In Vitro Antioxidant Activity Of The Hexane And Methanolic Extracts Of Cordia Wallichii And Celastrus Paniculata

H Makari, N Haraprasad, P Ravikumar

Citation

H Makari, N Haraprasad, P Ravikumar. *In Vitro Antioxidant Activity Of The Hexane And Methanolic Extracts Of Cordia Wallichii And Celastrus Paniculata*. The Internet Journal of Aesthetic and Antiaging Medicine. 2007 Volume 1 Number 1.

Abstract

In-vitro antioxidant effects of the hexane and methanolic leaf extracts of Cordia wallichii and Celastrus paniculata were tested. The methanolic extracts of C. wallichii had shown good DPPH (1, 1-diphenyl-2-picryl-hydrazyl) radical scavenging activity. The methanolic extract C. paniculata also exhibited promising result at higher concentration. BHA was used as standard antioxidant and positive control. The DPPH radical scavenging activity of the extract was increased with the increasing concentration. The methanolic extract of C. wallichii was found to be most effective than hexane extract. The Reducing power of extracts was carried out with ascorbic acid as a standard reducing agent. C. wallichii exhibited higher reducing power than C. paniculata. All the analysis was made with the use of UV-Visible Spectrophotometer (Systronics 117, INDIA). In these two plant leaf extracts there was a remarkable concentration dependent DPPH scavenging and reducing power was exhibited.

INTRODUCTION

Antioxidants are radical scavengers which protect the human body against free radicals that may cause pathological conditions such as ischemia, anaemia, asthma, arthritis, inflammation, neuro-degenertion, Parkinson's diseases, mongolism, ageing process and perhaps dementias (Polterat, 1997) Flavonoids and flavones are widely distributed secondary metabolites with antioxidant and antiradical properties. (Nakayoma and Yamada, 1995).

Plants are potent biochemical factories and have been components of phytomedicine since times immemorial; man is able to obtain from them a wondrous assortment of industrial chemicals. Plants based natural constituents can be derived from any part of plant like bark, leaves, flowers, roots, fruits, seeds, etc (Gordon and David, 2001) i.e. any part of the plant may contain active components. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. The medicinal actions of plants are unique to particular plant species or groups are consistent with this concept as the combination of secondary products in a particular plant is taxonomically distinct (Wink, 1999). Antioxidant-based dugs/formulations for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease, and cancer have appeared during the last 3 decades (Devasagayam et al 2004).

This has attracted a great deal of research interest in natural antioxidants. Subsequently, a worldwide trend towards the use of natural phytochemicals present in berry crops, tea, herbs, oilseeds, beans, fruits, and vegetables has increased. Several herbs and spices have been reported to exhibit antioxidant activity, including rosemary, sage, thyme, nutmeg, turmeric, white pepper, chili pepper, ginger, and several Chinese medicinal plants extracts (Lee et al 2003). The majority of the active antioxidant compounds are flavonoids, isoflavones, flavones, anthocyanins, coumarins, lignans, catechins, and isocatechins. In addition to the above compounds found in natural foods, vitamins C and E, beta-carotene, and tocopherol are known to possess antioxidant potential (Prior, 2003). The systematic record of the relative antioxidant activity in selected Iranian medicinal plant species extracts was recorded by Pourmorad et al in 2006. With this background and abundant source of unique active components harbored in plants, the present study was taken up on two alternative medicinal plants namely Cordia wallichii belongs to the family moraginaceae and Celastrus paniculata belongs to the family Celastraceae.

MATERIALS AND METHODS

PLANT MATERIAL

Plant samples of the selected species viz. Cordia wallichii and Celastrus paniculata were collected from Hassan, Mysore district, Karnataka. Further identified by DR.

Krishnappa, Deparment of Applied Botany and voucher specimens were deposited at the same department, Kuvempu University, Karnataka, India. Leaves were separated and dried under shade for three days. Dried leaf samples were ground into a uniform powder using a blender and stored in polythene bags at room temperature.

PREPARATION OF EXTRACTS

10 g of the dried powdered samples from plants Cordia wallichii and Celastrus paniculata species were taken separately in a paper cone and placed into Soxhlet apparatus. 100ml of Hexane a polar solvent was taken in the round bottom flask attached to the Soxhlet apparatus. A condenser was attached to this set up .Then the whole set up was placed on a heating mantle. The temperature was set in the range of 25-30o C. Hexane gets vaporized and rises up to the condenser where it condenses back into liquid. This liquid falls into the plant sample in the cone and extracts certain compounds and falls back into the round bottom flask. This process was continued till all the compounds that can be extracted from the plant by ether gets extracted and finally only clear liquid of ether starts falling into the round bottom flask. The same procedure was repeated with polar solvent such as methanol. The extracts got from the above process was evaporated over night and stored in screw cap vials.

ANTIOXIDANT ASSAY

The antioxidant activity of Plant extracts were determined by different in-vitro methods such as, the DPPH free radical scavenging assay and reducing power methods. The different extracts were dissolved in methanol at the concentration of 2mg/ml. all the assays were carried out in triplicate and average value was considered.

(A) DPPH RADICAL SCAVENGING ACTIVITY:

DPPH scavenging activity of the plant extract was carried out according to the method of Koleva et al 2002; Mathiesen et al 1995. 0.2 ml of methanolic solution of plant extract samples at different concentration (20- 100µg ml-l) was mixed with 0.8 ml of Tris Hcl buffer (100Mm, pH 7.4). One ml DPPH (500 M in methanol) solution was added to above mixture. The mixture was shaken vigorously and incubated for 30min in room temperature. Absorbance of the resulting solution was measured at 517nm UV-Visible Spectrophotometer (Systronics UV-Visible Spectophotometer 117, INDIA). All the assays were carried out in triplicates with BHA (Butylated Hydroxy Anisole) as a positive control. Blank was prepared without the addition of DPPH and for control 0.2 ml of methanol (without plant extract) was added. Percentage of DPPH scavenging activity determined as follows.

% DPPH radical-scavenging = [(Absorbance of control – Absorbance of test

Sample) / (Absorbance of control)] ? 100Control was the DPPH solution without plant extract.

Purified sample 2mg/ml in Methanol of Cordia wallichii and Celastrus paniculata extracts were taken for antioxidant activity with a standard BHA (Butylated Hydroxy Anisole) antioxidant. Decreased absorbance of the reaction mixture indicates stronger DPPH radical-scavenging activity. In this study, hexane and methanolic leaf extracts of both C. wallichii and C. paniculata were used.

(B) REDUCING POWER

This was carried out as described previously (Yildrim et al 2001; Lu and Foo). 1 ml of plant extract solution (final concentration 100-500 mg/l) was mixed with 2.5 ml phosphate buffer(0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide [$K_3Fe(CN_6)$] (10g/l), then mixture was incubated at 50 degree C for 20 minutes. Two and one-half, 2.5 ml of trichloroacetic acid (100g/l) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Finally, 2.5 ml of the supernatant solution was mixed with 2.5 ml of distilled water and 0.5 ml Fecl3 (1g/l) and absorbance measured at 700nm in UV-Visible Spectrophotometer (Systronics UV-Visible Spectophotometer 117, INDIA). As a control, ascorbic acid was used (final concentration 10 mg/ml).

Increased absorbance of the reaction mixture indicates stronger reducing power. In this study, hexane and methanolic leaf extracts of Cordia wallichii and Celastrus paniculata were used.

RESULT AND DISCUSSION

ANTIOXIDANT ASSAY

1. DPPH SCAVENGING ACTIVITY

The percentage of DPPH racial scavenging activity presented in Table 1(a). Like reducing power, the DPPH racial scavenging activity of the extract increases with increasing concentration, only 19.27% DPPH racial scavenging. Nevertheless, it was 72.32% in the presence of 100 mg/l BHA (Butylated Hydroxy Anisole). Although this plant extract shows lower scavenging activity in comparison

to BHA, it is remarkably higher than those of essential oils from the leaves of L.nobilis and the gum of A.cilicia (Alma et all 2003).

Figure 1

Table 1a: Antioxidant activity of hexane extract of

Concentration	OD 517nm			% of activity	
	Sample	Standard		Sample	Standard
50µl	1.048	2µl	0.873	5.58	21.35
100µl	1.014	4µl	0.679	8.64	38.83
150µl	0.983	6µl	0.482	11.40	56.58
200µl	0.944	8µl	0.359	14.95	67.66
250µl	0.896	10µl	0.274	19.27	75.32

Control OD AT 517nm - 1.110

The methanolic extract of Celastrus paniculata was found to most effective than hexane extract. The DPPH racial scavenging activity of the extract increases with increasing concentration, only 25.85% DPPH racial scavenging. Nevertheless, it was 75.32% in the presence of 100 mg/l BHA.

Figure 2

Table 1b: Antioxidant activity of Methanolic extract of

Concentration	OD 517nm			%of activity	
	Sample	Standard		Sample	Standard
50µl	1.030	2µl	0.873	7.20	21.35
100µl	1.010	4µl	0.679	9.00	38.83
150µl	0.923	6µl	0.482	16.84	56.58
200µl	0.910	8µl	0.359	18.01	67.66
250µl	0.823	10µl	0.274	25.85	75.32

Control OD AT 517nm - 1.110

The % of DPPH racial scavenging activity of hexane extract of C. wallichii presented in Table 1(c). Like reducing power, the DPPH racial scavenging activity of the extract increases with increasing concentration, only 16.75% DPPH racial scavenging was present for 250 l. This result found to be lower than that of hexane extract of C. paniculata. Nevertheless, it was 75.32% in the presence of 100 mg/l BHA. Although this plant extract shows lower scavenging activity in comparison to BHA. Plant extract exhibited antioxidative potential and increased concentration of plant extract has shown increased antioxidative potential.

Figure 3

Table 1c: Antioxidant activity of hexane extract of

Concentration	OD 517nm			%of activity	
	Sample	Standard		Sample	Standard
50µl	1.078	2µl	0.873	2.88	21.35
100µl	1.041	4µl	0.679	6.21	38.83
150µl	1.012	6µl	0.482	8.82	56.58
200µl	0.963	8µl	0.359	13.24	67.66
250µl	0.924	10µl	0.274	16.75	75.32

Control OD AT 517nm - 1.110

Figure 4

Table 1d: Antioxidant activity of methanolic extract of

Concentration	OD 517nm			%of activity	
	Sample	Standard		Sample	Standard
50µl	1.057	2µl	0.894	6.3	19.4
100µl	0.982	4µl	0.693	11.53	37.5
150µl	0.934	6µl	0.488	15.85	56.0
200µl	0.883	8µl	0.369	20.45	66.7
250µl	0.796	10µl	0.273	28.28	75.4

Control OD AT 517nm - 1.110

The methanolic extract of Cordia wallichii was found to most effective than hexane extract (1 (d)). The DPPH racial scavenging activity of the extract increases with increasing concentration exhibited only 28.28% DPPH racial scavenging activity was noted. Nevertheless, it was 75.40% in the presence of 100 mg/l BHA. These results suggest that methanolic extracts of C. wallicii exhibited little better than other extracts.

2. REDUCING POWER

Different extracts of Celastrus paniculata exhibited good reducing power. The reducing power of methanolic extract of Celastrus paniculata along with that of ascorbic acid at concentrations between 100-500 mg/ml. The reducing power of the plant extract was determined by the method of Ozaizu (1986). High absorbance indicates high reducing power. The reducing power of the plant hexane extract of C. paniculata leaf as the amount of extract increases Table 2(a). However, this reducing power is lower than that of ascorbic acid which was used as control. Therefore, the absorbance of ascorbic acid in a sample was (10 mg/l) 0.96 while at the 500mg/l methanolic extract concentration it was 0.44. Nevertheless the reducing power of methanolic extract of C. paniculata was 0.54 and it was considerably higher than those of hexane extract Table. 2(b).

Figure 5

Table 2a: Reducing power of hexane extract of

Sample	Concentration (mg/l)	Absorbance (700 nm)
*Control	0	0.07 ± 0.06
Celastrus paniculata	100	0.11 ± 0.013
	200	0.26 ± 0.047
	500	0.44 ± 0.07
Ascorbic acid	5	0.39 ± 0.006
	10	0.76 ± 0.006
	15	1.10 ± 0.006

*The control was test sample without plant extract. High absorbance indicates high reducing power.

Figure 6

Table 2b: Reducing power of methanolic extract of

Sample	Concentration (mg/l)	Absorbance (700 nm)
*Control	0	0.07 ± 0.06
Celastrus paniculata	100	0.18 ± 0.013
	200	0.38 ± 0.047
	500	0.53 ± 0.07
Ascorbic acid	5	0.39 ± 0.006
	10	0.76 ± 0.006
	15	1.10 ± 0.006

*The control was test sample without plant extract. High absorbance indicates high reducing power.

Figure 7

Table 2c: Reducing power of hexane extract of

Sample	Concentration (mg/l)	Absorbance (700 nm)
*Control	0	0.07 ± 0.06
Cordial wallichii.	100	0.16 ± 0.019
	200	0.31 ± 0.047
	500	0.52 ± 0.07
Ascorbic acid	5	0.27 ± 0.006
	10	0.43 ± 0.006
	15	0.96± 0.006

*The control was test sample without plant extract. High absorbance indicates high reducing power.

The reducing power of Cordia wallichii leaf extract has shown good reducing power than celastrus paniculata. As the amount of extract increase, the reducing power also increases. Table 2(c). However, this reducing power is lower than that of ascorbic acid which was used as control. Therefore, the absorbance of ascorbic acid in a sample was (10 mg/l) 0.96 while at the 500mg/l methanolic extract concentration it was 0.52. Neverthless the reducing power of extract of C. wallichii was higher (0.61) Table 2(d) than those of C. paniculata (0.53). In both cases of C. paniculata and C. wallichii there is a remarkable concentration dependent reducing power was exhibited. This variation in reducing activity may be due to crude nature of plant extracts and availability of different phytochemicals in these plants.

Figure 8

Table 2d: Reducing power of methanol extract of

Sample	Concentration (mg/l)	Absorbance (700 nm)
*Control	0	0.07 ± 0.06
Cordial wallichii.	100	0.19 ± 0.019
	200	0.42 ± 0.047
	500	0.61 ± 0.07
Ascorbic acid	5	0.27 ± 0.006
	10	0.43 ± 0.006
	15	0.96± 0.006

*The control was test sample without plant extract. High absorbance indicates high reducing power.

CONCLUSIONS

Against the backdrop of many known medicinal properties of these plants, results from the present work suggest that relatively low values of antioxidant and reducing power may not imply a low medicinal value. Emerging trends in antioxidant research point to the fact that low levels of phenolics (and other phytochemicals) and low value of antioxidant indices in plants do not translate to poor medicinal properties. The present investigation indicates that through Cordia wallichii and Celastrus paniculata has been described as plants of low economic values, these are not worthless. Use of these plants in traditional medicine attests to this. There is prospectus for the commercial utilization especially in the view abundant and widespread nature. The toxic compounds in these plants could be removed through appropriate extraction and processing methods making extracts and products of these plants safe for the utililization of animal and man.

ACKNOWLEDGEMENTS

The authors are grateful to Sri G M Lingaraju, Secretary and Dr. B T Patil, Principal GMIT, Davangere Karnataka, INDIA, for their financial assistance and lab facilities. The authors wish to thank all the staff members of the Dept of Biotechnology of GMIT and SJCE Mysore, Karnataka.INDIA.

CORRESPONDENCE TO

Makari H. K Department of Biotechnology, G M Institute of Technology Davangere-577006 Karnataka , INDIA . mac.gmit@gmail.com, Contact number - 91 8192 233345, Fax-918192 233344 Cell: 91 99642 66444

References

1. Alma, M.H., Mavi, A., Yildirim, A., Digrak, M., Hirata, T., 2003. Screening chemical composition and in vitro antioxidant and antimicrobial activities of the essential oils from Origanum syriacum L. growing in Turkey. Biol. Pharm. Bull. 26, 1725-1729.
2. Devasagayam T P A, Tilak J C, Boloor K.K. 2004. Review: Free radicals and antioxidants in human health:

Curr Stat Fut Prosp. JAPI, 52: 794-804.
3. Koleva I. I., Van Beek T. A., Linssen J.P.H., De Groot A, Evstatieva L.N. 2002. Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochemical Analysis. 13: 8-17.
4. Lee, S. E., Hwang, H. J. and Ha J. S 2003. Screening of medicinal plant extracts for antioxidant activity. Life Sci 73: 167-179,
5. Lu, Y. and Foo, Y. 2000. Antioxidant activities of polyphenols from sage (Salvia officinalis.)
Food Chem, 75: 197-202. Mathiesen, L., Malterud, K.E. and Sund R.B. (1995). Antioxidant activity of fruit exudate and methylated dihydrochalcones from Myrica gale. Planta Med., 61: 515-518.
6. Nakayoma, J and Yamada M. 1995. Suppression of active oxygen-indeed cyto toxicity by flavonoids. Biochem. Pharmcol. 45; 265-267
7. Oyaizu, M. 1986. Studies on products of browning reaction: Antioxidative activities of products of browning reaction prepared from glucosamine. Japan Journal of Nutrition. 44, 307-315.
8. Polterait O. 1997. Antioxidants and free-radical Scavengers of Natural origin Current Org. Chem. 1.415-440.
9. Prior, R.L. 2003. Fruit and vegetables in the prevention of cellular oxidative damage. American Journal of Clinical Nutrition, 78: 570S-578S.
10. Trease G. E. and Evans W. C. 1989. Trease and Evans Pharmacognosy: A Physicians's Guide to Herbal Medicine. 13th Edition. Bailliere, Tindall. London
11. Yildirim, A., Mavi, A., and Kara, A. 2001. J. Agric. Food Chem., 49, 4083-4089.

Author Information

H.K. Makari, M.Sc., M.Phil
Department of Biotechnology, G M Institute of Technology

N. Haraprasad, M.Sc., Ph.D
Department of Biotechnology, Sri Jayachamarajendra College of Engineering

Patil H. S. Ravikumar, M.Sc., Ph.D
Department of Biotechnology, G M Institute of Technology