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RESEARCH ARTICLE

EVALUATION OF ANTIOXIDANT POTENTIAL OF ANDROGRAPHIS ECHIOIDES AND BOERHAVIA DIFFUSA

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ABSTRACT

Free radical mediated oxidative stress is believed to be the primary cause of many disorders, such as cardiovascular diseases, cataract, arthritis, brain dysfunction, diabetes mellitus, cancer, ageing etc. In treatment of these diseases, antioxidant therapy has gained an utmost importance in the resent years. Current research is now directed towards finding naturally occurring antioxidants of plant origin. *Andrographis echioides* and *Boerhavia diffusa* are important medicinal plants, which have a wide range of application. In the present study, the antioxidant potential of ethanolic extract of *Andrographis echioides* and *Boerhavia diffusa* was evaluated by determining the levels of enzymatic and non-enzymatic antioxidants. Our results showed that both the plant extracts possessed significant levels of enzymatic and non-enzymatic antioxidants. However, *Andrographis echioides* showed higher levels of enzymatic and non-enzymatic antioxidants than *Boerhavia diffusa*.

INTRODUCTION

Free radicals and other reactive oxygen species are derived either from normal essential metabolic processes in the human body or from external sources such as exposure to radiation, ozone, cigarette smoking, air pollutants and industrial chemicals (Langseth, et al., 1996). The free radicals are major cause of human cancer and other diseases. That the risk of diseases can be reduced by increased consumption of antioxidants which are abundant in food (McLarty, 1997). Free radicals which have one or more unpaired electrons are produced in normal or pathological cell metabolism. Reactive oxygen species (ROS) react easily with free radicals to become radicals themselves. ROS are various forms of activated oxygen, which include free radicals such as superoxide anion radicals ($O_2^{\cdot -}$) and hydroxyl radicals ($OH^{\cdot}$), as well as non-free radical species (H_2O_2)and the singled oxygen ($^1O_2$) (Adeolu et al., 2009).

Free radicals can cause lipid peroxidation in foods, which leads to their deterioration (Sasaki et al., 1996 & Miller et al., 1995). In addition, reactive oxygen species have been implicated in more than 100 diseases, including malaria, acquired immunodeficiency syndrome, heart disease, stroke, arteriosclerosis, diabetes, and cancer (Tanizawa et al., 1992, Hertog et al., 1993, Alho et al., 1999 & Duh 1998).When produced in excess, ROSs can cause tissue injury. However, tissue injury can itself cause ROS generation (Auroma 1998).

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As a result of this, much attention has been focused on the use of antioxidants, especially natural antioxidants to inhibit lipid peroxidation and to protect from damage due to free radicals. A great number of aromatic and other medicinal plants contain chemical compounds that exhibit antioxidant properties. Sources of natural antioxidants are primarily, plant phenolics that may occur in all parts of plants such as fruits, vegetables, nuts, seeds, leaves, roots and barks (Pratt et al., 1990 & Mathew et al., 2006).

Andrographis echioides belongs to the family of Acanthaceae and commonly known as *False water willow*. It widely distributed in the dry districts of tropical India & Srilanka. In traditional medicine, the leaf juice of this plant is used as a remedy for fevers. *Boerhavia diffusa L.*, commonly known as purnarnava in Sanskrit, is an herbaceous plant of the family Nyctaginaceae. The medicinal value of this plant in the treatment of a large number of human ailments is mentioned in Ayurveda, Charaka Samhita, and Sushrita Samhita. It has many ethnobotanical uses (the leaves are used as vegetable; the root juice is used to cure asthma, urinary disorders, leukorrhea rheumatism, and encephalitis), and is medicinally used in the traditional, Ayurvedic system. Besides, the *B. diffusa* plant is reported to posses many pharmacological, clinical, and antimicrobial properties. Recently, the authors observed potent antiviral efficacy of this plant against phyotopathogenic viruses(Awasthi, 2000).

In the present study, we have evaluated the antioxidant property and potential of the ethanolic extract of *Andrographis echioides* and *Boerhavia diffusa* by

determining various enzymatic and non-enzymatic antioxidants.

MATERIALS AND METHODS

Collection and identification of plant materials

The *Andrographis echioides* and *Boerhavia diffusa* plant were collected from Kolli Hills, Tamil Nadu, India. The plant species were authenticated by the taxonomist, Consultant Central Siddha Research, ABS garden, Karipatty, Salem, Tamil Nadu, India.

Extraction of plant materials

The fresh leaves of *Andrographis echioides* and *Boerhavia diffusa* were collected and washed thoroughly with distilled water. The cleaned leaves are then allowed for the complete shade drying and then made to fine powder with homogenizer. About 1gm of clean dried leaves of *Andrographis echioides* and *Boerhavia diffusa* was taken along with 10 ml of ethanol and mashed well using a homogenizer and filtered and used for determining various enzymatic and non-enzymatic antioxidants.

Enzymatic antioxidants

Assay of Superoxide Dismutase (Kakkar et al., 1984**):** 0.5 ml of homogenate was diluted to 1 ml with water. Then 2.5 ml of ethanol and 1.5 ml chloroform (all reagents chilled) was added. This mixture was shaken for 1 minute at 4°C and then centrifuged. The enzyme activity in the supernatant was determined.

Assay of Catalase (Sinha et al., 1972**):** To 0.9 ml of phosphate buffer, 0.1 ml of homogenate and 0.4 ml of hydrogen peroxide were added. After 60 sec. 2.0 ml of dichromate acetic acid mixture was added. The tubes were kept in boiling water bath for 10 minutes and the colour developed was read at 620 nm.

Assay of Glutathione Peroxidase (Rotruck et al., 1973**):** To 0.2 ml of tris buffer, 0.2 ml of EDTA, 0.1 ml of sodium azide and 0.5 ml of homogenate were added. To the mixture, 0.2 ml of glutathione followed by 0.1 ml of hydrogen peroxide was added. The contents were mixed well and incubated at 37°C for 10 minutes along with a tube containing all the reagents except sample. After 10 minutes the reaction was arrested by the addition of 0.5 ml of 10% TCA, centrifuged and the supernatant was assayed.

Assay of Glutathione-S- Transferase (Habig et al., 1974**):** The reaction mixture contained 1.0 ml of phosphate buffer, 0.1 ml of CDNB, 0.1 ml of homogenate and 0.7 ml of distilled water. The reaction mixture was incubated at 37°C for 5 minute then the reaction was started by the addition of 0.1 ml of 30mM glutathione. The absorbance change was read at 340nm for 5 minutes. Reaction mixture without the enzyme was used as the blank.

Assay of Glucose-6-Phosphate Dehydrogenase (Ellis et al., 1961**):** The incubation mixture contained 1.0 ml of buffer, 0.1 ml of magnesium chloride, 0.1 ml of $NADP^+$, 0.5 ml of phenazine methosulphate, 0.4 ml of the dye solution and the requisite amount of the enzyme extract. The mixture was allowed to stand at room temperature for 10 min to permit the oxidation of endogenous materials. The reaction was initiated by the addition of 0.5 ml of glucose-6-phosphate. The absorbance was read at 640nm against water blank at one min intervals for 3-5 min in a UV Spectrophotometer. The activity of the enzyme was calculated in units by multiplying the change in OD/min by the factor 6/17.6, which is the molar extinction co-efficient of the reduced co-enzyme.

Non-enzymatic antioxidants

Estimation of Vitamin C (Baker et al., 1980**):** 0.5 ml of tissue homogenate was mixed thoroughly with 1.5 ml of 6% TCA and centrifuged for 20 minutes at 3500 g. To 0.5 ml of the supernatant, 0.5 ml of DNPH reagent was added and mixed well. The tubes were allowed to stand at room temperature for an additional 3 hours. Removed, placed in ice-cold water and added 2.5 ml of 85% sulphuric acid and allowed to stand for 30 minutes. A set of standards containing 10-50µg of ascorbic acid were taken and processed similarly along with a blank, containing 0.5 ml 4% TCA. The colour developed was read at 530 nm.

Estimation Of Vitamin E (Omaye et al., 1979**):** This method involves the reduction of ferric ion to ferrous ion by α-tocopherol and the formation of a red coloured complex with 2,2′-dipyridyl. absorbance of the chromophore was measured at 520 nm.

Estimation of Reduced Glutathione (Ellman et al., 1972**):** A known weight of tissue was homogenized in phosphate buffer. From this 0.5 ml was pipetted out and precipitated with 2.0 ml of 5% TCA. 1.0 ml of the supernatant was taken after centrifugation and added to it 0.5 ml of Ellman's reagent and 3.0 ml of phosphate buffer. The yellow colour developed was read at 412 nm. A series of standards were treated in a similar manner along with a blank containing 3.5 ml of buffer.

RESULTS

The antioxidant potential of *Andrographis echioides* and *Boerhavia diffusa* was evaluated and the data obtained was given in Table (2 and 3). The results revealed that the *Andrographis echioides* have a high antioxidant activity than *Boerhavia diffusa*.

DISCUSSION

Oxidative stress has been implicated in the pathology of many diseases and conditions including diabetes, cardiovascular diseases, inflammatory conditions, cancer and ageing (Marx, 1987). Antioxidants may offer resistance against the oxidative stress by scavenging the free radicals, inhibiting the lipid per oxidation and by many other mechanisms and thus prevent diseases(Braugghler,1986).

The table (1) p value ($p<0.05$) represents the enzymatic antioxidants content of *Andrographis echioides* and *Boerhavia diffusa.* Among the enzymatic antioxidants the level of SOD in *Andrographis echioides* is remarkable followed by GPx and Catalase. The GST and G6PD level of *Andrographis echioides* and *Boerhavia diffusa* varied moderately. The high content of enzymatic antioxidant store in *Andrographis echioides* is comprehensively validated from the table values. The non-enzymatic antioxidants level of *Andrographis echioides* and *Boerhavia diffusa* are presented in the table (2) represents the $p<0.05$ level. The Vit C and E level seems to be highly significant in *Andrographis echioides* than *Boerhavia diffusa,* while the level of GSH is markedly enhanced in *Boerhavia diffusa* than *Andrographis echioides.* Both *Andrographis echioides* and *Boerhavia diffusa* species possess a commendable level of non-enzymatic antioxidants.

The results of the enzymatic and non-enzymatic antioxidants in *Andrographis echioides* and *Boerhavia diffusa* exhibits that they possess preventive and productive role to maintain the cell survival, cellular interaction and

Table 1: Enzymatic antioxidant levels of *Andrographis echioides* and *Boerhavia diffusa.*

Medicinal Plants	SOD µg/g	Catalase µg/g	GPx µg/g	GST µg/mg	Glu-6 PhDase MIU/mg
Andrographis echioides (Mean ± SD)	791.62 ±3.9708*	568.224 ±3.1229*	637.5 ±2.1*	68.624 ±2.0*	0.752 ±0.14
Boerhavia diffusa (Mean ± SD)	10.41 ±0.282	10.6 ±3.318	19.27 ±4.436	52.54 ±4.504	0.856 ±0.1495

Values are mean ±S.D *p<0.05

Table 2. Non-enzymatic antioxidant levels of *Andrographis echioides* and *Boerhavia diffusa.*

Medicinal Plants	Vitamin C µg/g	Vitamin E µg/g	GSH µg/g
Andrographis Echioides (Mean ± SD)	88±21.35*	131.08±2.83*	9.23±1.52
Boerhavia diffusa (Mean ± SD)	26.2±4.53	16.6±4.07	18.66±4.24*

Values are mean ±S.D *p<0.05

maintenance of cell membrane architecture. Further investigation is needed in order to fully elucidate the mechanism of action. *Andrographis echioides* and *Boerhavia diffusa* have effective and therapeutic antioxidant potential against various inflammatory diseases. Based on the results from the present study, it can be concluded that *Andrographis echioides* and *Boerhavia diffusa* were found to be a good natural antioxidant sources, with *Andrographis echioides* have higher antioxidant potential than *Boerhavia diffusa*.

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