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In vitro Antioxidant Potential of Ethanolic Contents of *Eclipta alba* and *Wedelia chinensis*

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ABSTRACT

Ethano botanical search reveals use of many traditional herbs in the treatment of cancer, which are usually free from side effects, are economical and also easily accessible to humans.Using DPPH, Nitricoxide, Superoxide radical scavenging activity and Ferric reducing antioxidant power assay to investigate the antioxidant potential of crude ethanolic extracts from the leaves of *Eclipta alba* and *Wedelia chinensis*, it was found that the ethanolic extracts exhibited a stronger antioxidant activity. The extracts showed the inhibition in a dose dependant manner. The results were expressed as IC_{50}. *Eclipta alba* showed higher reducing activity in DPPH assay and FRAP assay. *Wedelia chinensis* showed potent radical scavenging activity for Nitricoxide and Superoxide radicals. Both the medicinal plants are a potential source of natural antioxidants.

Keywords: *Eclipta alba, Wedelia chinensis*, Radical scavenging activity, Reducing activity.

INTRODUCTION

Plants have formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years and continue to provide mankind with new remedies[1]. Free radicals are chemical species, which contains one or more unpaired electrons due to which they are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability[2]. The oxygen molecule produces a highly reactive oxygen species (ROS) by some exogenous factors and endogenous metabolic processes in human body. ROS include a number of chemically reactive molecules such as hydrogen peroxide (H_2O_2), superoxide (O_2^-) and hydroxyl radical (·OH) [3]. Reactive oxygen species and reactive nitrogen species (RNS) have been widely implicated in the pathogenicity of several degenerative diseases such as Ulcer, Liver cirrhosis, Atherosclerosis, Alzheimer's disease, Parkinson's disease and Cancer[4]. Antioxidants are reducing agents and limit oxidative damage to biological structures by passivating free radicals[5]. Antioxidant compounds may function as free radical scavengers, completers of prooxidant metals, reducing agents and quenchers of singlet oxygen formation[6]. The medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent antioxidant activities, no side effects and economic viability[7].

Eclipta alba and *Wedelia chinensis* belong to the family Asteraceae. *Eclipta alba* commonly known as Bhringraj in Hindi is a small and erect annual herb. *Eclipta alba* is the main herb for the hair care and cirrhosis in Ayurveda. It is believed to maintain and rejuvenate hair, kidneys, liver, teeth, bones, memory, vision and hearing. In Ayurveda, the root powder is used for treating hepatitis, enlarged spleen and skin disorders. Mixed with a little oil when applied to the head, the herb relieves headache. The extract of its leaves is mixed with honey and given to infants, for the expulsion of worms. *Eclipta alba* is also given to children in case of urinary tract infections.

Wedelia chinensis is a tender, spreading and hairy herb, with light camphor like odour. The leaves are used in dyeing grey hair and in promoting the growth of hair. They are considered tonic, alternative and useful in coughs, skin diseases. The seeds and flowers, as well as the leaves, are used in decoction, in the quantity of half of tea cupful twice daily, as deobstruent. In decoction, the plant is used in uterine haemorrhage and menorrhagia. Therefore, the aim of the present study is to analyze free radical scavenging potential of ethanolic extracts of *Eclipta alba* and *Wedelia chinensis* by various methods in *in vitro* condition.

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MATERIALS AND METHODS

Plant materials

The leaves of *Eclipta alba and Wedelia chinensis* were collected from Coimbatore (Tamil Nadu) respectively. The plants were identified and authenticated by the Botanical Survey of India - Tamilnadu Agricultural University, Coimbatore. The leaves were washed, shade dried, powdered and stored in air tight containers separately under refrigeration.

Extraction of the plant materials

50g of powdered leaves of *Eclipta alba* and *Wedelia chinensis* was taken in a conical flask. To this 250ml of 95% ethanol was added. The contents of the flask were soaked overnight. This suspension was filtered and residue was resuspended in an equal volume of 95% ethanol for 48hr and filtered again. The two filtrates were pooled and the solvents were evaporated in a rotatory evaporator at temperature below 50°C and the extracts were freeze-dried. The residue was used to analyse the various *in vitro* free radical scavenging parameters.

Evaluation of antioxidant activities

DPPH radical scavenging assay

The free radical scavenging activity of the fractions was measured *in vitro* by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay. About 0.3 mM solution of DPPH in 100% ethanol was prepared and 1 ml of this solution was added to 3.0 ml of the fraction dissolved in ethanol at different concentrations. The mixture was shaken and allowed to stand at room temperature for 30 min and the absorbance was measured at 517nm. The percentage scavenging inhibition was determined and was compared with that of ascorbic acid which was used as the standard. The percentage inhibition was calculated by

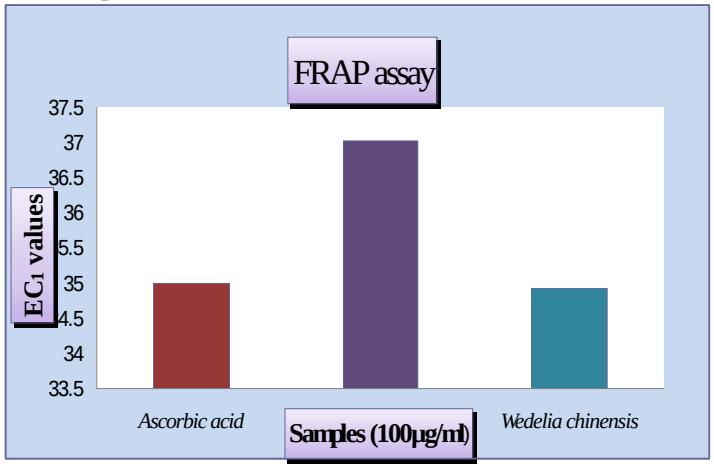
$$\text{Inhibition (\%)} = \frac{(A_{cont} - A_{test})}{A_{cont}} \times 100$$

Where A_{cont} is the absorbance of the control reaction and A_{test} is the absorbance in the presence of the sample of the extracts. The antioxidant activity of the extract was expressed as IC_{50}. The IC_{50} value was defined as the concentration (in µg/ml) of extracts that inhibits the formation of radicals by 50%.

FRAP (Ferric reducing antioxidant power) assay

The FRAP assay was done by the method of Pulido (2000)[9]. The assay mixture contained 2.5ml of 300mM acetate buffer at pH 3.6, 0.25ml of 10mM TPTZ solution in 40mM HCl, 0.25ml of 20mM $FeCl_3$ and test substances in 0.1ml ethanol. The absorbance was measured after 30 min incubation at 593nm. All tests were run on triplicate and mean values were used to calculate EC_1 values. EC_1 is defined as concentration of an antioxidant having a ferric reducing ability equivalent to that of 1mM ferrous salt.

Figure 1: The ferric reducing ability of the ethanolic extracts of selected medicinal plants and standard ascorbic acid.



Nitricoxide radical scavenging activity[10]

Nitricoxide was generated from sodium nitroprusside and measured by the Griess reaction. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitricoxide which interacts with oxygen to produce nitrite ions that can be estimated by use of Griess reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide. Nitric oxide scavenging activity was measured spectrophotometrically.

Sodium nitroprusside (5 mM) in phosphate buffered saline was mixed with different concentration of the extract (20-100µg/ml) dissolved in ethanol and incubated at 25°C for 30min. A control without the test compound but with an equivalent amount of ethanol was taken. After 30 min, 1.5ml of the incubated solution was removed and diluted with 1.5ml of Griess Reagent (1% Sulphanilamide, 2% Phosphoric acid and 0.1% N-1-naphthylethylenediamine dihydrochloride). The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with N-1-naphtylethylenediamine dihydrochloride was measured at 546nm and percentage scavenging activity was measured with reference to standard. The percentage inhibition was calculated as in DPPH radical scavenging activity.

Superoxide radical scavenging activity

Superoxide activity of the extract was determined by McCord and Fridovich method[11] which depends on light induced superoxide generation by riboflavin and the corresponding reduction of NBT. The assay mixture contained 0.3ml of different concentrations of the extract and 0.2ml ethylene diamine tetra acetic acid (6µM containing 3µg NaCN), 0.1ml nitro blue tetrazolinium (NBT) (50µM), 0.05ml riboflavin (2µM) and 2.35ml phosphate buffer (58mM, pH 7.8) to give a total volume of 3.0ml. The tubes were uniformly illuminated with an incandescent light for 15 min and the optical density was measured at 560nm. The percentage inhibition by the extract of superoxide production was evaluated by comparing the absorbance values of control and experimental tubes. The percentage inhibition was calculated as in DPPH radical scavenging activity.

Statistical analysis

Tests were carried out in triplicates. Statistical analyses were carried out using the statistical package SPSS version (10). Differences among the tested antioxidants were analysed by using one-way ANOVA. Values are expressed as the Mean ± SD and differences between groups were considered to be significant if $p<0.05$.

RESULTS AND DISCUSSION

Compelling evidence indicates that increased consumption of dietary antioxidants or fruits and vegetables with antioxidant properties may contribute to the improvement in quality of life by delaying onset and reducing the risk of degenerative diseases associated with aging.

There is extensive evidence to implicate free radicals in the development of degenerative diseases[12]. Reactive oxygen species such as superoxide anion, hydroxyl radical and nitric oxide inactivate enzymes and damage important cellular components causing tissue injury through covalent binding and lipid peroxidation[13].

DPPH radical scavenging activity

ROS produced *in vitro* include superoxide radical, hydrogen peroxide and hypochlorous acid. Hydrogen peroxide and superoxide can interact in the presence of certain transition metal ions to yield a highly-reactive oxidising species, the hydroxyl radical. The antioxidants react with the stable free radical DPPH (deep violet colour) and convert it to 1, 1-diphenyl-2-picryl hydrazine with decolouration. The scavenging effects of extract increased with their concentrations to similar extents.

Eclipta alba (83.47%) showed potent DPPH radical scavenging activity than *Wedelia chinensis* (77.33%) at the concentration of 100µg/ml. The IC_{50} values

Table 1. Scavenging effects of ethanolic extracts of selected medicinal plants and standard ascorbic acid on DPPH radical.

Sample Concentration (µg/ml)	% of inhibition Ascorbic acid	*E.alba*	*W.chinensis*
20	34.56 ±1.02[a]	32.65±3.06[a]	32.57 ±0.22[a]
40	55.00 ±0.05[a]	47.95 ±1.02[a]	45.23 ±0.21[a]
60	72.80 ±0.09[a]	69.72 ±2.56[a]	58.40 ±0.42[a]
80	79.23 ±1.82[a]	79.93 ±3.11[a]	65.04 ±1.89[a]
100	82.61 ±2.00[a]	83.47 ±2.16[a]	77.33 ±2.52[a]

Results are expressed as mean ± SD of the three parallel measurements.
SD values followed by common superscript letter (a) are significant at 5% level when compared to control.

showed a maximum inhibition value for *Eclipta alba* (42µg/ml) when compared to *Wedelia chinensis* (45µg/ml). Standard ascorbic acid showed an IC_{50} value of 36µg/ml as a positive control illustrated in table 1.

FRAP (Ferric reducing antioxidant power) assay

Ferric reducing antioxidant power measures the ability of antioxidants to reduce ferric 2, 4, 6-tripyridyl-s-triazine complex to intensively blue colored ferrous complex in acidic medium[14]. Hence any compound which is having redox potential lower than that of redox pair Fe (III)/Fe (II) can theoretically reduce Fe (III) to Fe (II). The reducing capacity of compound may serve as significant indicator of its potential antioxidant activity. The presence of reductants such as antioxidant substance causes the reduction of the Fe^{3+}/ ferricyanide complex to the ferrous form[15].

The tests were run in triplicate and mean values were used to calculate EC_1 values. EC_1 is defined as concentration of an antioxidant having a ferric reducing ability equivalent to that of 1mM ferrous salt. The EC_1 values of the plant extracts and standard ascorbic acid are indicated in the figure 1, showed that the ferric reducing antioxidant potential of ethanolic extracts were very much near to the standard vitamin C.

The FRAP EC_1 values of ethanolic extracts of *Eclipta alba* and *Wedelia chinensis* were found to be 37.03 and 34.92 whereas the standard vitamin C was found to be 35.00 µg/ml respectively. *Eclipta alba* exhibited greater ferric reducing power than the standard ascorbic acid at 100µg/ml.

Nitricoxide radical scavenging activity

Nitricoxide (NO) is a potent pleiotropic mediator of physiological processes such as smooth muscle relaxation, neuronal signalling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical which plays major roles as an effector molecule in diverse biological systems including neuronal messenger, vasodilation, antimicrobial and antitumor activities[16].

Nitricoxide is generated from the amino acid L-arginine by vascular endothelial cells, phagocytes and certain cells in the brain. Nitric oxide reacts with superoxide and forms peroxynitrite radicals and is responsible for the inflammatory response by the release of prostaglandin. Some scientists believe that repeated infections throughout life cause an excessive production of NO, which over time, diseases such as Heart disease, Alzheimer's disease and Diabetes. In the present study, nitricoxide was generated from sodium nitroprusside, at physiological pH (7.4) liberates nitric acid. This nitric acid gets converted to nitrous acid and further forms nitrite ions on contact with air. The nitrite ions diazotize with sulphanilic acid and couple with naphthylethylenediamine forming pink colour complex, which was measured at 546nm[17].

Table 2. Scavenging effects of ethanolic extracts of selected medicinal plants and standard ascorbic acid on nitricoxide radical.

Sample Concentration (µg/ml)	% of inhibition Ascorbic acid	*E.alba*	*W.chinensis*
20	36.86 ±0.50[a]	35.34 ±0.50[a]	35.15 ±0.52[a]
40	59.76 ±2.10[a]	41.91 ±1.51[a]	55.54 ±1.54[a]
60	69.52 ±2.54[a]	62.12 ±2.02[a]	67.09 ±1.27[a]
80	76.59 ±0.02[a]	73.56 ±2.95[a]	77.33 ±2.52[a]
100	90.40 ±2.60[a]	87.70 ±2.54[a]	90.73 ±3.28[a]

Results are expressed as mean ± SD of the three parallel measurements.
SD values followed by common superscript letter (a) are significant at 5% level when compared to control.

Table 3. Scavenging effects of the ethanolic extracts of selected medicinal plants and standard ascorbic acid on superoxide radical.

Sample Concentration (µg/ml)	% of inhibition Ascorbic acid	*E.alba*	*W.chinensis*
10	34.01 ±1.05[a]	25.29 ±1.57[a]	35.22 ±0.51[a]
20	47.20 ±0.06[a]	41.02 ±2.05[a]	46.89 ±1.02[a]
30	65.36 ±0.09[a]	55.55 ±1.64[a]	63.04 ±2.56[a]
40	82.30 ±0.10[a]	69.74 ±0.51[a]	81.91 ±1.56[a]
50	93.41 ±1.62[a]	86.66 ±1.35[a]	94.55 ±2.13[a]

Results are expressed as mean ± SD of the three parallel measurements.
SD values followed by common superscript letter (a) are significant at 5% level when compared to control.

The plant extracts inhibited nitricoxide radical scavenging activity in dose dependant manner. This may be due to antioxidant principles present in the extract, which compete with oxygen to react with nitricoxide[18]. *Wedelia chinensis* (90.73%) showed potent nitricoxide radical scavenging activity than *Eclipta alba* (87.70%) at the concentration of 100µg/ml. The IC_{50} values showed a maximum inhibition for *Wedelia chinensis* (35µg/ml) when compared to *Eclipta alba* (50µg/ml). Standard ascorbic acid showed a IC_{50} value of 32µg/ml as a positive control illustrated in table 2.

Superoxide radical scavenging activity

Superoxide is a highly reactive molecule that can react with many substances, produced in various metabolic processes, including phagocytosis. It causes oxidation or reduction of solutes depending on their reduction potential. Both aerobic and anaerobic organisms possess superoxide dismutase enzyme, which catalyses the breakdown of superoxide radical[19].

The formation of superoxide radical leads to a cascade formation of other ROS in the cell[20]. Endogenously, superoxides could be produced in large amounts by various metabolic and physiological processes[21].

From the table 3, the scavenging effects of the ethanolic extracts on the superoxide radical was higher for *Wedelia chinensis* than *Eclipta alba* and the values were found to be 94.55% and 86.66%. Increasing the sample concentration range from 10 to 50µg/ml, the scavenging effect also increased in the dose dependant manner. The IC_{50} value of ethanolic extracts of *Eclipta alba* and *Wedelia chinensis* were found to 26µg/ml, 22µg/ml when compared to the standard ascorbic acid whose IC_{50} value were found to 21.5µg/ml at a concentration of 50µg/ml.

CONCLUSION

The reactive oxygen species or oxidants, which are formed in the human body due to exogenous and endogenous factors, are found to be responsible for many diseases. Day by day, a lot of researches have shown the potential of phytochemical antioxidants as health benefactors because of their ability to neutralize free radicals, reactive oxygen species, or oxidants responsible for the onset of cell damage.

On the basis of our results of the present study, it is concluded that the ethanolic extracts of selected medicinal plants have significant antioxidant activity compared to other well characterized, standard antioxidant systems *in vitro*.

From the above antioxidant parameters assayed, *Eclipta alba* was found to better antioxidant in DPPH radical scavenging activity and FRAP assay when compared to *Wedelia chinensis*, whereas *Wedelia chinensis* showed potent nitricoxide radical and superoxide radical scavenging activity when compared to *Eclipta alba.*

Finally the two selected medicinal plants (*Eclipta alba, Wedelia chinensis)* could be considered for preparation of Nutraceuticals with potent antioxidant activity suitable for the prevention of human disease.

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