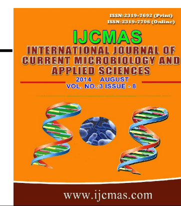

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Original Research Article

In-vitro Screening of an Antioxidant Potential of *Byttneria herbacea* Roxb

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A B S T R A C T

Keywords

Byttneria herbacea, antioxidant activity, nitric oxide, catalase and superoxide dismutase.

Current advances in an antioxidant investigation have shown that the potential antioxidant properties of many medicinal plants could be used as a source for the treatment of oxidative stress related diseases in human beings. We have not cited any work done with an antioxidant activity of *Byttneria herbacea* hence, we have undertaken this study to evaluate an antioxidant properties of this plant species and for the first time, we were reported its antioxidant capacity. The present study was carried out to investigate the *in vitro* antioxidant activities of aqueous extract from the leaves, stem and root of *B. herbacea*. To evaluate an antioxidant activity of three favorable *in vitro* test methods was used, including nitric oxide, catalase and superoxide dismutase. The results indicated that *B. herbacea* exhibits a good antioxidant activity.

Introduction

Antioxidants are micronutrients that have gained importance in recent year due to their ability to neutralize free radicals, or their actions (Mishra *et al.,* 2006). Reactive oxygen species (ROS) are an entire class of highly reactive molecules derived from the metabolism of oxygen. Moreover, ROS can cause extensive damage to cells and tissues, during infections and various degenerative disorders, such as cardiovascular disease, aging, and neurodegenerative diseases like Alzheimer's disease, mutations and cancer (Ames1998; Cox and Cohen 1996; Finkel and Holbrook 2000; Harman 1994).

Although many anti-oxidant defense systems consisting of enzymatic (Superoxide dismutase (SOD), Catalase (CAT) and Glutathione peroxidase (GPx) and non enzymatic (ascorbic acid, glutathione, and α-tocopherol) compounds can maintain the balance between ROS generation and protection from damage by ROS, these anti-oxidant systems do not provide complete protection from ROS attack under conditions of severe oxidative stress (Cesaratto, *et al.,* 2004).

Excessive amounts of ROS may overwhelm

natural antioxidant defenses promoting DNA, lipid and protein oxidative damage and oxidative stress, which may lead to cell injury and death (Ames, *et al*., 1983). Long term oxidative stress has been associated with numerous diseases and disorders in higher organisms. The negative effects of oxidative stress may be mitigated by the consumption of antioxidants. Antioxidants are compounds that can delay or inhibit the oxidation of lipids and other molecules and by doing so inhibits the initiation and propagation of oxidative chain reactions. They act by one or more of the following mechanisms: reducing activity, free radical scavenging and potential complexation of pro-oxidant metals and quenching of singlet oxygen. Many epidemiological studies have shown that numerous phytonutrients found in fruits and vegetables are able to protect the human body against damage by ROS. In fact, it is well established that the consumption of natural antioxidant phytochemicals was reported to have many potential health benefits (Sumino, *et al*., 2002). 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 (Auddy *et al*., 2003).

Plants play a significant role in the development of novel drugs and in many developing country's attention has been paid to explore natural substances as substitutes for synthetic compounds. The commonly used anti-oxidants, butylated hydroxyanisol and butylated hydroxytolune are synthetic chemicals and the possible toxicity of these anti-oxidants has resulted in their reduced usage (Ito *et al*.,1985). Due to health concerns, natural anti-oxidants have been extensively employed in recent years (Yen *et al*., 2003). Plants and other natural products contain hundreds of compounds those acts as a natural antioxidant. Therefore, several methods have been developed to quantify these compounds individually. The techniques are different in terms of mechanism of reaction, effectiveness and sensitivity (Khal *et al*., 1986, Frankel *et al*., 1993, and Koleva *et al*., 2002).

Recent research finds interest in natural antioxidants from ethnomedicinal plant sources. Increased use of different chemicals, pesticides, pollutant, some of synthetic medicine, smoking and alcohol intake enhances the chance of free radicals based diseases. The present study is a compilation of three different *in vitro* assay methods used in determining the antioxidant activity of extracts of different plant parts of *B. herbacea*. The selected plant species is branched (sometimes unbranched) procumbent spreading herb with a perennial, 4 -10 cm long woody root-stocks. Wildlife Institute of India, Dehradun's Envis Bulletin (Envis Bulletin 2008) has recognized *B. herbacea* is an endemic to the Indian Peninsular region and categorized into the IUCN threatened category. Previous studies have revealed that the *B. herbacea* crude drug is used in health care by indigenous cultures as a medicine in various ailments. In traditional herbal medicine, various parts of *B. herbacea* is used as an ethnoveternary medicine to cure dysentery, impaction (Reddy, *et al*., 1998), to treat leprosy (Jain, S P *et al*.,) to promote retention of placenta (Patel, *et al*., 2003), for treatment of fracture of limbs (Prusti 2007) to get relief against asthma (Chandra Babu, *et al*., 2010), in leucorrhoea and inflammation (Ashutosh, *et al*., 2010). Parrotta (2001) reported *B. herbacea* as a healing plant of Peninsular India, National Medicinal Plant Board (2012) enlisted *B. herbacea* as medicinal

plant of India, while Envis. (Frlht.) documented *B. herbacea* as a medicinal plant of West Bengal. Day *et al*.,(2012) reported the use of *B. herbacea* root paste on the wound, Mallik *et al*.,(2012) documented that the *B. herbacea* root paste is taken orally to get relief from body pain, Sreeramulu *et al.* (2013) have found that *B. herbacea* leaf is used against dysentery and impaction while recently Suthari *et al.* (2014) observed *B. herbacea* root used against swellings.

Materials and Methods

Plant Material and Preparation of Extracts

Fresh plant parts from four different plants of *Byttneria herbacea* were collected in chilled container from Satnavari (Nagpur) forest in the month of October. The various plant parts, i.e. leaves, stem and root was thoroughly washed in cold water to remove the earthy material. 1 mg of each plant part was weighed and dissolved in 10 ml of 0.1 N Phosphate buffer pH 6.8 and homogenized separately (Kar, and Mishra, 1976). The homogenized extract was then centrifuged at 10,000 g for 20 minutes at 4˚C. All samples were recentrifuged at same condition to avoid the carryover of plant materials. The clear supernatant was used for an enzymatic analysis.

Enzymatic Analysis

An activity of catalase was estimated following the methodology given by Aebi *et al*., (1976). Total 3ml reaction cocktail contains 1.980 ml of 50mM phosphate buffer, 30mM H_2O_2 1ml and 0.020 ml enzymatic sources. Blank was prepared without enzymatic source. The rate of H_2O_2 decomposition was measured at 240nm. Activity of an enzyme was expressed as units/mg protein.

Superoxide dismutase was estimated according to Marklund *et al*., (1974). The reaction cocktail contains 1.480ml of 50mM Tris HCL buffer, 20µl enzyme source, 6 mM 0.5ml EDTA and 0.2 mM 1ml pyrogallol. Control was prepared without enzyme source. The absorbance was recorded at 420 nm for 3 min in spectrophotometer. Activity was expressed as units/mg protein.

The concentration of nitric oxide was estimated by using methodology provided by Green *et al*. (1982). An equal volume of Greiss reagent (0.1% Napthyl Ethylene Diamine Dihydrochloride and 1% Sulpholylamide in 5% Ortho-Phosphoric

Fig.1 ***Byttneria herbacea*** **Roxb.**

Fig.2 Plant Extracts

acid) and nitric oxide reacts to form a pink colored complex which was estimated at 535nm. Concentration was calculated using standard graph of 0.1mM sodium nitrate solution.

Protein was estimated as per Lowery *et al.*, (1951) methodology. Reaction mixture contains 0.010 µL protein source, 990 µL distilled water, 5 ml Alkaline copper sulphate solution and 0.5 ml Folin Ciocalteu reagent. Blank was prepared without protein source. The concentration of an unknown protein sample was estimated using 200µGm of the standard BSA solution.
All samples were run in triplicates.

Statistical Analysis: All statistical analysis was done using Med Calc and Epi Info statistical software. Students "T" test for two tailed probabilities assuming unequal variance was used for significant differentiation. The criterion for significant difference was 0.05.

Results and Discussion

This study compared an activity of catalase, superoxide dismutase and concentration of nitric oxide in leaf to root and stem samples of *Byttneria herbacea*. We also compared stem and root samples of the same plant for significant differentiation for the above mentioned parameters; to evaluate an antioxidant capacity of this plant. The result shows that there was a more significant activity of catalase in leaf than root ($P<0.05$). An activity of catalase was significantly more in stem samples as compared to leaf ($p<0.001$) and root sample ($p<0.001$) [Table 1, Fig 3 (A)].

This study has been demonstrated that there was no significant difference of superoxide dismutase activity in leaf and root samples ($p>0.05$), but there was a more significant activity of this enzyme in stem than leaf ($p<0.001$) and root ($p<0.001$) [Table 1, Fig 3 (B)]. Likely, we reported significantly more concentration of nitric oxide not only in root when it was compared to leaf ($p<0.001$) but also in stem as compared to leaf ($p<0.001$). This study also shows a significantly more concentration of nitric oxide in stem samples when it was compared to the root sample ($p<0.05$) [Table 1, Fig 3 (C)].

Oxidative stress is mainly because of an imbalance between the concentration of antioxidant enzymes and the free radicals. To maintain a proper redox potential within the cell it is an important to maintain the balance between free radicals and its scavenging systems. Now-a-days medical industries have shifted their interest of research on an antioxidant property of various herbs, shrubs and plants so that industries could generate the new biological based formulas to eradicate free radicals based damages within the cells. This study demonstrates an antioxidant enzyme levels in *Byttneria herbacea*.

Catalase is an antioxidant enzyme widely distributed in all animal and plant tissues. An increased concentration of hydrogen peroxide inside of biological cell can increase an oxidative stress, which may result in serious complications within the cells. Hydrogen peroxide if not removed from the cell, it could convert into a hydroxyl radical. Hydroxyl radicals are the most deadliest free radicals, which have ever been found (Tiedge, *et al*., 1998 and Chelikani, *et al*., 2004). Catalase decomposes hydrogen peroxide and protects the tissues or cells from highly reactive hydroxyl radicals (2003). Hence, catalase enzyme can play a pivotal role in maintaining the redox reaction within the cells.

Table.1 A statistical analysis of catalase, superoxide dismutase and nitric oxide in *B.herbacea.*

ENZYME	SAMPLES	MEAN±SD
CATALASE	LEAF	6.23±0.37
(UNITS/ MG PROTEIN/ML)	ROOT	5.40±0.13*
	STEM	9.28±0.44***
Superoxide Dismutase (SOD)	LEAF	1.20±0.11
(UNITS/ MG PROTEIN/ML)	ROOT	1.30±0.23**
	STEM	5.26±0.23***
Nitric Oxide (NO)	LEAF	3.67±0.60
(μM / ML)	ROOT	6.42±0.37***
	STEM	7.20±0.29***

*$p<0.05$, **$p>0.05$, ***$p<0.001$; p-values of catalase, SOD and NO for the stem and root samples are $p<0.001$, $p<0.001$ and $p<0.05$ respectively.

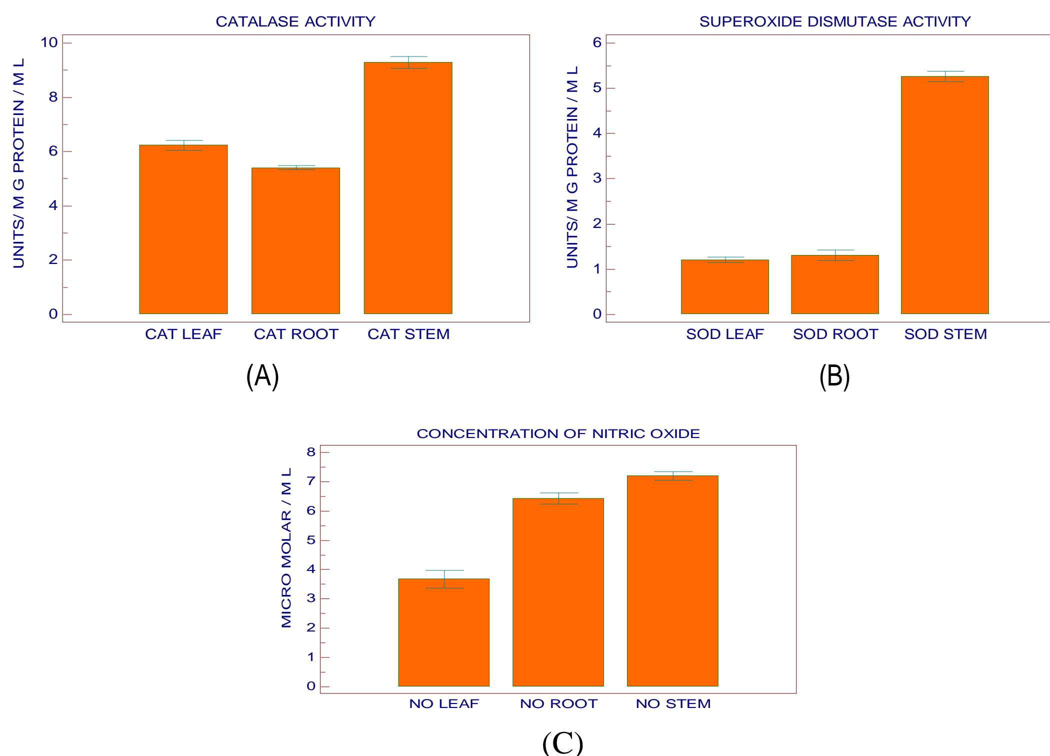

Fig.3 An activity of Catalase (A), Superoxide Dismutase (B) and concentration of Nitric Oxide (C) in leaf, root and stem of *Byttneria herbecea*.

Zong *et al*., (2013) demonstrates an increase levels catalase in *Ambrosia artemisiifolia* L (belonging to the family Malvaceae, same family of *B. herbacea*) at different irradiance level.

Superoxide dismutase is another important antioxidant enzyme involved in the defense mechanism against free radicals (Zelko, *et al*., 2002). Superoxide dismutase constitutes an important link in the biological defense mechanism through dismutation of endogenous cytotoxic superoxide radicals to H_2O_2 (Hassan 1988). Hence, to eradicate a superoxide anion is a prime important function of superoxide dismutase enzyme. Shuge *et al*., (2014) prior studies done on *Althaea rosea* seeds demonstrate a potent scavenger for superoxide anion radicals.

Nitric oxide radical inhibition assay proved that some medicinal plants have a good potential to scavenge nitric oxide. The nitric oxide generated from sodium nitro prusside reacts with oxygen to form nitrite. The extract of aerial plants inhibits nitrite formation by competing with oxygen to react with nitric oxide directly and also to inhibit its synthesis. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide (Marcocci, *et al*., 1994). Nitric oxide can act as a free radical in biological system. Therefore, an evaluation of nitric oxide concentration is important in biological samples (Green *et al*., 1982). In this study, the concentration of nitric oxide was reported more in stem samples rather than leaf and root samples, but root samples exhibited more concentration of nitric oxide than leaf samples.

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