in the defense mechanisms, damage to cell structures, DNA, lipids and proteins [3] occur which increases risk of more than 30 different disease processes. [4] The most notorious among them being neurodegenerative conditions like Alzheimer's disease [5-6], mild cognitive impairment (MCI) and Parkinson's disease (PD). Other neurodegenerative diseases significantly associated with oxidative stress include multiple sclerosis, Creutzfeldt–Jacob disease and meningoencephalitis.

Other diseases include highly disabling vascular pathologies like cardiovascular disease (CVD) and cardiac failure [7], alcohol-induced liver disease (ALD) [8] and ulcerative colitis and cancer caused by a complex of different causes, of which RNS/ROS is a component.

In Ayurveda, an ancient Indian form of medicine that deals with plants and plant extracts, several herbal formulations have been described for liver disorders. [9] The role of free radicals in hepatic injury has been recognized. In the present study attempt is made to evaluate antioxidant property of few hepatoprotective drugs.

MATERIAL AND METHODS:

Plant material

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The drugs were collected from Penta Care Ayur Pharma (Bangalore) and authenticated Dr Niranjan, CEO of Penta Care Ayur Pharma, Bangalore. Prior to use, it was insured that the all drugs were free from contamination, sand and had no microbial growth.

Preparation of Extracts

50 g of the shredded drugs powder was macerated with distilled water for four days in sterilized sample bottles. The solution was filtered and the filtrate obtained was used immediately for analyses of antioxidant capacity.

Antioxidant activity

DPPH radical scavenging activity [10-11]

The free radical scavenging activity of the extracts, based on the scavenging activity of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical was determined by the method described by Braca *et al*, (2001). Plant extract (0.1 ml) was added to 3 ml of a 0.004% methanol solution of DPPH. Absorbance at 517 nm was determined after 30 min, and the percentage inhibition activity was calculated from [(A0–A1)/A0] × 100, where A0 is the absorbance of the control, and A1 is the absorbance of the extract/ standard. A blank is the absorbance of the control reaction (containing all reagents except the test compound). The % scavenging activity and EC_{50} value of extracts of 10 different drugs were calculated for the various concentrations and compared with standard Gallic acid.

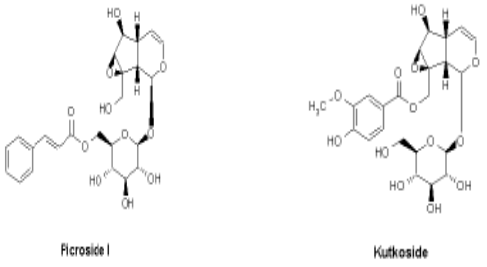
Reducing Power method [12]

Extracts of 10 different drugs (5 µg, 10 µg, 15 µg, and 20 µg) in 1 ml of appropriate solvents were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide [$K_3Fe(CN)_6$] (1%), and then the mixture was incubated at 50°C for 30 min. Afterward, 2.5 ml of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Finally, 2.5 ml of the upper layer solution was mixed with 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ (0.1%), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicates increased reducing power.

Active constituents responsible for both Hepatoprotective and anti oxidant activity:

1. *Picrorrhiza kurroa* [13]

It is a small perennial herb from the Scrophulariaceae family. The rhizome of Picrorrhiza has been traditionally used to treat worms, constipation, low fever, scorpion sting, asthma and ailments affecting the liver. Kutkin, a bitter glycosidal principle a stable mixed crystal of two C-9 iridoid glycosides-Picroside I and Kutakoside which is isolated and shown to have Hepatoprotective activity



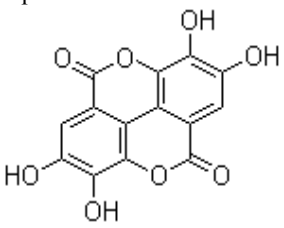
Kutkin (Kutakoside and Picrosides)

2. *Tephrosia purpurea* [14]

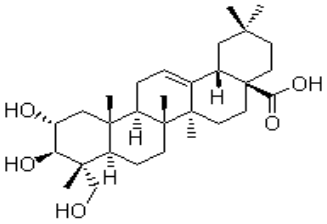
Tephrosia purpurea Pers. is a pan tropical coastal shrub that grows up to 1 m in height. It occurs throughout the Indian subcontinent. Previous phytochemical investigations on this plant have shown the presence of coumarins, flavonoids and rotenoids, flavanones and isoflavanones and quercetin.

3. *Terminalia Arjuna* [15]

The bark of *Terminalia arjuna* (family *Combretaceae*) is one such Ayurvedic remedy that has been mentioned in many ancient Indian medicinal Ellalic acid,Arjunolic acid, a new triterpene isolated from the bark of T. Arjuna have been reported to have antioxidant.



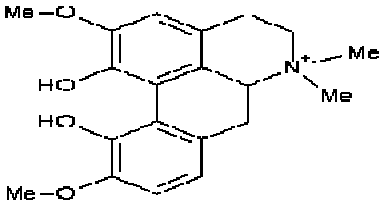
Ellalic acid



Arjunolic acid

4. *Tinospora cordifolia* [16]

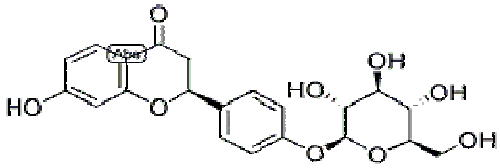
Tinospora cordifolia (Wild) Miers (*Menispermaceae*) is a common climbing shrub, found throughout India in forests. It is one of the most valuable Traditional Indian medicinal herbs and has been used in Ayurvedic preparations for the treatment of various ailments throughout the centuries.



Magnoflorine

5. *Glycyrrhiza glabra* [17]

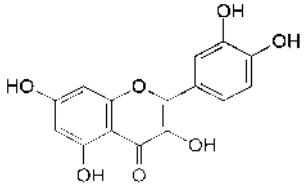
Glycyrrhiza glabra Linn. of the family *Fabaceae* is a tall Perennial under shrub. Its underground stems and roots are used medicinally.



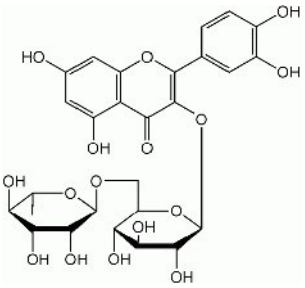
Liquiritin

6. Azadirachta indica [18]

Azadirachta indica is well known for its wide range of therapeutic uses. Different parts of the neem tree have been used as traditional ayurvedic medicine in India.



Quercetin

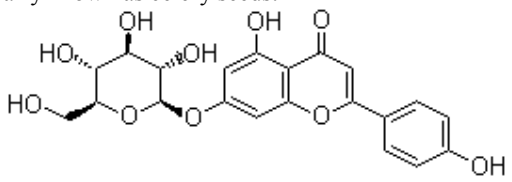


Rutin

7. *Apium graveolens* [19]

Apium graveolens (*Apiaceae*) is one of the most well known plants used in the history of mankind as a medicament or a

spice. It is commonly known as 'Ajmod' and the fruits are popularly known as celery seeds.



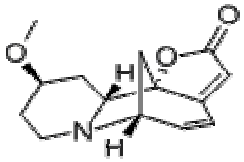
Apigenin 7-glucoside

8. *Swertia chirata* [20]

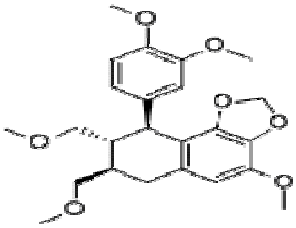
The genus Swertia (Gentianaceae) is an annual or perennial herb comprising more than 170 species. 1,5-dihydroxy-3,8-dimethoxy xanthone (chiratol).

9. *Phyllanthus amarus* [21]

Phyllanthus amarus Schum. et Thonn. (Bhuia amla) is a medicinal herb used in connection with secondary hepatitis and other ailments, in ayurvedic medicine for over 2000 years.



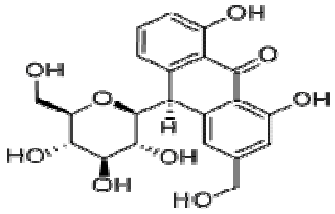
Phyllanthin



Hypo Phyllanthin

10. Aloe vera [22]

Aloe barbadensis Mill. Syn. Aloe vera Tourn. Ex Linn. (Liliaceous) has been used in variety of diseases in traditional Indian system of medicine in India and its use for hepatic ailments is also documented.



Barbaloin

Statistical Analysis: Each datum represents the mean of three different experiments in each of which two measurements were made. Values of $P < 0.05$ were considered to be significant and values of $P < 0.01$ very significant.

RESULTS AND DISCUSSION

Antioxidant activity of extracts

Extracts of 10 different drugs (*Picrorrhiza kurroa, Tephrosia purpurea, Terminalia Arjuna, Tinospora cordifolia, Glycyrrhiza glabra, Azadirachta indica, Apium graveolens, Swertia chirata, Phyllanthus amarus, and Aloe vera*) were subjected to screening for their possible antioxidant activity. Two complementary test systems, namely DPPH free radical-scavenging and reducing power, were used for the analysis. DPPH, a stable free radical with a characteristic absorption at 517 nm, was used to study the radical scavenging effects of extracts. As antioxidants donate protons to these radicals, the absorbance decreases. The decrease in absorbance is taken as a measure of the extent of radical scavenging. Free radical-scavenging capacities of the extracts and standard (Gallic acid), measured by DPPH assay, are shown in Fig. 1.

It was observed, that in line with the increase seen in the amount of *Azadirachta indica Picrorrhiza kurroa*, *Phyllanthus amarus, Terminalia Arjuna* and standard, an increase in DPPH free radical-scavenging occurred. Inhibition values in the concentrations of 50, 100,150, 200, 250µg/ml were, respectively given in Table 1and 2.

Reducing Power

The reducing power of all 10 different drug extracts was increased as the amount of extract increased (Table 3 and Fig. 2). This difference was statistically significant, $P < 0.05$.

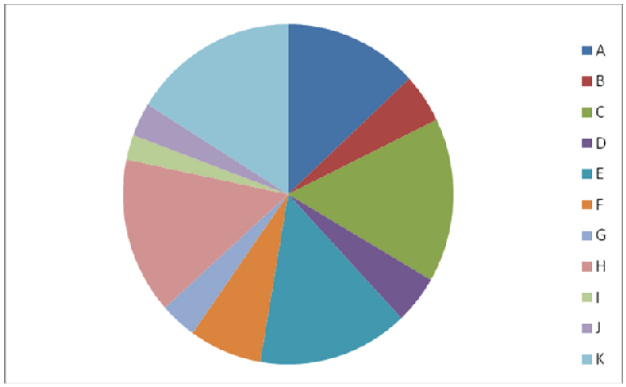


Fig. 1: Free radical-scavenging capacities of the extract A-*Picrorrhiza kurroa*, B-*Tephrosia purpurea*, C-*Terminalia Arjuna*, D-*Glycyrrhiza glabra*, E- *Azadirachta indica*, F- *Apium graveolens*, G- *Swertia chirata*, H- *Phyllanthus amarus*, I- *Tinospora cordifolia*, J-*Aloe Vera*,K-std.

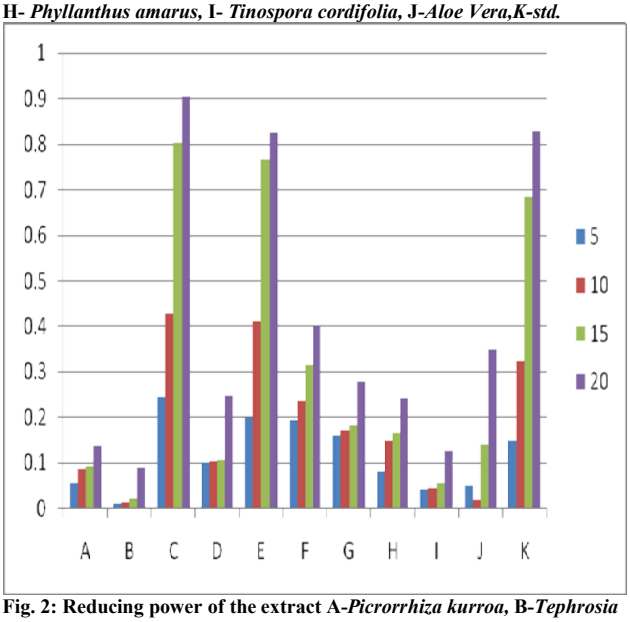


Fig. 2: Reducing power of the extract A-*Picrorrhiza kurroa*, B-*Tephrosia purpurea*, C-*Terminalia Arjuna*, D-*Glycyrrhiza glabra*, E- *Azadirachta indica*, F- *Apium graveolens*, G- *Swertia chirata*, H- *Phyllanthus amarus*, I- *Tinospora cordifolia*, J-*Aloe Vera*,*K-std* measured absorbance at 700 nm

The study clearly indicates that the extract of *Picrorrhiza kurroa, Tephrosia purpurea, Terminalia Arjuna, Tinospora cordifolia, Glycyrrhiza glabra, Azadirachta indica, Apium graveolens, Swertia chirata, Phyllanthus amarus, and Aloe vera* possesses antioxidant. Thus, this investigation is the first report on the comparative analysis of the antioxidant properties of 10 important Hepatoprotective drug. These findings justify the traditional uses of these plants. Further research is necessary for elucidating the active principles.

Table 1: % scavenging activity of different extract in DDPH assay method

S. No	Conc. µg/ml	% scavenging activity										
		A	B	C	D	E	F	G	H	I	J	STD
1.	50	35.14	3.32	87.46	4.97	77.70	2.84	6.09	30.28	1.89	6.24	94.46
2.	100	73.50	13.50	91.45	15.76	82.80	23.12	33.44	87.73	12.04	10.8	94.94
3.	150	77.41	27.64	92.20	27.15	86.93	41.97	21.09	88.00	14.00	18.97	95.32
4.	200	93.41	40.53	93.19	43.59	93.24	52.56	59.80	92.61	14.76	30.28	95.99

Extracts of: A-*Picrorrhiza kurroa,* B-*Tephrosia purpurea,* C-*Terminalia Arjuna,* D-*Glycyrrhiza glabra,* E- *Azadirachta indica,* F- *Apium graveolens,* G- *Swertia chirata,* H- *Phyllanthus amarus,* I- *Tinospora cordifolia,* J-*Aloe Vera*

Table 2: EC_{50} values (µg/ml) of different extract in DDPH assay method

S. No	Extract	EC_{50} (µg/ml)	S. No	Extract	EC_{50} (µg/ml)
1.	*Picrorrhiza kurroa*	85.77	7.	*Swertia chirata*	315.83
2.	*Tephrosia purpurea*	410.36	8.	*Phyllanthus amarus*	83.18
3.	*Terminalia Arjuna*	67.97	9.	*Tinospora cordifolia*	1609.91
4.	*Glycyrrhiza glabra*	331.47	10.	*Aloe Vera*	397.30
5.	*Azadirachta indica*	71.51	11.	Blank	-
6.	*Apium graveolens*	366.37	12.	Standard	65.56

Table 3: Reducing powers activity of various extract

S. No	Conc. µg/ml	Absorbance in 700nm										
		A	B	C	D	E	F	G	H	I	J	STD
1.	5	0.0553	0.0121	0.244	0.101	0.199	0.195	0.160	0.082	0.042	0.050	0.150
2.	10	0.0880	0.0141	0.428	0.103	0.410	0.238	0.173	0.149	0.046	0.021	0.324
3.	15	0.0930	0.0212	0.803	0.108	0.767	0.316	0.182	0.167	0.056	0.140	0.685
4.	20	0.1382	0.0909	0.904	0.248	0.825	0.400	0.278	0.242	0.128	0.349	0.827

Extracts of: A-*Picrorrhiza kurroa,* B-*Tephrosia purpurea,* C-*Terminalia Arjuna,* D-*Glycyrrhiza glabra,* E- *Azadirachta indica,* F- *Apium graveolens,* G- *Swertia chirata,* H- *Phyllanthus amarus,* I- *Tinospora cordifolia,* J-*Aloe Vera*

ACKNOWLEDGMENTS

This research was supported by Dr Niranjan, CEO of Penta Care Ayur Pharma, Bangalore by providing the drug sources and to my juniors helped me to carry out this research work.

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