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Research Article

A Combinative Evaluation Of Antioxidant Potential In *Tridax procumbens* and *Boerhavia diffusa*

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Abstract

Free radicals are substances that are capable of inducing oxidative damage to human body. This free radical reaction can be terminated effectively by the antioxidants thus reducing the risk of diseases. The present study was designed to develop safer and protective herbal combination to prove the free radical scavenging effect as a new alternative. The ethanolic extracts of *Tridax procumbens* leaves and *Boerhavia diffusa* roots individually and in combination were tested for their radical scavenging ability like DPPH, Hydrogen peroxide, nitric oxide and ferrous ion. The activities of the medicinal plants were compared with standard antioxidant ascorbic acid. All the free radicals were effectively scavenged by all the three plant extracts. The Combinative ethanolic extract showed maximum scavenging activity, followed by the *Boerhavia diffusa* extract and *Tridax procumbens* extract. The results of the present study showed that the combinative plant extract exhibited synergistic radical scavenging activity thus proving its efficacy to be used in pharmacological industries.

Keywords: *Tridax procumbens*, *Boerhavia diffusa*, DPPH, free radical scavenging activity, antioxidant activity, hydrogen peroxide, Nitric oxide.

INTRODUCTION

Oxidative stress caused by Reactive oxygen Species (ROS) such as superoxide anion, hydroxyl radical and hydrogen peroxide play a central role in the development of various chronic and degenerative disorders such as atherosclerosis, ischemic heart disease, ageing and neurodegenerative diseases [1]. The free radicals in the human body are generated as a byproduct of biological reactions and from exogenous sources [2].

Medicinal plants possess an abundant source of phytochemicals that exhibit significant antioxidant properties. The presence of naturally occurring antioxidant compounds has developed a complex anti oxidative defense mechanism against free radicals[3]. Since antioxidants provide significant free radical scavenging activity from oxidative damage,

researchers are having profound interest in isolating new compounds from medicinal plants with antioxidant activity. High intake of antioxidant rich food can reduce the risk of degenerative diseases particularly cardiovascular diseases and cancer [4].

Tridax procumbens Linn. belongs to the family Asteraceae is a common herb found in India. It is denoted by different names, Mexican Daisy in English, Jayanti in Ayurvedic medicine, Vettukkaayathalai in Siddha/Tamil language. The whole plant was reported to treat various ailments, such as bronchial catarrh, dysentery, diarrhea, preventing hair loss, and to check hemorrhage from cuts [5].

In the Indian systems of medicine *Tridax procumbens* is used either as a single drug or in combination with other drugs. Traditionally, it is used for the treatment of bronchial catarrh, dysentery, malaria, stomach ache, diarrhoea, high blood pressure. The leaf extract has been extensively used as an anticoagulant, anticancer, antifungal and insect repellent [6].

Boerhavia diffusa is an herbaceous plant belongs of the family Nyctaginaceae. It is widely distributed in the tropics and subtropics[7]. The plant has been called by many names like "punarnava" in Sanskrit, "mukaratte" in Tamil and "thazhuthama" in Malayalam.

It has a long history of being used as an indigenous medicinal plant by the tribal people and also in Ayurveda or herbal medicines [8]. The plant roots have been widely used for the treatment of dyspepsia, jaundice, enlargement of spleen, and abdominal pain [9].

An attempt has been made in the present study to determine free radical attenuating activity of *Tridax procumbens* and *Boerhavia diffusa* individually and in combination.

Materials and Methods

Sample Collection: Freshly grown leaves of *Tridax procumbens* and roots of *Boerhavia diffusa* were collected from the local villages around Tiruchirappalli and got identified from plant taxonomist at the Department of Botany, St.Joseph's College, Tiruchirappalli.

Preparation of extractions

The collected plant parts were dried at room temperature for two weeks and were powdered. About 1 g of the powdered material was then subjected to extractions using Soxhlet apparatus using ethanol for 6 hours. The extracts were finally filtered and used for analysis.

Preparation of Herbal Combinations: Herbal combination was prepared by appropriately mixing the ethanolic extracts of *Tridax procumbens* leaves and *Boerhavia diffusa roots* in the ratio of 1:1.

Preparation of extract for free radical study

Different concentrations of Samples (20, 40, 60 and 80 μg/ml) were chosen for *in vitro* antioxidant activity. L-Ascorbic acid was used as the standard.

DPPH free radical activity assay

The antioxidant activity of the plant extracts was examined on the basis of the scavenging effect on the stable DPPH free radical activity [10]. Ethanolic solution of DPPH (0.05 mM) (300μl) was added to 40 μl of extract solution with different concentrations (0.02 - 2 mg/ml). DPPH solution was freshly prepared and kept in the dark at 4°C. Ethanol 96% (2.7 ml) was added and the mixture was shaken vigorously. The mixture was left to stand for 5 min and absorbance was measured spectrophotometrically at 517 nm. Ethanol was used to set the absorbance zero. A blank sample containing the same amount of ethanol and DPPH was also prepared. All determinations were performed in triplicate. The radical scavenging activities of the tested samples, expressed as percentage of inhibition were calculated according to the following equation.

Percent (%) inhibition of DPPH activity = [(AB – AA) / AB] x 100

Ferrous ion chelating activity assay

The chelating activity of the extracts for ferrous ions Fe^{2+} was measured according to the method of [11].To 0.5 ml of extract, 1.6 ml of deionized water and 0.05 ml of $FeCl_2$ (2mM) was added. After 30 s, 0.1 ml ferrozine (5mM) was added. Ferrozine reacted with the divalent iron to form stable magenta complex species that were very soluble in water. After 10 min at room temperature, the absorbance of the Fe^{2+}-Ferrozine complex was measured at 562 nm. The chelating activity of the extract for Fe^{2+} was calculated as:

Chelating rate (%) = $(A_0 - A_1) / A_0 \times 100$

Nitric oxide scavenging activity assay

Nitric oxide radical scavenging activity was determined according to the method [12].Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be determined by the use of the Griess reaction. 2 ml of 10mM sodium nitroprusside in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of extract at various concentrations and the mixture incubated at 25°C for 150 min. From the incubated mixture 0.5 ml was taken out and added into 1.0 ml sulfanilic acid reagent (33% in 20% glacial acetic acid) and incubated at room temperature for 5 min. Finally, 1.0 ml N-1-naphthylethylene diamine dihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30 min. The absorbance at 540 nm was measured with a spectrophotometer. The nitric oxide scavenging activity was calculated according to the following equation:

$$\% \text{ Inhibition} = ((A_0 - A_1) / A_0 \times 100)$$

Hydrogen peroxide scavenging activity assay

Hydrogen peroxide scavenging activity of the extract was estimated by method[13].Aliquot of 1.0 ml of 0.1 mM H_2O_2 and 1.0 ml of various concentrations of extracts were mixed, followed by 2 drops of 3% ammonium molybdate, 10 ml of 2M H_2SO_4 and 7.0 ml of 1.8M KI. The mixed solution was titrated with 5.09mM NaS_2O_3 until yellow color disappeared. Percentage of scavenging of hydrogen peroxide was calculated as:

$$\% \text{ Inhibition} = (V_0 - V_1) / V_0 \times 100$$

Statistical analysis

Tests were carried out in triplicate for 3 separate experiments. The scavenging activity of sample was expressed as 50% effective concentration (EC_{50}), which represented the concentration of sample having 50% of radical scavenging effect. The amount of extract needed to inhibit free radicals concentration by 50%, IC_{50}, was graphically determined by a linear regression method using Ms- Windows based graphpad Instat (version 3) software.

Results and Discussion

Lipid peroxidation leads to the generation of free radicals which ultimately results in extensive damage to tissues and biomolecules. Medicinal plants act as an excellent drug source to alleviate diseases associated with oxidative stress [14].

DPPH free radical scavenging activity

DPPH radical scavenging activity was observed in all the extracts, the combined extract showed dominant activity followed by *Boerhavia diffusa* and *Tridax procumbens* extract (Figure-1). The IC_{50} values were calculated and are depicted in Table 1.

Ferrous ion chelating activity

Ferrous ion radical scavenging activity shown by the three extract was concentration dependent with an IC_{50} value of 88.84±6.21, 76.92±5.38mg /mL and 68.46±4.79respectively (Table 2 and Figure 2).The free radical activity was found to be maximum at 80µg/ml.Among the three extracts ,the combine extract showed significant scavenging activity.

Nitric oxide chelating activity

The Nitric oxide scavenging activity was shown in Table 3and figure 3.The ethanolic extract of the three plants caused a moderate dose-dependent inhibition of nitric oxide generation based on previous reports [15]. Inhibition of NO generation was exhibited by the aqueous extract of *Wasabia japonica* [16].

Hydrogen peroxide chelating activity

H_2O_2, a cellular metabolic substance causes cell dysfunction and generation of diseases when accumulated in large amount[17].The ethanolic extract of the three medicinal plants scavenged H_2O_2 in a dose-dependent manner.(Table 4 and figure 4).The strong antioxidant activity and H_2O_2 scavenging effect of water and ethanolic extract of *Ocimum basilicum* was reported by Gulcin[18]

The ethanolic extract of the three medicinal plants scavenged H_2O_2 in a dose-dependent manner. The results of the study revealed that the ethanolic combined extract exhibited the highest H_2O_2 scavenging activity. The antioxidant potential studied in *Trigonella foenum graecum* L. varied considerably among different radical scavenging assay, and the results showed promising source of natural antioxidants [19].

Table 1: DPPH Radical scavenging activity of plant extracts standard as ascorbic acid

Test samples	20 (µg/ml)	40 (µg/ml)	60 (µg/ml)	80 (µg/ml)	IC_{50} (µg/ml)
*Tridax*extract	16.37±1.14	31.82±2.22	54.10±3.78	79.55±5.56	54.28
*Boerhavia*extract	20.46±1.43	39.55±2.76	61.37±4.29	87.28±6.10	48.05
Combined extract	23.64±1.65	42.73±2.99	74.10±5.18	93.64±6.55	42.93
Standard (Ascorbic acid)	25.6±1.79	61.26±4.28	88.98±6.22	99.34±6.95	34.89

Values were expressed as mean ± SD for triplicates

Figure 1

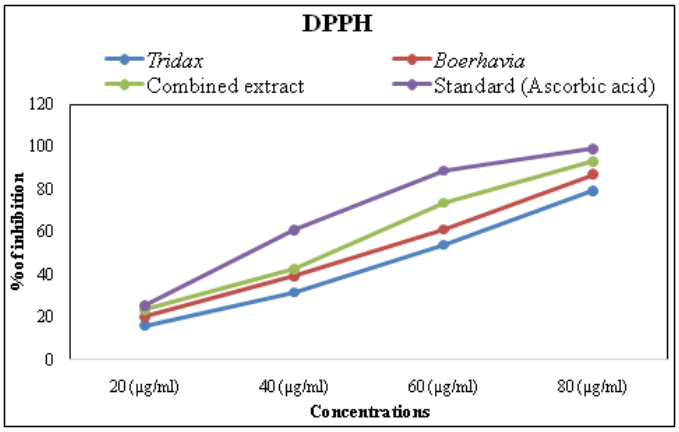


Table 2: Ferrous iron chelating activity of plant extracts

Test samples	20 (µg/ml)	40 (µg/ml)	60 (µg/ml)	80 (µg/ml)	IC_{50} (µg/ml)
*Tridax*extract	15.38±1.07	26.92±1.88	53.84±3.76	68.46±4.79	59.50
*Boerhavia*extract	21.53±1.50	39.61±2.77	62.30±4.36	76.92±5.38	49.90
Combined extract	24.23±1.69	41.15±2.88	76.92±5.38	88.84±6.21	43.21
Standard (Ascorbic acid)	35.23±2.46	65.21± 4.56	78.51± 5.49	98.65± 6.90	30.939

Values were expressed as mean ± SD for triplicates

Figure 2:

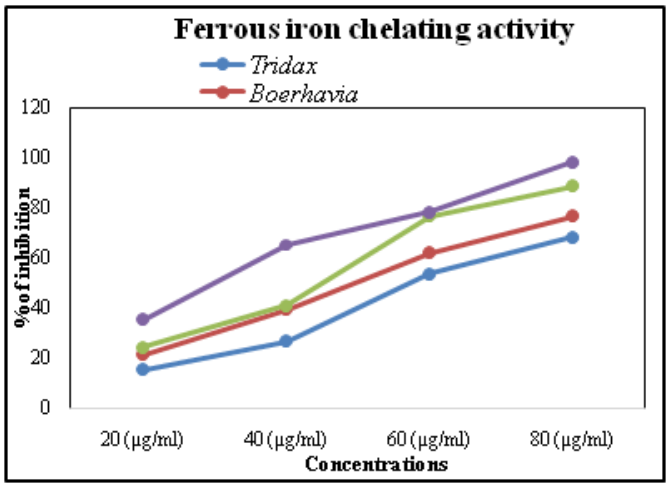


Table 3 Nitric oxide scavenging activity of plant extracts

Test samples	20 (µg/ml)	40 (µg/ml)	60 (µg/ml)	80 (µg/ml)	IC_{50} (µg/ml)
*Tridax*extract	19.04±1.33	34.76±2.43	62.38±4.36	78.57±5.49	51.27
*Boerhavia*extract	20.47±1.43	38.09±2.66	71.42±4.99	83.80±5.86	46.91
Combined extract	22.85±1.59	43.33±3.03	77.61±5.43	92.38±6.46	42.55
Standard (Ascorbic acid)	26.21 ±1.83	59.62±4.17	84.23 ±5.89	96.45 ±6.75	35.86

Values were expressed as mean ± SD for triplicates

Figure 3:

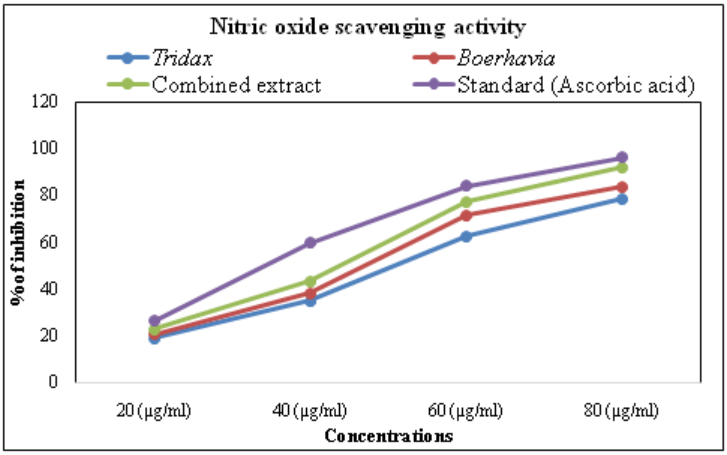
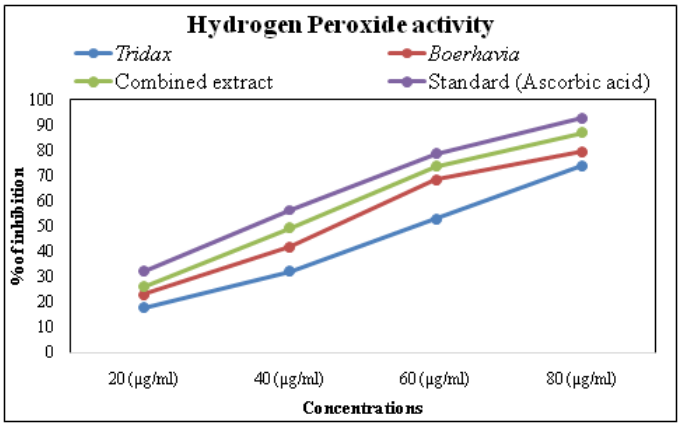


Table 4 Hydrogen Peroxide activity of plant extract

Test samples	20 (µg/ml)	40 (µg/ml)	60 (µg/ml)	80 (µg/ml)	IC_{50} (µg/ml)
*Tridax*extract	17.50±1.22	32.08±2.24	52.91±3.70	74.16±5.19	56.11
*Boerhavia*extract	22.91±1.60	41.66±2.91	68.33±4.78	79.58±5.57	46.82
Combined extract	25.83±1.80	49.16±3.44	73.75±5.16	87.08±6.09	41.40
Standard (Ascorbic acid)	32.21± 2.25	56.45± 3.95	78.65±5.50	92.75±6.49	35.26

Values were expressed as mean ± SD for triplicates

Figure 4:



Conclusion

The three different plant extracts exhibited good radical quenching ability against a battery of radicals namely DPPH, Ferrous ion, H_2O_2 and Nitric oxide. It can be concluded that the synergistic potential of *Tridax procumbens* and *Boerhavia diffusa* combination possessed enormous antioxidant potential and an effective radical scavenger, indicating the potential of the extracts as a source of natural antioxidants or nutraceuticals to reduce oxidative stress and therefore slow down the degenerative diseases.

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