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Antioxidant and Anticancer Study of *Boerhavia erecta*

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A B S T R A C T

Boerhavia erecta, an annual herb, has been used in folklore for managing of a wide range of diseases including cancer. However, the safety and effectiveness of this medicinal plant was better evaluated. The intention of this study was to evaluate the *in-vitro* antioxidant and anticancer activities of whole plant extract of *Boerhavia erecta* well as the qualitative phytochemical constituents. The DPPH and nitric oxide scavenging assays were used to evaluate the plant's antioxidant potential. The *in-vitro*3-(4, 5- Dimethylthiazol-2-yl)-2, 5-Diphenyltetrazolium Bromide (MTT) assay was exploited for determination of *in-vitro* anticancer activity against two cell lines: Mouse fibroblastic cell lines (L929), it's a normal cell line and human brain tumor cell lines (U87), it's a cancer cell line. Ascorbic acid and quercetin were used a standard antioxidant compound. The IC50 and CTC50 values were also estimated for antioxidant and anticancer studies respectively.

Introduction

Over the past two decades, medicinal plant has gained attention as natures cure for human cancer, is a major reason for the human mortality rate [1], especially with a growing number of population in the world.

In 2012, 40% of the developed antitumor agents are either natural products or modified natural products. Some of these have been correlated with antioxidant activities [2].

Antioxidants are the major plant products that play a major role in anticancer agents through acting as reducing agents, hydrogen donators, DPPH, nitric oxide free radical scavenging activity, etc. [3].

Anticancer agents mainly exhibit a preventative or curative role in a damaged system. Under normal conditions, the cells in which the DNA or other components were irreversibly damaged by various causes, undergo programmed cell death (apoptosis) [4].

The aim of anticancer agents is to trigger the apoptosis signaling system in these cancer cells which controls their proliferation [5].

Current research therefore seeks for novel natural products from most known medicinal plant sources that exhibit anticancer activities based on ethnobotanical or ethnomedical

studies which reveal scared prescriptions and folkloric beliefs inherited through generations [6].

Boerhaviaerecta, commonly known as the erect spiderling, erect boerhavia, Hindi Shweta and Marathi pandharipunarnava. It is an herbaceous member of the family Nyctaginaceae and mainly used as a traditional medicinal plant in Africa [7].

Boerhaviadiffusa is also an herbaceous member of the family Nyctaginaceae which has some similar properties, so from the pharmacological studies it is demonstrated that it possesses a diuretic [8], anti-inflammatory [9], antifibrinolytic [10], anticonvulsant [11] and hepatoprotective activities [12and13] these all properties are very useful to make a perfect medicinal plant.

In moderate doses, it was useful in the treatment of asthma [14]. In West and East Africa, the leaves are eaten as a vegetable or used for the preparation of sauces [15].

The focus of this research was to access *in-vitro* antioxidant and anti-cancer activities of crude methanolic extract of whole plant *Boerhaviaerecta*.

Materials and Methods

Cell lines and reagents

Mouse fibroblastic cell lines (L929) and Human brain glioblastoma cell lines (U87) were obtained from ATCC (Animal Tissue Cell Culture). Dimethyl sulphoxide (DMSO), 2, 2-Diphenly 1-picryl hydroxyl solution (DPPH), Methanol, Sodium nitroprusside solution, Naphthyl ethylene diaminedihydrochloride (NEDD), Sulphanilic acid, PBS (pH 7.4), 3-(4, 5-dimethylthiazol-2-yl) 2, 5-diphenyltetrazolium bromide (MTT) dye, etc.

Cell culture

The cell lines were cultured as slightly described modifications [16]. The L929 cell lines were cultured in MEM medium and U87 cell lines were cultured in Ham's F12 medium. All culture media were supplemented with 1% penicillin streptomycin L-glutamine (PSG) and 8% fetal bovine serum (FBS). The cells were maintained in a 5% CO_2 incubator at 37°C and sub-cultured on reaching about 70-80% confluency.

Plant material and extraction

Boerhavia erecta plant was collected from the district of Tamil Naidu. *Boerhaviaerecta* plant was dried and weighed approximately 270 gm along with 2.5 L methanol solvent. The extraction process was done by hot continuous extraction method using soxhlet apparatus (Figure 1). The process of transferring the partially soluble components of a solid to the liquid phase using a soxhlet extractor. The solid was placed in a filter paper thimble which was then placed into the main chamber of the soxhlet extractor. The solvent (heated to reflux) travels into the main chamber and the partially soluble components are slowly transferred to the solvent.

In-vitro antioxidant activity

DPPH scavenging activity

The effect of crude extract of *Boerhavia erecta* on the scavenging activity of DPPH were determined as earlier slightly modification [17]. Carry out the assay in a 96 well microtiter plate. Add 200 µl of methanolic DPPH solution in triplets as test and control and add 200 µl of methanol as test blank and control blank in singlet. To that add 10 µl of each of the test substance or the standard from the lowest concentration and 10

µl DMSO serves as control and control blank. Use 1000, 500, 250, 125, 62.5 µg/ml as final concentration of the test and standard solutions. Incubate the plates at 37^0 C for 30 minutes. Take the absorbance of each solution at 490 nm and record the values [18].

Nitric oxide free radical scavenging activity

The effect of crude extract of *Boerhavia erecta* on the scavenging activity of nitric oxide were determined as earlier slightly modification [17]. Incubate the reaction mixture (0.6 ml) in 1.5 ml centrifuge tubes containing sodium nitroprusside (10 mM, 0.4 ml), phosphate buffer saline (PBS, pH 7.4, 0.1 ml) and 0.1ml of test substance or standard in DMSO of various concentrations as test and 0.1ml of DMSO as control at 25°C for 150 minutes.

Use 1000, 500, 250, 150, 62.5 µg/ml as final concentration of the test and standard solution. Follow the same procedure for test blank and control blank. In place of sodium nitroprusside, take distilled water. After incubation, pipette out 0.05 ml of the reaction mixture containing nitrite ion from centrifuge tubes and transfer to microtiter plate in triplets as test, control. Make control blank and test blank in singlet. Add 0.1 ml of sulphanilic acid reagent to all the wells, mix well and allow it to stand for 5 min for completion of diazotization. Then, add 0.1 ml of NEDD, mix and allow it to stand for 30 minutes in diffused light. A pink colored chromophore will be formed. Take the absorbance of these solutions at 540 nm and record the values [18].

In-vitro cytotoxicity (MTT) assay

The *in-vitro* cytotoxicity of the crude extract was performed on L929 cell lines and U87 cell lines as described [19].

Day 1: Seed the cells in a 96 well plate in three rows with the average cell density of 10,000 cells per well. Incubate the 96 well culture plate at 37°C in CO_2 incubator overnight.

Day 2: Made the stock solution of plant extract (drug) in DMSO with concentration of 10mg/100µl. Dissolve it properly in the sonicator. From this stock solution, prepared five different dilution of plant extract in the complete Ham's F12 media (U87 cell lines) and MEM media (L929 cell lines) using multichannel reservoir. Dilutions were 1000, 500, 250, 125 and 65.2 µg/ml. Removed the media from the 96 well seeded plate. Added the media containing different dilutions of plant extract as made above. For control wells, add only media. Incubate the 96 well culture plate at 37°C in CO_2 incubator overnight.

Day 3: Observed the treated cells under inverted microscope. Prepared MTT reagent by adding MTT powder in phosphate buffer saline (pH 7.2) in concentration of 5mg/10ml. Removed the media and add 100 µl MTT reagent in all 96 well plate. Incubate it at 37°C in CO_2 incubator for 3-4 hrs.

Removed the MTT reagent and add 100 µl DMSO for solubilizing the formazan produced. Shake the plate well and took the absorbance reading at 490 nm.

Inhibition percentage was calculated by the following formula-

% inhibition = (control cells absorbance-drug cells absorbance)/ control cells absorbance * 100

Results and Discussion

In vitro antioxidant activity

In DPPH assay, the test sample showed moderate activity when compared to the standard antioxidant quercetin (Table 1).

Nitric oxide free radical scavenging activity

In nitric oxide assay, the test sample showed very poor antioxidant activity as compared to the standard ascorbic acid (Table 2).

In vitro cytotoxicity (MTT) assay

The *Boerhavia erecta* extract showed CTC50 value of 902.99 µg/ml and 945.65 µg/ml respectively in a dose-dependent manner on the growth in U87 cell lines and L929 cell lines respectively(Tables 3 and 4). The search for novel natural antioxidants constitutes as geared by safety considerations, public's perception and risk reduction of chronic diseases by consumption of edible fruits and vegetables.

Antioxidants can protect the body by preventing the formation of free radicals through interruption of free radical's attack, or by scavenging the reactive metabolites or converting then to less reactive molecules [21].

Table.1 DPPH assay results

Concentration (µg/ml)	Average % inhibition
1000	77.11
500	45.71
250	21.83
150	13.49
62.5	5.41
Control	0.00
IC50 value	567.501

Table.2 Nitric oxide scavenging results

Concentration (µg/ml)	Average% inhibition
1000	51.04
500	36.80
250	25.52
150	18.87
62.5	12.71
Control	0.00
IC50 value	965.8748

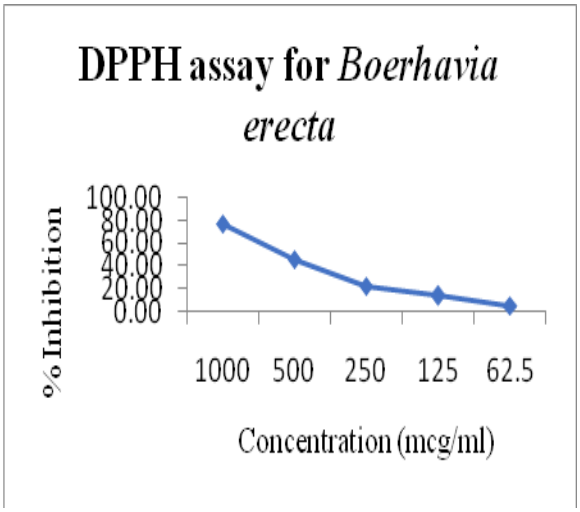
Table.3 Cytotoxicity effect of *Boerhavia erecta* plant extract on U87 cell lines

Conc. (µg/ml)	Average % inhibition
1000	56.50
500	22.99
250	11.19
150	5.88
62.5	1.30
Control	0.00
CTC50 value	902.99

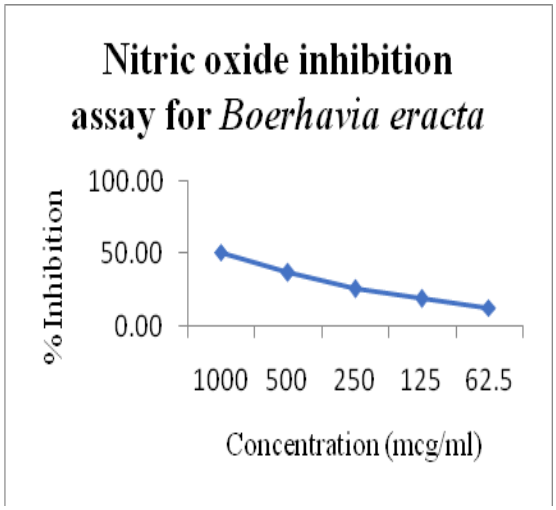
Table.4 Cytotoxicity effect of plant extract on L929 cell lines

Conc. (µg/ml)	Average% inhibition
1000	52.82
500	26.93
250	18.74
150	8.99
62.5	3.58
Control	0.00
CTC50 value	945.65

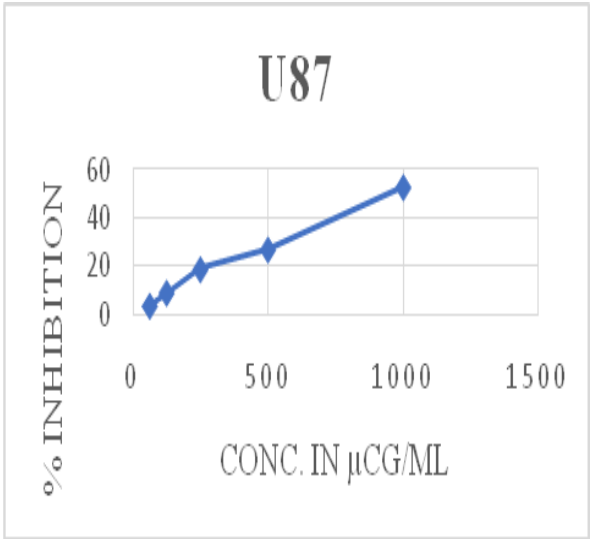
Graph.1 % inhibition of plant extract



Graph.2 % inhibition of plant extract



Graph.3 % inhibition of plant extract on U87 cell line



Graph.4 % inhibition of plant extract on L929 cell lines

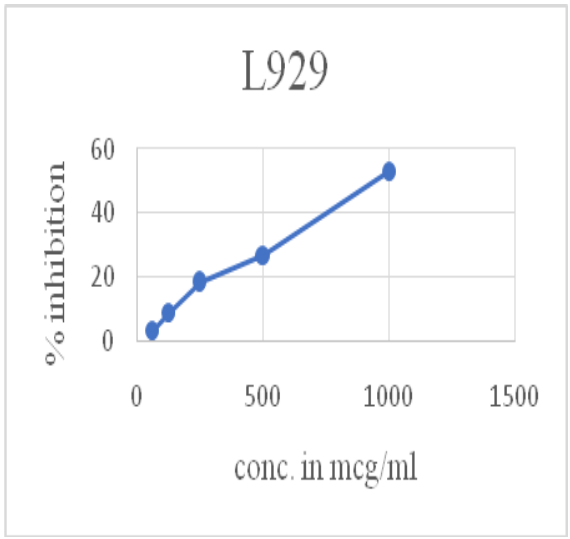
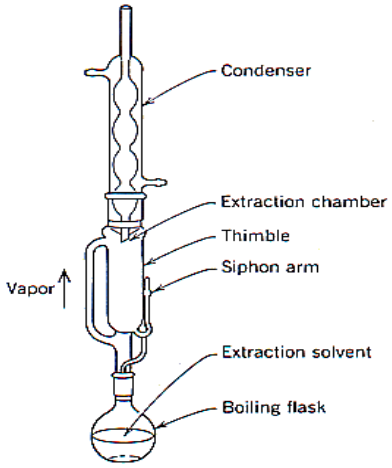


Fig.1 Soxhlet extraction apparatus



The present study, *in-vitro* antioxidant and anticancer activities of *B. erecta*, using crude methanolic extract of the whole plant, were reported for the first time.

The results of the present investigations have shown that whole plant of methanolic extract of *B. erecta* possesses better antioxidant results in DPPH as compared to nitric oxide free radical scavenging activity. Anticancer activity showed better results in both the cell lines i.e. U87 and L929.

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