IN VITRO ANTIOXIDATIVE ACTIVITY OF SOME VIETNAMESE MEDICINAL PLANTS

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Abstract:

Biomolecules can be oxidized by free radicals. This oxidative damage has an etiological aging and development of disease like cancer, antherosclerosis and other inflamotory disorder. Using synthetic antioxidant can be carcinogenic therefore there is an interesting in researching for antioxidant of natural origin. We report here the result of screening for antioxidant activity of 20 plants with 60 extracts. The antioxidant activity *invitro* was tested for their free radical scavenging activity in the DPPH (1,1-diphenyl-2-picrylhydrazyl) screening assay. In the DPPH method showed that methanol extract of *Adenosma bracteosum* (IC_{50} = 3.4 μg/ml) possessed stronger antioxidant properties than acid ascorbic (IC_{50}= 3.46 μg/ml).

INTRODUCTION

Oxidative stress is defined as the state in which the level of toxic reactive oxygen species (ROS) overcomes the endogenous antioxidant defences of the host. This state results in an excess of free radicals, which can react with various components of a living cell such as lipids, protein, and nucleic acids, leading to local injury and eventual organ dysfunction. Such damages have been linked to various degenerative diseases including cancer, cataracts, cardiovascular disease and the aging process itself (Ames et al. 1993, Priscilla and Heather 2000).

The additional use of natural antioxidants is an effective way for the prevention and treatment of these conditions. The use of synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene(BHT), due to their toxicity (Namiki,1990) can be at risk of carcinogenicity, therefore, there is a trend in finding of antioxidants of natural origin (Liu and Wang 2000).

The aim of the present study was to evaluate the antioxidant activity of medicinal plants which were selected based on their ethnomedical use for he treatment of rheumatism, fever, inflammation, tonic, hepatitis and cancer. Selected 60 extracts of 20 medicinal plants were evaluated for antioxidant activity using the ferric reducing/antioxidant power assay (FRAP), the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging assay

MATERIALS AND METHODS

Plant material

Most of samples were freshly collected and carefull dried in shade or oven at not higher than 60°C. *Gentiana scabra, Equisetum debile, Periploca sepium, Tetrapanax papyriferus*, *Oroxylum indicum* were purchased from local herbal market in District 5, Ho Chi Minh City, VietNam.

Sample preparation and extraction

Dried plant samples were milled with the grinder to medium size powders. 50 grams of powder were extracted with dichloromethane, methanol 95% and water

successively to obtained dichloromethane, methanol 95% and water extracts, respectively using for antioxidant tests.

Determination of FRAP

The working FRAP reagent was prepared by mixing 10 volumes of 300 mM acetate buffer, pH 3.6, with 1 volume of 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) in 40mM hydrochloric acid and with 1 volume of 20mM ferric chloride. Freshly prepare FRAP reagent (1.5ml) was warmed to 37^{o}C. Subsequently, 50µl of sample and 150 µl of deionized water was added to the FRAP reagent. Absorbance readings were taken after 1.5 h. Standard curve was prepared using different concentrations (100-1000 µM) of $FeSO_4.7H_2O$. All solutions were used on the day of preparation. The results were corrected for dilution and expressed in µM Fe^{2+}/L. Acid ascorbic was measured within 1 h after preparation. FRAP assay measures the change in absorbance at 595 nm owing to the formation of a blue colored Fe^{II}-tripyridyltriazine compound from colorless oxidized Fe^{III} form by the action of electron donating antioxidants (Benzie and Strain 1996, Pulido et al. 2000). All determinations were performed in triplicate

Free radical scavenging capacity

The free radical scavenging capacity of samples and pure compound was analyzed by DPPH assay. Aliquots (50 µl) of the tested samples were mixed with 2 ml of 6.10^{-5} M methanolic solution of DPPH radical. Methanolic solution of pure compounds were tested too. Absorbance measurements commenced immediately. The decrease in absorbance at 515 nm was determined after 30 min for all samples. Methanol was used to zero spectrophotometer .

All determinations were performed in triplicate. The percent inhibition of the DPPH radical by the samples was calculated according to the formula proposed of Yen an Dul (1994):

$$\% \text{ inhibition} = [(A_{C(0)} - A_{A\,(t)})/A_{C(0)}]x100$$

where $A_{C(0)}$ is the absorbance of the control at t = 0 min and $A_{A\,(t)}$ is the absorbance of the antioxidant at t = 30 min

For determination of IC_{50}, ascorbic was used as reference

RESULTS AND DISCUSSION

Total antioxidative capacity of 60 medicinal plant extracts

The yield of crude extracts (grams of extract/ 100 grams of sample), antioxidative capacity, free radical scavenging activity and in vitro XO inhibition of extracts were shown in table 1. There were big differences in the antioxidative capacity FRAP beween the selected medicinal plant extracts. The FRAP values varied from 147 to 4333 µmol Fe^{2+}/L of 1 mg/ml sample concentrations.

According to their reducing ability/antioxidative power, the antioxidative effect of these thirty six medicinal plant extracts can be divided into four groups: (a) low (< 1 mM), n = 29; (b) average (1-2.5 mM), n = 21; (c) good (2.5-4 mM), n = 7; and (d) high (> 4 mM), n = 3.

As shown in table 1 and Fig.1, the extracts with the strongest antioxidative properties when measured with the FRAP assay (in order) were: *Adenosma bracteosum* (M extract) > *Phyllanthus amarus* (H extract) > *Periploca sepium* (M extract) > *Periploca sepium* (H extract) > *Oroxylum indicum* .(M extract) >

Table 1: Antioxidative activities of 60 plant extracts on FRAP, DPPH˙ free radical

STT	Plant name	Local name	Parts used	Extracted yield			FRAP (μmolFe^{2+}/L)			% DPPH inhibition		
				D	M	W	D	M	W	D	M	W
1.	*Acanthus ilicifolius*	Ô rô nước	Leaves, stems	2.34	8.2	20.02	527	671	512	11.48	17.41	13.49
2.	*Achyranthes bidentata*	Ngưu tất	Whole plant	1.51	7.97	22.1	433	1073	1170	18.63	22.36	52.29
3.	*Adenosma bracteosum*	Nhân trần Tây Ninh	Whole plant	1.56	10.78	20.36	555	4333	3019	26.7	90.62	36.93
4.	*Artemisia vulgaris*	Ngải cứu	Whole plant	2.01	10.87	17.45	837	2335	3001	11.27	28.38	25.91
5.	*Crescentia cujete*	Đào tiên	Fruits	4.36.	10.07	19.3	438	1344	725	24.28	31.93	30.53
6.	*Eclipta prostrate*	Cỏ mực, nhọ nồi	Whole plant	0.89	11.84	28.31	916	1429	2316	9.99	42.81	59.2
7.	*Eleusine indica*	Mần trầu	Whole plant	2.17	8.53	20.56	526	147	1195	13.87	19.51	13.26
8.	*Equisetum debile*	Mộc tặc	Whole plant	3.27	8.93	23.1	742	1780	1029	35.53	66.11	29.04
9.	*Gentiana scabra*	Long đởm thảo	Roots	4.95	12.8	23.6	729	1893	1104	64.66	69.23	40.58
10.	*Glinus oppositifolius*	Rau đắng đất	Whole plant	1.89	8.83	18.9	483	1233	877	18.44	26.24	49.95
11.	*Momordica charantia*	Mướp đắng	Fruits	1.23	12.9	20.3	378	1079	682	38.98	31.79	26.94
12.	*Morinda persicaefolia*	Nhàu nước	Roots	0.98	13.6	23.9	634	1347	657	65.27	54.86	50.51
13.	*Oroxylum indicum*	Núc nác, hoàng bá nam	Stem bark	1.01	9.94	15.3	2527	3139	2575	82.03	68.02	67.83
14.	*Oxalis corniculata* L.	Chua me đất	Whole plant	3.71	6.04	21.18	811	848	937	12.46	21.8	10.97
15.	*Periploca sepium*	Hương gia bì	Stem bark	5.95	14.56	21.34	1045	4088	3393	21.24	71.8	36.51
16.	*Phyllanthus amarus*	Diệp hạ châu	Whole plant	4.36	9.14	20.64	553	2008	4146	27.92	24.18	72.13
17.	*Plumbago zeylanica*	Bạch hoa xà	Whole plant	3.89	14	22.3	719	2513	1687	15.13	31.14	25.02
18.	*Polygonum aviculare*	Rau đắng	Whole plant	2.07	8.67	28	660	1690	1894	27.54	29.74	33.75
19.	*Scutellaria barbata*	Bán chi liên	Whole plant	1.51	7.97	22.1	656	2349	1868	44.77	67.41	48.79
20.	*Tetrapanax papyriferus*	Thông thảo	Core of stems	0.32	2.5	40.49	824	882	613	13.35	26.61	9.52

Abbreviation of extracts. D: dicloromethane; M: methanol; H: aqueous extracts

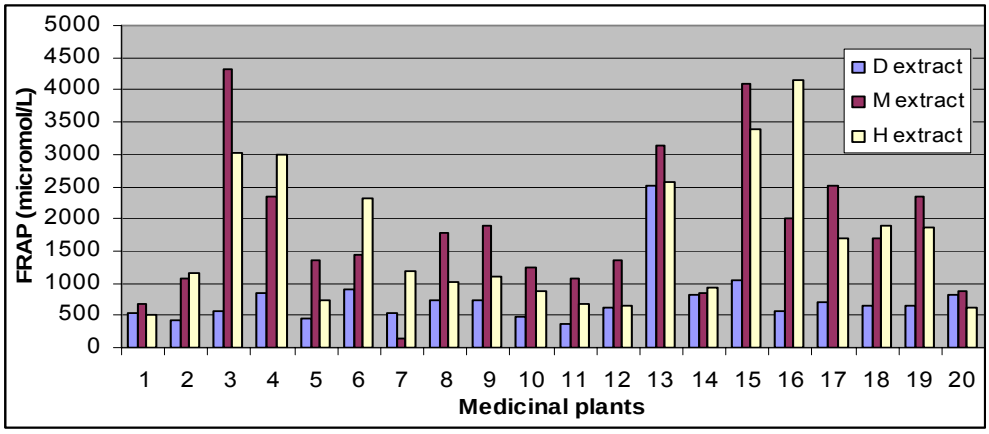

Fig. 1. Total antioxidant capacity determined as FRAP of 60 extracts
Note: Abbreviation of N^0 is plant extracts in table 1.

Adenosma bracteosum (H extract) > *Artemisia vulgaris* (H extract)> *Oroxylum indicum* (H extract) > *Oroxylum indicum* (D extract) > *Plumbago zeylanica* (M extract) > *Scutellaria barbata* (M extract).

In general, values of methanolic or water extracts were stronger than that of dichloromethane ones. Depending on samples, the methanolic or aqueous can be stronger than the other.

Free Radical Scavenging Ability of Medicinal Plant Extracts

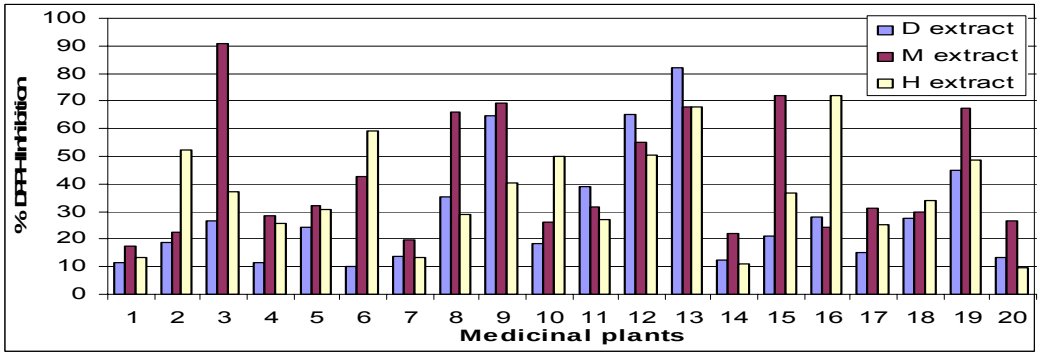

Fig. 2. Free radical DPPH$^{\bullet}$ scavenging activity of 60 extracts
Legend: as that of Fig. 1

Table 2. IC_{50} values of 5 extracts and reference compounds

Names	Extract	IC_{50} (µg/ml)
Phyllanthus amarus	W	15.55
Oroxylum indicum	D	13.02
Adenosma bracteosum	M	3.4
Periploca sepium	M	15.65
Ascorbic Acid		3.46

As shown in table 1 and fig.2, there were three different kinds of extracts according to their activities: 12 plant samples showed activity of M extracts were stronger than that of D and H extracts. On the other hand, activity of H extracts of other 5 plant samples were stronger than M and D extracts. Finally, 3 plant samples had D extract activity were the strongest ones.

The highest 4 active extracts in DPPH assay among tested ones (in order) were: *Adenosma bracteosum* (M extract) > *Oroxylum indicum* (D extract) > *Phyllanthus amarus* (H extract) > *Periploca sepium* (M extract). These extracts were further determined their IC_{50} values in comparison with reference ascorbic acid.

The result was shown in Fig. 3 and Table 2. Each IC_{50} value was achieved from a linear regression analysis showing good correlation coefficient ($r^2 \geq 0.9$).

IC_{50} of crude M extract of *Adenosma bracteosum* which showed IC_{50} of 3.4 μg/ml has better *in vitro* antioxidative activity than that of ascorbic acid (IC_{50}= 3.46 μg/ml)

Comparison of Total Antioxidant FRAP and DPPH˙ Radical Scavenging Properties

The FRAP mechanism is totally electron transfer. Hence, in combination with DPPH assay, the FRAP can be very useful in distinguishing dominate mechanisms with different antioxidants. The DPPH assay is considered to be mainly based on an electron transfer reaction and hydrogen atom abstraction is marginal reaction pathway (Ronald et al. 2005). Some extracts had the different levels of antioxdative capability in each method. This may be explained to be FRAP reaction activity take place in aqueous solution, for this reason, the polarized compounds easily react in this solution, and the orthers meet a prolem when reaction in polarized solvent. In DPPH assay, reaction happen in methanolic solvent, consequently, compounds is not very polarized react well in this solution.

CONCLUSIONS

The results of the study demonstrate that some medicinal plants are promising sources of natural antioxidants. The strongest antioxidative properties when measured with FRAP, DPPH among sixty test samples were methanolic extracts of *Adenosma bracteosum* and H extract of *Phyllanthus* These extract will be further for isolating of active compounds.

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