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(Research Article)

In-vitro Antioxidant Activity of Eight Traditionally Used Indian Medicinal Plants

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ABSTRACT

In the present study free radical scavenging activities of eight medicinal plants were evaluated. Free radicals are involved in many disorders like neurodegenerative diseases, cancer and AIDS. Antioxidants due to their scavenging activity are useful for the management of those diseases. The antioxidant activity of all the extract by different method was found to be increasing in dose dependent manner. Phylanthus emblica extract and Curcuma longa extract showed best free radical scavenging activity by DPPH (IC_{50}- 148.76 and 198.0 µg/ ml respectively), superoxide scavenging (IC_{50}- 162.28 and 249.66 µg/ ml respectively) and by hydrogen peroxide scavenging activity method (IC_{50} -249.42 and 269.42 µg/ ml respectively) and were found to be most potent amongst all the extracts. The IC_{50} values of all the extract was compared with standard IC_{50} value and found that all the extract was having significant antioxidant activity ($P<0.01$). This study supports the contention that traditional medicines remain a valuable source in the potential discovery of natural product pharmaceuticals. The significant antioxidant activity showed by all the selected plant extracts provides a scientific validation for the traditional use of these plants.

Key Words: Antioxidant, DPPH, Superoxide scavenging, H_2O_2 Scavenging.

INTRODUCTION

There are a lot of antioxidants that are introduced to minimize actions of reactive oxygen species (ROS) [1, 2]. Phenol compounds can trap the free radicals directly or scavenge them through a series of coupled reactions with antioxidant enzymes. Thus efforts have been made to search for novel natural antioxidants from fruits, vegetables, herbs and spices. External supplementation through antioxidants is recommended to protect cells from the deleterious effects of such oxidative stress conditions. In the search of novel antioxidant substances, the herbal extracts used in ayurvedic preparation received considerable attention due to their natural origin and lesser side effects. The importance of ROS and free radicals has attracted increasing attention over the past decade. ROS which include free radicals such as superoxide anion radicals ($O_2^{\cdot-}$), hydroxyl radicals ($OH^{\cdot}$) and non-free radical species such as H_2O_2 and singlet oxygen ($^1O_2$) are various forms of activated oxygen. ROS is continuously produced during normal physiologic events and they can easily initiate the peroxidation of membrane lipids, leading to the accumulation of lipid peroxides. Under pathological conditions, ROS is overproduced and results in oxidative stress. There is also a considerable amount of evidence revealing an association between individuals who have a diet rich in fresh fruits and vegetables and the decreased risk of cardiovascular diseases and certain forms of cancer. There is currently immense interest in natural antioxidants and their role in human health and nutrition. Considerable amount of data have been generated on antioxidant properties of food plants around the globe[3, 4]. However, a traditionally used medicinal plant awaits such screening. On the other hand, the medicinal properties of plants have also been investigated in the light of recent scientific developments throughout the world, due to their potent pharmacological activities, low toxicity and economic viability. Some of these plants have shown potent antioxidant activity However, majority of plants have not yet been screened for such activity. So, in order to contribute further to the knowledge of Indian traditional plants, our present study is focussed on eight plants namely *Phyllanthus emblica, Terminalia ballerica, Terminalia chebula ,Curcuma longa, Hemidesmus indicus, Eclipta alba, Glycyrrhiza glabra* and *Aloe vera* to determine their antioxidant and free radical scavenging properties. The literature survey showed scanty information available on these plants and thus prompted us to analyze these common ayurvedic plants.

MATERIALS AND METHODS

Collection, Extraction and Phytochemical Screening

The selected plant parts were identified and authenticated at Dept. of botany, Payyannur College, Payyannur, Kannur. The drugs were washed several times with water to remove soil and extraneous matters, the drugs were spread in trays and air dried for two weeks. The method employed for extraction was Cold water extraction. Fresh plant materials are collected washed thoroughly and air dried and powdered. The powder is weighed and subjected to the process of extraction using 70% ethanol under cold condition (15-25^{0}C) for 48-96 hours. The extracts were subjected to the qualitative tests to reveal the presence of Alkaloids, Triterpenes, Sterols, Cabohydrates, Glycosides, Saponins, Flavanoids, Tannins and Phenolic compounds[6, 7, 8].

Evaluation of Antioxidant Activity

DPPH radical scavenging assay[11, 12]

To the 2.5 ml of various concentrations of extracts, 1ml of solution of DPPH 0.1 mM (0.39 mg in 10 ml methanol) was added to the test tube. After 30 minutes incubation in the dark, absorbance was recorded at 517 nm against methanol as blank. Experiment was performed in triplicate and IC_{50} values were calculated by linear regression of the plot.

$$\% \text{ Scavenging} = \frac{\text{Control-Test}}{\text{Control}} \times 100$$

Where, Control: Methanol + DPPH; Test: Extracts + Methanol + DPPH; Blank: Methanol.

Superoxide Scavenging Activity[13, 14]

From the stock solution of 100 mg/ml of the extracts in methanol, 100, 150, 200, 250 and 300 µg/ml of various concentrations of extracts were prepared. To 0.3 ml of various concentrations of extracts, 1 ml of alkaline DMSO and 0.1 ml of NBT was added and sample absorbance was measured immediately at 560 nm.

$$\% \text{ Scavenging} = \frac{\text{Control-Test}}{\text{Control}} \times 100$$

Where, Control: DMSO+ NBT; Blank: Distilled Water; Test: Extracts+ DMSO+ NBT.

Hydrogen peroxide scavenging activity[15, 16]

Different concentrations of various extracts were prepared and to 2 ml of extracts 2 ml of phosphate buffer saline were added and 1ml of 4mM H_2O_2 was added. After 10 min, the absorbance value of the reaction mixture was recorded at 230 nm. Blank solution was recorded at 230 nm. Blank solution was containing the phosphate buffer without H_2O_2. The percentage of scavenging activity was calculated by using the formula given below:

$$\% \text{ Scavenging} = \frac{\text{Control-Test}}{\text{Control}} \times 100$$

Where, Control: Phosphate buffer + Hydrogen peroxide, Test: Phosphate buffer + Hydrogen peroxide + Extracts, Blank: Phosphate buffer.

RESULTS AND DISCUSSION

The results of the phytochemical screening of the extracts are given in table no.1. We have chosen different methods as it has been suggested that results will be inconclusive if one dimensional method is used to evaluate the natural biological antioxidants which are multifunctional. But at the same time, that these assays differ from each other in terms of substrates, probes, reaction conditions and quantification methods. It is extremely difficult to compare the results from different assays. However, we have attempted to correlate the very diverse results obtained in the different methods.

DPPH Radical Scavenging Activity

Phylanthus emblica extract and *Curcuma longa* extract showed best free radical scavenging activity by DPPH method and were found to be most potent amongst all the extracts (IC_{50}- 148.76 and 198.0 µg/ ml respectively) as shown in table no 2.

Superoxide Scavenging Activity

Phylanthus emblica extract and *Curcuma longa* extract showed best free radical scavenging activity by superoxide scavenging method and were found to be most potent amongst all the extracts (IC_{50}- 162.28 and 249.66 µg/ ml respectively) as shown in table 3.

Hydrogen Peroxide Scavenging Activity

The best H_2O_2 scavenging potential was shown by *Phylanthus emblica* extract and *Curcuma longa* extract and with IC_{50} values 249.42 and 269.42 µg/ ml respectively as shown in table 4. The antioxidant activity of all the extract by different method was found to be increasing in dose dependent manner. The IC_{50} values of all the extract was compared with standard IC_{50} value and found that all the extract was having significant antioxidant activity ($P<0.01$) and the comparison of IC_{50} of various extracts showed in table 5 and figure 1.

CONCLUSION

In the present study free radical scavenging activities of eight medicinal plants were evaluated. Free radicals are involved in many disorders like neurodegenerative diseases, cancer and AIDS. Antioxidants due to their scavenging activity are useful for the management of those diseases. The extracts, which showed the strongest radical scavenging activity, are *Phylanthus emblica* and *Curcuma longa* while the others also showed significant antioxidant properties. The antioxidant activity of all the extract by different method was the activity was found to be increasing in dose dependent manner. The IC_{50} values of all the extract was compared with standard IC_{50} value and found that all the extract was having significant antioxidant activity ($P<0.01$). This study supports the contention that traditional medicines remain a valuable source in the potential discovery of natural product pharmaceuticals. Significant antioxidant activity showed by all the selected plant extracts provides a scientific validation for the traditional use of these plants. Further work on isolation and identification of active compounds and its efficacy needs to be done.

Table 1: Phytochemical Screening of the Extracts

Sl No.	Chemical Test	P.E. Extract	E.A. Extract	T.C. Extract	T.B. Extract	G.G. Extract	C.L. Extract	H.I. Extract	A.V. Extract
1	Tests for Sterols	+	-	+	-	+	-	+	-
2	Tests for Triterpenes	-	+	-	-	+	-	+	-
3	Tests for Saponins	-	-	-	+	++	-	+	-
4	Tests for Alkaloids	-	+	-	-	-	-	+	-
5	Tests for Carbohydrates	+	-	+	-	+	-	+	+
6	Tests for Reducing sugars	+	-	+	-	-	-	-	+
7	Tests for Tannins	++	+	++	+	-	-	+	++
8	Tests for Phenolic compounds	+	+	+	-	+	-	+	+
9	Test for Glycosides	+	-	+	-	+	-	+	+

Note: +: Present, - : Absent,P.E. –*Phylanthus emblica*, E.A .– *Eclipta alba*, T.C.– *Terminalia chebula*, T.B. – *Terminalia ballerica*, G. G. – *Glycyrrhiza glabra*, C.L .– *Curcuma longa*, H.I .– *Hemidesmus indicus*, A.V. – *Aloe vera*.

Table 2: DPPH radical scavenging activity of selected plant extracts

% Scavenging									Ascorbic Acid	
Conc. (µg/ml)	T.C. extract	A.V. extract	G.G extract	H.I. extract	T.B. extract	P.E extract	E.A. extract	C.L. extract	**Conc. µg/ml**	**% Scavenging**
100	23.98 ±2.11	33.39 ± 1.87	6.21 ± 1.29	28.78 ± 1.99	4.49 ± 1.47	48.06±0.58	37.9± 0.74	36.99±0.97	2	21.6±1.64
150	30.46 ±2.34	36 ± 2.37	10.44 ± 1.20	30.89 ± 1.07	8.12 ± 1.43	51.95±0.51	38.78 ± 0.77	41.21±0.15	2.5	31.70+1.52
200	32.74 ±3.32	49.98 ± 2.90	36.84 ± 2.99	46 .00 ± 2.88	35.12 ± 2.10	55.18±0.47	41.11 ± 0.74	51.47±0.34	3	49.02+2.35
250	38.36 ±3.71	56.01 ± 2.52	38.14 ± 1.93	55.58 ± 2.73	36.63 ± 1.08	57.32±0.5	45.04 ± 1.47	71.76±0.11	3.5	54.30+2.65
300	45.73 ±4.11	61.70 ± 3.35	49.74 ± 2.68	59.20 ± 3.64	46.81 ± 2.76	61.13±0.43	47.92 ± 0.65	78.42±0.22	4	68.20+3.03

Values are Mean±SEM, n=3**,** P.E. –*Phylanthus emblica*, E.A. – *Eclipta alba*, T.C. – *Terminalia chebula*, T.B. – *Terminalia ballerica*, G. G. – *Glycyrrhiza glabra*, C.L. – *Curcuma longa*, H.I. – *Hemidesmus indicus*, A.V. – *Aloe vera*.

Table 3: Superoxide scavenging activity of selected plant extracts

% Scavenging									Ascorbic Acid	
Conc. (µg/ml)	P.E extract	E.A extract	C.L extract	T.C extract	T.B extract	A.V extract	H.I extract	G.G extract	Conc. µg/ml	% scavenging
100	42.21±2.22	32.56 ± 1.19	32.36 ± 1.70	23.12 ± 1.36	21.53 ±1.23	26.35 ±1.92	27.17±1.61	23.98 ±2.11	10	44.47± 2.37
150	45.48±2.77	34.88 ± 1.51	42.00 ± 2.05	26.78 ± 1.18	25.67 ±1.90	29.79 ±2.52	31.91 ±1.97	30.46 ±2.34	20	53.15±2.53
200	56.48± 6.26	36.04 ± 1.78	43.74 ± 2.06	27.36 ± 1.18	28.77 ±2.57	38.22 ±2.94	35.42 ±2.02	32.74 ±3.32	30	57.29±2.77
250	62.45±6.40	38.54 ± 1.99	46.04 ± 2.51	31.98 ± 1.96	33.93 ±5.86	48.80 ±2.57	40.21 ±2.84	38.36 ±3.71	40	63.47±3.41
300	73.08±11.00	40.27 ± 2.85	58.19 ± 3.66	35.65 ± 2.01	37.29 ±6.27	54.08 ±2.38	51.32 ±1.92	45.73 ±4.11	50	71.48±3.87

Values are Mean±SEM, n=3.

P.E –*Phylanthus emblica*, E.A – *Eclipta alba*, T.C – *Terminalia chebula*, T.B – *Terminalia ballerica*, G. G – *Glycyrrhiza glabra*, C.L – *Curcuma longa*, H.I – *Hemidesmus indicus*, A.V – *Aloe vera*.

Table 4: Hydrogen peroxide scavenging activity of selected plant extracts

% Scavenging									Ascorbic Acid	
Conc. (µg/ml)	A.V extract	T.C extract	G.G extract	E.A extract	P.E extract	T.B extract	C.L extract	H.I extract	Conc. µg/ml	% scavenging
100	25.46 ± 1.94	7.11 ± 0.90	8.07 ± 1.13	2.34 ± 0.25	8.52 ± 0.64	4.66 ± 0.38	1.93 ± 0.29	1.57 ± 0.4	10	28.39±2.43
150	26.28 ± 1.95	9.86 ± 0.92	10.64 ± 1.08	2.51 ± 0.33	15.48 ± 1.58	5.37 ± 1.40	6.97 ± 1.76	6.2 ± 0.78	20	39.62±2.19
200	33.96 ± 1.33	12.31 ± 1.12	13.64 ± 1.14	2.78 ± 0.47	21.71 ± 2.09	6.59 ± 1.01	15.89 ± 1.78	7.76±0.53	30	46.74± 2.36
250	35.29 ± 1.40	14.11 ± 1.14	17.55 ± 1.22	11.95 ± 1.94	50.77 ± 2.52	12.40± 1.24	45.73 ± 2.2	10.47 ± 1.17	40	55.63±2.77
300	37.75 ± 2.33	15.93 ± 1.19	22.78 ± 1.24	22.6 ± 0.97	68.98 ± 3.23	22.49 ± 1.45	65.11 ± 3.81	23.45 ± 1.95	50	63.57± 3.48

Values are Mean±SEM, n=3.

P.E –*Phylanthus emblica*, E.A – *Eclipta alba*, T.C – *Terminalia chebula*, T.B – *Terminalia ballerica*, G. G – *Glycyrrhiza glabra*, C.L – *Curcuma longa*, H.I – *Hemidesmus indicus*, A.V – *Aloe vera*.

Table 5: IC_{50} of selected plant extracts

Extract Used/Standard Drug	IC_{50} (µg/ml)		
	By Superoxide Scavenging Method	By DPPH Method	By Hydrogen Peroxide Scavenging Method
P.E. extract	162.28**	148.76**	249.42**
E.A. extract	554.31**	309.68**	741.38**
C.L. extract	249.66**	198.0**	269.42**
T.C. extract	547.40**	353.16**	900.48**
T.B. extract	458.75**	305.01**	523.41**
A.V. extract	270.90**	216.83**	472.98**
H.I. extract	293.07**	234.49**	494.83**
G.G. extract	353.16**	304.67**	694.45**
Standard (Ascorbic acid)	16.0	3.17	34.79

t-test- ****-** Significant**,** P‹0.01.
P.E –*Phylanthus emblica*, E.A – *Eclipta alba*, T.C– *Terminalia chebula*, T.B – *Terminalia ballerica*, G. G – *Glycyrrhiza glabra*, C.L – *Curcuma longa*, H.I– *Hemidesmus indicus*, A.V – *Aloe vera*.

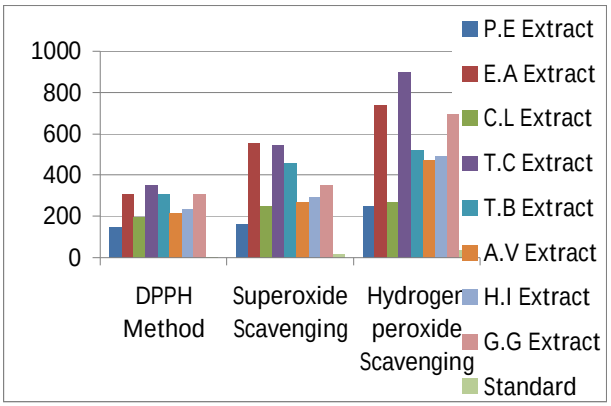

Figure-1: IC_{50} of selected plant extracts

P.E. –*Phylanthus emblica*, E.A. – *Eclipta alba*, T.C. – *Terminalia chebula*, T.B. – *Terminalia ballerica*, G. G. – *Glycyrrhiza glabra*, C.L. – *Curcuma longa*, H.I.. – *Hemidesmus indicus*, A.V – *Aloe vera*.

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