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Boerhaavia erecta-A potential source for phytochemicals and antioxidants

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ABSTRACT:
To estimate phenols and flavonoids, enzymic antioxidants in the leaves of *Boerhaavia erecta* (LBE). The sample was extracted with 0.1M phosphate buffer for the analysis of phenols, flavonoids and antioxidants. The total phenolic content and total flavonoids in LBE were measured using Folin-Ciocalteau and aluminium chloride colorimetric methods respectively. The activity of Superoxide Dismutase (SOD) was assessed by the formation of nitrite from hydroxylamine hydrochloride, Catalase (CAT) activity was determined by reduction of dichromate in acetic acid to chromic acetate, the peroxidase (POD) activity was assessed by the formation of oxidised pyrogallol colorimetric methods. Methanolic extraction of the sample was done for the HPTLC analysis. The presence of phenols and flavonoids was analysed using HPTLC method. The amount of total phenols and total flavonoids present in the leaves was found to be 85.90 ± 1.97 mg CEQ /100mg and 11.39 ± 0.86 mg CE/100mg respectively. The HPTLC fingerprinting has proved the presence of four phenolic and two flavonoid compounds. The activities of SOD, CAT and POD were 0.086 ± 0.009, 0.440 ± 0.350, 40.95 ± 12.60 units/mg protein respectively. This investigation demonstrates that the leaves of *Boerhaavia erecta* have therapeutic properties and hence could be used as source for nutraceuticals.
Keywords - phenols, flavonoids, HPTLC, Nyctaginaceae.

INTRODUCTION

Herbs have been used in many domains including medicine, nutrition, flavoring, etc and other industrial purposes. Since the prehistoric era, herbs have been the basis for nearly all medicinal therapy until synthetic drugs were developed in the nineteenth century [1,2]. Traditional knowledge of medicinal plants has always guided the search for new cures [3]. The possible beneficial effects of foods are due to micronutrients and to functional food ingredients and antioxidant nutraceuticals, "Phytochemical Substances" [4]. Phytochemicals, secondary plant metabolites which are biologically active non-nutrients and antioxidants constituents in plant have raised interest among Scientists, food manufacturers, producers and consumers for their roles in protection and maintenance of human health [5]. Hence medicinal plants could be a potential source for nutraceuticals. The phytochemical substances namely, phenols and flavonoids are the major important substances responsible for the medicinal values of the plants including antioxidant, anticancer, antimicrobial activities, etc.

The main objectives of the present study were to investigate the total phenols, total flavonoids and antioxidant status in the leaves of *Boerhaavia erecta*. It is a weedy herb of the family Nyctaginaceae (fig.1) and is commonly available in almost all places. It was used as a traditional medicinal plant in Africa [6].

Fig.1 *Boerhaavia erecta*

MATERIALS AND METHODS

Chemicals: All the chemicals and solvents used in the present study were of highest purity and analytical reagent grade. Methionine, riboflavin, sulphanilamide, naphthylethylene diamine dihydrochloride and pyrogallol, DTNB, catechol and catechin were procured from Himedia laboratories, Mumbai, India. All other reagents were purchased from Ranbaxy Chemicals Ltd, Mumbai, India.

Plant material: The plant (*Boerhaavia erecta*) sample was collected from the plain regions around Thirumurthi hills, Udumalpet, Tamilnadu, India. Taxonomic authentication was done by Dr.V.S.Ramachandran, Associate Professor, Department of Botany, Bharathiar University, Coimbatore, Tamilnadu, India. The healthy leaves of the plant were separated, surface cleaned by washing with water, shade dried and powdered. The powdered sample was ground with 0.1M phosphate buffer (1g in 10ml buffer) and used for the phytochemical analysis and the sample was extracted (10g) with 100ml of 80% methanol using Soxhlet apparatus, concentrated and dried under reduced pressure and controlled temperature (40-50˚C) in rotary evaporator. The extract yielded a greenish brown residue solid which was used for the HPTLC analysis.

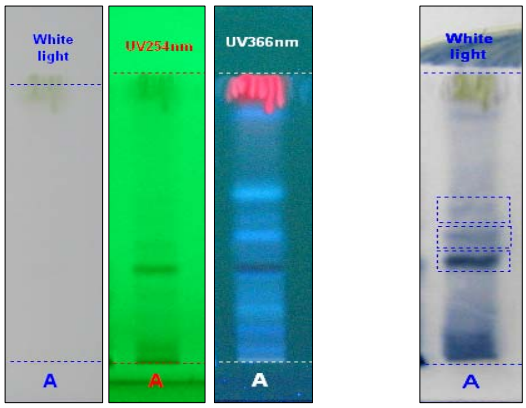
Estimations:

Phytochemical analysis: Total phenolic content was determined by the Folin-Ciocalteau method Singleton and Rossi, [7], using catechol as a standard phenolic compound and the result is expressed as mg catechol equivalents (CEQ)/100mg sample. The determination of total flavonoids was performed according to the colorimetric assay of Jia *et al.*,[8]. The result is expressed as mg catechin equivalents (CE)/100mg sample. SOD activity was ascertained using the nitrite method of Das *et al.,* [9]. And its activity is expressed as the inhibition of 50% nitrite formation/min/mg protein. Catalase activity was assayed by the method of Sinha [10]. The activity of catalase is expressed in terms of 1µmole of H_2O_2 decomposed/min/ mg protein. Peroxidase activity was assayed by the method of Addy and Goodman [11]. The difference in OD change per minute with and without enzyme addition was a measure of peroxidase activity and is expressed as 1µmole of pyrogallol oxidized/min/ mg protein.

***HPTLC determination of phenols and flavonoids*:** 50mg of methanolic extract of *Boerhavia erecta* (MEBE) was dissolved in methanol, filtered and made up to 5ml with methanol. 5µl of the made up solution was loaded in the 5 x 5 Silica gel $60F_{254}$ TLC plate using Hamilton syringe and LINOMAT 5 instrument. The sample loaded TLC plate was kept in TLC twin trough developing chamber with respective mobile phase (Phenolic compounds & Flavonoids) for 15min for Chamber saturation. The plate was developed using above mobile phase up to 80mm.The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in Photo-documentation chamber and captured the Plate images at White light, UV 254nm and UV366nm. For the derivatization of phenols and flavonoids, the plate was sprayed with respective spray reagent (Phenolic compounds & Flavonoids) and dried at 110˚C in Hot air oven. The plate was photo-documented at White light using Photo-documentation chamber. Finally, the plate was fixed in scanner stage and scanning was done at respective wavelength. The Peak table and densitogram were noted.

RESULTS AND DISCUSSION:

Table 1 shows the level of total phenols and total flavonoids in the powdered leaves of *Boerhaavia erecta*. In the present investigation, chromatographic fingerprint of MEBE has been developed by HPTLC method. HPTLC is a well defined analytical technique based on thin layer chromatography, but with enhancements intended to increase the resolution of the compounds to be separated and to allow quantitative analysis of the compounds. The blue bands in fig.2 (a) and the yellow bands in fig.3 (a) represent the presence of phenolic compounds and flavonoids respectively. By virtue of the different elution pattern (after derivatization) of MEBE showed autogenerated peaks under white light at 366 nm, which confirmed the presence of four types of phenolics, fig.2(b) and two types of flavonoids, fig. 3 (b) and their peak height and Rf values are shown correspondingly in table 2 and table 3. HPTLC method has been found to be rapid, sensitive, precise, and accurate and it has been applied for simultaneous quantitation of phytoconstituents [12].



Before Derivatization **After derivatization**

A – Methanol extract of *Boerhaavia erecta* sample

Fig.2(a) Chromatogram of Phenolic compounds

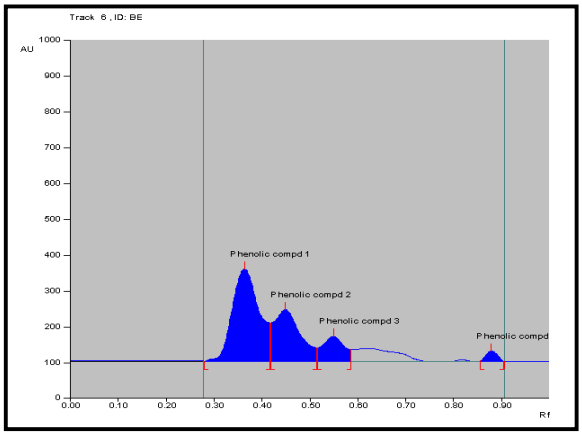
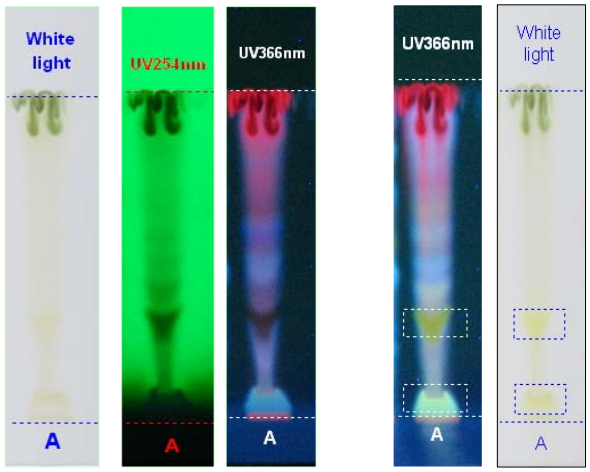


Fig 2 (b): Track A - Peak densitogram display of phenols



Before Derivatization **After derivatization**

A – Methanol extract of *Boerhaavia erecta* sample

Fig.3(a) Chromatogram of Flavanoids

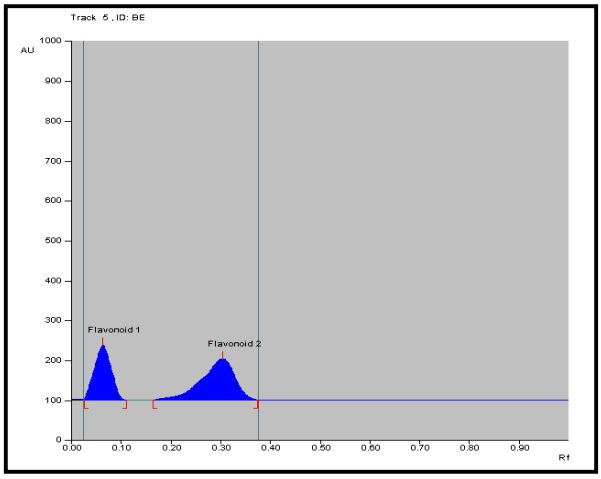


Fig 3 (b): Track A - Peak densitogram display of Flavanoids

The phenolic compounds such as flavonoids, phenolic acids and tannins are considered to be major contributors to the antioxidant capacity of plants. These antioxidants also possess diverse biological activities such as anti-carcinogenic, anti-atherosclerotic and anti-inflammatory activities. These activities may be related to their antioxidant activity [13]. So far as phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators, it was reasonable to

determine their total amount in the selected plant extract [14].

Table 1: Levels of total phenols and flavonoids

Phytochemical	Quantity
Total phenols	85.90 ± 1.97 mg CEQ /100mg
Total flavonoids	11.39 ± 0.86 mg CE/100mg

Values are expressed as mean ± SD of 3 replicates of sample

Table 2: Peak Table of Phenols

Track	Rf	Height	Area	Assigned substance
A	0.36	258	11755.8	Phenolic compound 1
A	0.45	145.4	6476.6	Phenolic compound 2
A	0.55	70.2	2568.6	Phenolic compound 3
A	0.88	29.4	566.1	Phenolic compound 4

Table 3: Peak Table of Flavonoids

Track	Rf	Height	Area	Assigned substance
A	0.06	136.9	3763.6	Flavonoid 1
A	0.3	103.5	5618.1	Flavonoid 2

Flavonoids as one of the most diverse and widespread group of natural compounds are probably the most important natural phenolics [14]. These compounds possess a broad spectrum of chemical and biological activities including radical scavenging properties [15]. A number of flavonoids have been shown to suppress carcinogenesis in various animal models. Anticancer effects may also be exhibited through selective cytotoxicity, antiproliferative actions and apoptosis [16]. From the experimental result and HPTLC analysis, it is clear that the leaves of the sample contain considerable amount of phenols and flavonoids and hence the leaves of *Boerhaavia erecta* could be used for therapeutic purposes.

Evidences obtained from laboratory and human studies suggested that antioxidants play a critical role in the maintenance of health and prevention of many diseases. Table.4 clearly shows that the leaves of *Boerhaavia erecta* were found to exhibit enzymic antioxidant activities such as Superoxide Dismutase (SOD), Catalase (CAT) and Peroxidase (POD). Plants used in traditional medicine are revalued for their therapeutic principles in several laboratories all over the world. Experimental results are suggestive that free radicals and reactive oxygen species are linked with the causation and deterioration of diseases. Reactive oxygen species (ROS) are formed constantly in living organisms as metabolic byproducts or as a result of many different environmental influences. As plants produce a lot of antioxidants to control the oxidative stress caused by sunbeams and oxygen, they can represent a source of new compounds with antioxidant activity [17]. Antioxidants are the biological compounds, which have the ability to neutralize the reactive oxygen species (ROS).

Superoxide Dismutase, Catalase and Peroxidase are involved in the clearance of superoxide and hydrogen peroxide radicals. SOD catalyses the dismutation of superoxide into H2O2, which has to be eliminated by CAT and/or peroxidase [18]. These enzymes are modulated in various diseases caused by free radical attacks [19]. Thus maintaining the balance between the rate of generation of radicals and scavenging of radicals is an essential part of biological homeostasis [20]. SOD is therapeutically used in the treatment of antioxidative and anti-inflammatory reaction [21].

Catalase was found to be important in the inactivation of many environmental mutagens. Plant catalases are reported to be very sensitive to environmental condition and have a rapid turnover rate [22]. CAT catalyses the reduction of hydrogen peroxides and thereby protect the cellular constituents from oxidative damage by highly reactive hydroxyl radicals. Catalase uses H_2O_2 as a substrate as well as hydrogen acceptor

[23]. Since catalases were found to be important in the inactivation process of environmental mutagens, they are gaining much industrial and therapeutical importance.
Peroxidase activity in plants can eliminate H2O2 by catalyzing the oxidation of wide variety of electron donors and thereby prevent the human system from many diseases [24]. In recent years, herbal medicines are gaining much importance in the prevention of diseases and several antioxidants of plant origin have been identified and used as protective agents against oxidative injury. Based on the experimental results, it is confidently inferred that the crude extract of the fresh leaves of Boerhavia erecta possesses the antioxidant potentials, Superoxide dismutase (SOD), Catalase (CAT) and Peroxidase (POD). There is considerable scientific and public interest in the important role that antioxidants may play in health care, such as by acting as cancer chemopreventive and anti-inflammatory agents and by reducing risk of cardiovascular mortality [25]. Due to the presence of antioxidant potentials, the leaves of the plant *Boerhaavia erecta* could be recommended to be used as a potent antioxidant source to prevent the oxidative stress and other diseases caused by ROS, like cancer and cardiovascular diseases.

Table 4: Antioxidant activity of *Boerhaavia erecta* leaves

Antioxidant	Activity
Superoxide Dismutase (SOD)[a]	0.086 ± 0.009
Catalase (CAT)[b]	0.440 ± 0.350
Peroxidase (POD)[c]	40.95 ± 12.60

Values are expressed as mean±SD of 3 replicates

Units:

a - Inhibition of 50% nitrite formation/min/mg protein

b - 1µmole of H_2O_2 decomposed/min/ mg protein

c - 1µmole of pyrogallol oxidized/min/ mg protein

CONCLUSION:

The phytoconstituents are the major important compounds which are responsible for the medicinal properties of the herbs. Most of the researchers suggest that the medicinal plants contain many of the phytoconstituents such as phenolic compounds, could be used for therapeutic purposes as they often exhibit a huge amount of medicinal properties such as antioxidant, anticarcinogenic, antitumor, antidiabetic, anti-inflammatory activities that are non-lethal and most valuable to the living system. The results shown in this work demonstrate that the leaves of *Boerhaavia erecta* bearing a considerable amount of phenols, flavonoids and antioxidant potentials. Since the leaves of *Boerhaavia erecta* possess phenolics, flavonoids, antioxidant activities, it could be suggested that the leaves could be used as a potential source of antioxidants in the nutraceuticals or dietary supplements to contribute the health benefit of the consumers.

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