INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com ISSN 2230 – 8407
Research Article

IN-VITRO PHARMACOLOGICAL INVESTIGATIONS OF THE PLANT *BOERHAVIA REPENS* (FAMILY: NYCTAGINACEAE)

Avijit Dey[1], S. M. Abdur Rahman[2]*, Rezwana Nasreen Chowdhury[1], Md. Hamiduzzaman[3], Mohammad Razi-Ul-Hasan Alvi[4]
[1]Department of Pharmacy, BRAC University, Dhaka
[2]Department of Clinical Pharmacy and Pharmacology, [3]Department of Pharmacy, ASA University, [4]Faculty of Pharmacy, University of Dhaka
*Corresponding Author Email: shila_pharm@yahoo.com

Article Received on: 13/05/13 Revised on: 07/06/13 Approved for publication: 18/07/13

DOI: 10.7897/2230-8407.04708
IRJP is an official publication of Moksha Publishing House. Website: www.mokshaph.com
© All rights reserved.

ABSTRACT
Boerhavia repens is an important medicinal plant having application in jaundice, fever and various other disorders. The whole plant *Boerhavia repens* was sun dried and extracted using methanol. Later the crude methanolic extract was fractionated into four different fractions using Petroleum ether, carbon tetra-chloride, Di-chloromethane and Ethyl acetate. The anti-oxidant activity of the different fractions was measured by the DPPH free radical scavenging activity. The ethyl acetate and the di-chloro methane soluble fractions showed very potent anti-oxidant activity by the DPPH free radical scavenging method. The anti-microbial activity of the different fractions was measured by disc diffusion method. The antimicrobial screening of the plant showed that the carbon tetra-chloride soluble fractions and the Petroleum ether soluble fractions showed mild antimicrobial activity. Evaluation of cyto-toxic activity was done using the brine-shrimp lethality bio-assay. The petroleum ether soluble fraction and the ethyl acetate soluble fractions showed significant cyto-toxic activity.
Keywords: Anti-oxidant, DPPH, Brine shrimp, cyto-toxicity, Disc-diffusion, antimicrobial activity.

INTRODUCTION

Plants represent a rich source of antimicrobial agent[1] and natural antioxidants.[2] Many of the plant materials used in traditional medicines are readily available in rural areas at relatively cheaper than modern medicines.[3] Plants generally produce many secondary metabolites which constitute an important source of microbicides, anti-oxidants. Many natural substances having anti-oxidant and anti-microbial properties have been used in health foods for medicinal and preservative purposes.[4] Many plants derived natural products are also used as anti-cancer agents like vincristine and vinblastine. The use of natural products to prevent cancer is becoming increasingly popular. *Boerhavia repens* (Family: Nyctaginaceae) is a terrestrial annual erect herb. The plant distributed in the tropical areas. It is found in India, Hawaii islands, Taiwan, Madagascar, Southern Africa, North Australia and a wide range of places. *Boerhavia repens* is an important medicinal plant having application in jaundice[5], fever[6] and constipation[7]. It is also known to be a blood purifier and is also reported to have anti-viral use[6]. The present study was designed to investigate the anti-oxidant, anti-microbial and cyto-toxic potential of the different partitionate fractions of the whole plant *Boerhavia repens*.

MATERIAL AND METHOD

Collection of the Plant Sample

Fresh plant of *Boerhavia repens* was collected from Sherpur, Mymensingh, Bangladesh in October, 2010. This plant was identified by the taxonomist of the Botany Department of the University of Dhaka. The reference sample for the plant was DUSH Accession Number 3615 and Call no 01.

Preparation of Plant Extract

The stem-bark, roots and leaves were sundried for 5 days. The plant materials were then oven dried for 24 h at low temperature. 1.0 kg of powdered material (Stem-bark and leaves) was macerated with 2.5 L of methanol in two 4 L round bottom flask. The containers were sealed with cotton plug and aluminum foil at room temperature for 15 days with occasional shaking. The mixture was filtered through cotton and then evaporated to dryness (45°C) under reduced pressure by rotary evaporator. The obtained crude extract was 20.54 g. 15 g of methanolic extract was triturated with 270 ml of methanol containing 30 ml distilled water. The crude extract was dissolved completely to obtain the mother solution. This solution was partitioned successfully by four solvents of different polarity. The mother solution was taken in a separating funnel. 100 ml of Petroleum Ether was added here and the funnel was shaken and kept undisturbed. Then the organic portion was collected and repeated thrice. Carbon tetrachloride (CCl_4) dichloromethane (CH_2Cl_2) and ethyl acetate extract was collected with the help of aqueous mother fraction adding 38 ml, 48 ml and 55ml of distilled water respectively keeping the other procedure unchanged. Finally Petroleum ether, carbon tetra-chloride, dichloromethane, ethyl acetate and aqueous extract were obtained.

Evaluation of Antioxidant Activity

Brand-Williams method[8] was used to estimate free-radical scavenging activities of the methanolic extracts of stem-bark and leaves of the plant on stable radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH). 2.0 mg of the extracts was dissolved in methanol for the experiment. Solution of different concentrations such as 500 µg / ml, 250 µg / ml, 125 µg / ml, 62.50 µg / ml, 31.25 µg / ml, 15.62 µg / ml, 7.8125 µg / ml, 3.91 µg / ml, 1.95 µg / ml and 0.98 µg / ml were obtained by serial dilution technique. 50 µl of methanol solution of the extract of each concentration was mixed with 5 ml of a DPPH-methanol solution (40 µg / ml). The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 minutes. The absorbance of the mixture was measured spectrophotometrically at 517 nm and from these values the corresponding percentage of inhibitions were calculated by using the following equation:

% inhibition = [1- (ABS sample / ABS Control)] x 100 %

Where ABS sample is the absorbance of the sample material and ABS control is the absorbance of the control reaction (containing all reagents except the test material).

Then percent inhibitions were plotted against respective concentrations. IC_{50} values were calculated as the concentration of each sample required to give 50 % DPPH radical scavenging activity from the graph. Tert-butyl-1-hydroxytoluene (BHT) was used as positive control. The experiment was performed thrice and the result was expressed as mean± Standard Error of Mean (SEM) in every case.

Antimicrobial Screening

Antimicrobial screening was performed using disc-diffusion method.[9] 8 mg of samples from different extract were dissolved in methanol to obtain desired concentration in aseptic condition. Sterilized filter paper discs were taken in a blank Petridis under laminar hood. Then discs were soaked with solutions of test samples and dried. Standard Ciprofloxacin (5 µg / disc) discs were used as positive control for bacterial strains and kanamycin (30 µg / disc) for fungal strains and blank discs were used as negative controls. The sample discs, standard antibiotic discs and control discs were placed gently on marked zones in the agar plate's pre-inoculated with test bacteria and fungi. The plates were then kept in a refrigerator at 4°C for about 24 h to allow sufficient diffusion of materials from discs to surrounding agar medium. The plates were then inverted and kept in an incubator at 37°C for 24 h. The bacterial and fungal strains used for the experiment were collected as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka. Both gram positive and gram-negative organisms were taken for the test and they are listed in Table 1.

Evaluation of Cyto-toxic Activity

The evaluation of cyto-toxic activity was done by the Brine shrimp lethality bio-assay.[10] In this experiment simulated sea water is prepared by dissolving 38 g of sea salt in 1 L of distilled water. Brine shrimp eggs were collected and hatched in a tank containing sea water. Two days were allowed to hatch the shrimp and to be matured as nauplii. Constant oxygen supply was carried out throughout the hatching time. With the help of a Pasteur pipette 10 living shrimps were added to each of the test tubes containing 5 ml of seawater. Clean test tubes were taken. These test tubes were used for preparing ten different concentrations (one test tube for each concentration) of test samples. Again ten test tubes were taken for ten concentrations of standard drug Vincristine and another one test tubes for negative control test. 4 mg of all the test samples (Pet ether soluble fraction, carbon tetrachloride soluble fraction, DCM and the ethyl acetate soluble fraction) were taken and dissolved in 200 µl of pure di methyl sulfoxide (DMSO) in vials to get stock solutions. Then 100 µl of Solution was taken in test tube each containing 5 ml of simulated seawater and 10 shrimp nauplii. Thus, final concentration of the prepared solution in the first test tube was 400 µg / ml. Then a series of solutions of varying concentrations were prepared from the stock solution by serial dilution method. In each case 100 µl sample was added to test tube and fresh 100 µl DMSO was added to vial. Thus ten different test tubes had different concentrations of test samples. The concentrations in ten different test tubes were 400 µg / ml, 200 µg / ml, 100 µg / ml, 50 µg / ml, 25 µg / ml, 12.5 µg / ml, 6.25 µg / ml, 3.125 µg / ml, 1.5625 µg / ml and 0.78125 µg / ml respectively. In the present study Vincristine Sulfate was used as the positive control. Measured amount of the Vincristine Sulfate is dissolved in DMSO to get an initial concentration of 40 µg / ml from which serial dilutions are made using DMSO to get 20 µg / ml, 10 µg / ml, 2.5 µg / ml, 1.25 µg / ml, 0.625 µg / ml, 0.3125 µg / ml, 0.15625 µg / ml and 0.078125 µg / ml. Then the positive control solutions are added to the pre marked vials containing ten living brine shrimp nauplii in 5 ml simulated sea water to get the positive control groups. For the preparation of negative control, 100 µl of DMSO was added to each of three pre marked glass vials containing 5 ml of simulated sea water and 10 shrimp nauplii was added to each vial. If the brine shrimps in these vials show a rapid mortality rate, then the test is considered as invalid as the nauplii died due to some reason other than the cyto-toxicity of the compounds. After 24 h, the vials were inspected using a magnifying glass and the number of survivors were counted. The percent (%) mortality was calculated for each dilution. The concentration-mortality data were analyzed statistically by using Microsoft Excel program. The effectiveness or the concentration-mortality relationship of plant product is usually expressed as a median lethal concentration (LC_{50}) value. This represents the concentration of the chemical that produces death in half of the test subjects after a certain exposure period.

RESULTS

***In vitro* Antioxidant Activity**

The antioxidant activity of the different fractions of *Boerhavia repens* was measured on the basis of its DPPH scavenging activity. The concentration of Petroleum Ether soluble fraction, Carbon tetra-chloride fraction, DCM fraction and the ethyl acetate soluble fractions needed for 50 % scavenging (IC50) of DPPH was found to be 1831.82 ± 18.87 µg / ml, 23.29 ± 2.83 µg / ml, 7.67 ± 0.86 µg / ml, 4.61 ± 1.24 µg / ml respectively. The positive control used was- Butyl hydroxyl toluene (BHT) and for which the IC_{50} values were found to be 34.75 ± 2.36 µg / ml.

Table 1: List of Gram positive and Gram negative Bacteria and Fungi

Gram positive bacteria	Gram negative Bacteria	Fungi
Bacillus cereus	*Escherichia coli*	*Aspergillus niger*
Bacillus megaterium	*Salmonella paratyphi*	*Candida albicans*
Bacillus subtilis	*Salmonella typhi*	*Sacharomyces cerevacae*
Sarcina lutea	*Shigella boydii*	
Staphylococcus aureus	*Shigella dysenteriae*	
	Pseudomonas aeruginosa	
	Vibrio mimicus	
	Vibrio parahemolyticus	

Table 2: Measurement of Zone of Inhibition for Different Experimental Samples

Test Microorganisms	Diameter of the zone of inhibition (mm)				
	CTCSF	PESF	DCMSF	EASF	Standard
Gram positive Bacteria					
Bacillus cerus	7	9	-	-	40
Bacillus megaterium	7	8			45
Bacillus subtilis	8	8	-	-	42
Staphylococcus aureus	7	8	-	-	42
Sarcina lutea	8	8	-	-	42
Gram negative Bacteria					
Escherichia coli	7	8	-	-	41
Pseudomonas aeruginosa	7	8	-	-	42
Salmonella paratyphi	7	8	-	-	44
Salmonella typhi	7	8	-	-	41
Shigella boydi	8	8	-	-	42
Shigella dysenteriae	8	8	-	-	42
Vibrio mimicus	7	8	-	-	42
Vibrio parahemolyticus	7	8	-	-	42
Fungi					
Candida albicans	8	8	-	-	46
Aspergillus niger	8	8			42
Saccharomyces cerevaceae	*8*	8			46

Antimicrobial Activities

In vitro antimicrobial screening of the whole plant *Boerhavia repens* was evaluated. Ciprofloxacin (5 µg / disc) was used as a standard for bacterial strains while kanamycin (30 µg / disc) was used as a Standard for fungal strains. Petroleum ether soluble fraction (PESF), Carbon tetrachloride soluble fractions (CTCSF), Dichloromethane soluble fraction (DCMSF) and Ethyl acetate soluble fractions (EASF) were used as sample to measure the zone of inhibition. The result is shown in Table 2.

Cyto-toxic Activity

The Pet Ether soluble fraction showed the greatest cyto-toxic activity with a LC_{50} value of 2.24 µg / ml. The Crude methanol extract, Carbon Tetrachloride and Ethyl acetate fractions also showed significant cytotoxic activity with LC50 values of 4.07 µg / ml, 10.90 µg / ml and 3.57 µg / ml respectively.

DISCUSSION

The current study established that the various fractions of the whole plant *Boerhavia repens* showed strong anti-oxidant activity. The anti-oxidant activities of plant extracts are mainly attributed to the presence of phenolic compounds. Therefore there is a probability that these fractions are rich in phenolic compounds. The slight anti-microbial activity present in the carbon-tetrachloride soluble fraction and the Petroleum ether soluble fraction may be caused by the disruption of the cell membrane or may be due to the inhibition of protein synthesis. However the anti-microbial activities of these two fractions were very low and therefore the minimum inhibitory concentration was not determined. The strong cyto-toxic activities present in the Petroleum ether fractions and the ethyl-acetate fractions may be due to the presence of cyto-toxic compounds. Therefore further study may be recommended to find out that the anti-cancer potential of the plant.

REFERENCES

1. B Mahesh and S Satish. Antimicrobial activity of some important medicinal plant against plant and human pathogens. World Journal Agricultural Science 2008; 4: 839-843.
2. Halliwell B, Aeschbach R, Liger J and Aruoma O. The characterization of antioxidant, Food Chem and Toxicol 1995; 33: 601-617. http://dx.doi .org/10.1016/0278-6915(95)00024-V
3. Mann A, Banso A, Clifford L. An antifungal property of crude plant extracts from *Anogeissus leiocarpus* and *Terminalia avicennioides*. Tanzania Journal of Health Research 2008; 10(1): 34-38. http://dx.doi.org/10.4314/thrb.v10i1.14339 PMid:18680963
4. Reynolds T. The compounds in Aloi leaf exudates. Botanical Journal Linn Society 1985; 90: 157. http://dx.doi.org/10.1111/j.1095-8339.1985.tb00378.x
5. Purkayastha J and Nath SC. Biological activities of ethnomedicinal claims of some plant species in Assam, Indian Journal of Traditional knowledge 2006; 5(2): 229-236.
6. Bhosle SV, Ghule VP, Aundhe DJ and Jagtap SD. Ethnomedical Knowledge of Plants used by the Tribal people of Purandhar in Maharashtra, India. Ethnobotanical Leaflets 2009; 13: 1353-61.
7. Mitra S, Mukhherjee SK. Ethnomedicinal usage of some wild plants of North Bengal plains for gastro- intestinal problems, Indian Journal of Traditional knowledge 2010; 9(4): 705-712.
8. Brand Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT – Food Science and Technology 1995; 28: 25–30.
9. Rizvi SMD, Biswas D, Arif JM. *In-vitro* antibacterial and antioxidant potential of leaf and flower extracts of *Vernonia cinerea* and their phytochemical constituents. International Journal Pharmaceutical Science Review and Research 2011; 9(2): 164-169.
10. Meyer BN, Ferrigni NR, Putnam JE. Brine shrimp: a convenient general bioassay for active plant constituents. Planta Medica 1982; 45(5): 31-34. http://dx.doi.org/10.1055/s-2007-971236

Cite this article as:

Avijit Dey, S. M. Abdur Rahman, Rezwana Nasreen Chowdhury, Md. Hamiduzzaman, Mohammad Razi-Ul-Hasan Alvi. In-vitro pharmacological investigations of the plant Boerhavia repens (Family: Nyctaginaceae). Int. Res. J. Pharm. 2013; 4(7):36-38 http://dx.doi.org/10.7897/2230-8407.04708

Source of support: Nil, Conflict of interest: None Declared