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Pharmaceutical Sciences **Research Article……!!!**

QUANTITATIVE ESTIMATION AND ANTIOXIDANT ACTIVITY OF *BOERHAAVIA DIFFUSA* LEAF

DR.S. SENTHILKUMAR

Karur, Tamilnadu, India.

KEYWORDS:

Free radicals, Scavenging activity, *Boerhaavia diffusa*, phytochemical, Ethanol extracts.

For Correspondence:
DR.S.SENTHILKUMAR.
Address:
Karur, Tamilnadu, India.

ABSTRACT

Medicianl plants are a source of great economic value in the Indian sub continent. For most the disease. Plants materials are used as drugs because of its active compounds. In many disorders the free radicals mediated damage may play an important role. Free radicals are responsinle for causing a wide number of health problems which include cancer, aging, heart diseases and gastric problems etc.

INTRODUCTION:

Free radicals arising from metabolism or environmental sources interact continuously in biological systems and their uncontrolled generation correlates directly with molecular level of many diseases (1). Lots of research has clearly showed that free radicals would damage nearby structures including DNA, proteins or lipids. Radical scavenging antioxidants are particularly important in antioxidant are particularly important in antioxidant-defense in protection cells from the injury of free-radical (2).

Plants are the good source of biologically active compounds known as phytochemicals. The phytochemicals have been found to act as antioxidants by scavenging free radicals and may have therapeutic potential for free radicals associated disorders (3). It is well known that free radicals are the major causes of various chronic and degenerative diseases, such as coronary heart disease, inflammation, stroke, diabetes mellitus and cancer. (4).

MATERIALS AND METHODS:

PREPARATION OF ETHANOL EXTRACT:

The leaves of *Boerhaavia diffusa* was shade dried at room temperature. The dried material was then homogenized to obtain coarse powder and stored in air-tight bottles for further analysis. The shade dried, powdered leaves were extracted with ethanol by lot extraction using soxhlet apparatus collected and stored in a vial for further analysis.

CHEMICALS:

All chemicals used including the Solvents, were analytical grade. Folin-ciocalteu phenol reagent, 1,1-diphenyl-2-picryl hydrazyl (DPPH), polyvinyl polypyrrolidone (PVPP), Ammonium acetate, glacial aceticacid, 2,4,6- tripyridyl-s-triazine(TPTZ) and Ethanol were purchased from merck co (Germany).

Determination of total phenolics and tannins

The total phenolic content was determined according to the method described by Siddhuraju and Becker (2003). Ten microlitre aliquots of the extracts (2mg/2ml) were taken in test tubes and made up to the volumes of 1 ml with distilled water. Then 0.5 ml of Folin-Ciocalteu phenol reagent and 2.5 ml of sodium carbonate solution (20%) were added sequentially in each tube. Soon after vortexing the reaction mixture, the test tubes were placed in dark for 40min and the absorbance was recorded at 725 nm against the reagent blank. The analysis was performed in triplicate and the results were expressed as tannic acid equivalents

Using the same extracts the tannins were estimated after treatment with polyvinyl polypyrrolidone (PVPP) (Siddhuraju and Manian,2007). One hundred milligrams of PVPP was weighed into a 100*12 mm test tube and to this 1 ml distilled water and then 1 ml of the sample extracts were added. The content was vortexed and kept in the test tube at 4^{o} C for 4h. Then the sample was centrifuged (3000 rpm for 10 min at room temperature) and the supernatant was collected. This supernatant has only simple phenolics other tannins. The phenolic content of the supernatant was measured as mentioned above and expressed as the results, the tannin contenat of sample was calculated as follows:

Tannin (%) = Total phenolics (%) – Non-tannin phenolics (%)

Determination of total flavonoid content

The flavonoid content was determined by the use of a slightly modified method 5% $NaNO_2$ solution. After 6 min, 0.15ml of 10% $AlCl_3$ solution was added and allowed to stand for 6 min, and then 2ml of 4% NaOH solution was added to the mixture. Immediately, water as added to bring the final volume to 5ml, and then the mixture was thoroughly mixed and allowed to stand for another 15min. Absorbance of the mixture was determined at 510 nm versus water blank. The analysis was performed in triplicate and the results were expressed as rutin equivalent.

DPPH radical scavenging activity (Blois, 1958)

The sample extracts at various concentrations was added to 5ml of a 0.1 mM methanolic solution of DPPH and allowed to stand for 20 min at 27^{0} C. The absorbance of the sample was measured at 517 nm. Radical scavenging activity was excpressed as the inhibition percentage of free radical by the sample and was calculated using the formula

% DPPH radical scavenging activity = (Control OD-Sample OD / Control OD) × 100

IC_{50} values of the extract i.e., concentration of extract necessary to decrease the initial concentration of DPPH by 50% was calculated.

Nitric oxide radical scavenging activity (Sreejayan and R1997)

3ml of 10mM sodium nitroprusside in 0.2 M phosphate buffered saline ($p^{H\ 7.4}$) was mixed with different concentrations of extract and incubated at room temperature for 150 min. After incubation time, 0.5 ml of Griess reagent (1% sulfanilamide, 0.1% naphthylethylene diamine dihydrochloride in 2% $H_3\ PO_4$) was added. The absorbance of the chromophore formed was read at 546 nm. Percentage radical scavenging activity of the sample was calculated as follows:

% NO radical scavenging activity = (control OD-sample OD / control OD) × 100

Superoxide radical scavenging activity (Beauchamp and Fridovich, 1971)

Each 3 ml reaction mixture contained 50 mM sodium phosphate buffer (p^H 7.6) , 20 mg riboflavin, 12mM EDTA, 0.1 mg NBT and various concentrations of sample extracts. Reaction was started by illuminating the reaction mixture with sample extract for 90 seconds. Immediately after illumination the absorbance was measured at 590 nm. The entire reaction assembly was enclosed in a box lined with aluminium foil Identical tubes with reaction mixture kept in dark served as blank. The percentage inhibition of superoxide anion generation was calculated as: % Inhibition = control OD – sample OD / control OD) × 100

Hydroxyl radical scavenging activity (Klein et al., 1991)

Various quantities of extract were added with 1ml of iron-EDTA solution (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5 ml of EDTA solution (0.018%) and 1ml of DMSO (0.85%V/V in 0.1M Phosphate buffer pH 7.4). The reaction initiated by adding 0.5ml of ascorbic acid (0.22%) and incubated at 80-90^o C for 15 min in a water bath. After incubation the reaction was terminated by the addition of 1ml of ice cold TCA (17.5 w/v). Three milliliters of Nash reagent (75.0g of ammonium acetate, 3ml of glacial acetic acid were mixed and raised to IL with distilled water) was added and left at room temperature for 15 min. The reaction mixture without sample was used as control. The intensity of color formed was measured spectroscopically at 412 nm against reagent blank. The % hydroxyl radical scavenging activity by the following formula

% HRSA = 1 – (difference in absorbance of sample / difference in absorbance of blank) × 100

Ferric reducing/antioxidant power (FRAP) assay

The antioxidant capacities of extracts of samples were estimated according to the procedure described by Pulido et al. (2000). FRAP reagent (900) µl), prepared freshly and incubated at 37^0 C, was mixed with 90 µl of distilled water and 30µl of test sample. The test sample was incubated at 37^0 C min in a water bath. The final dilution of the sample in the reaction mixture was 1/34. The FRAP reagent contained 2.5 ml of 20 mmol/l 2,4,5-tripyridyl-S-triazine (TPTZ) solution in 40 mmol/l HCL plus 2.5 mL of 20 mmol/l $FeCl_3$. 6H2O and 25ml of 0.3 mol/l acetate buffer (pH 3.6) described by Siddhuraju and Beaker, (2003). At the end of incubation, the absorbance readings were taken immediately at 593 nm, using a spectrophotometer. The values are expressed as mmol Fe (II) per milligram extract.

TABLE-1. Table phenol, flavonoids and tannin content of Ethanolic leaf extract of *Boerhaavia diffusa*

Boerhaavia diffusa	Total phenolics content(Mg/g extract)	Total flavonoid content (Mg/g extract)	Total tannin content (Mg/g extract)
Leaf	78.64 ± 0.19	1.12± 0.09	38.26±0.57

TABLE-2. Antioxidant activity of *Boerhaavia hiffusa* leaf

S.L. NO	Antioxidant activity	Sample concentration (Mg)	Percentage activity	IC 50(Mg)
1.	DPPA radical scavenging activity	100 200 300 400 500	10.17±0.11 20.81±0.19 34.59±0.16 44.22±0.15 67.69±0.20	409.83
2.	Nitric oxide radical scavenging activity	100 200 300 400 500	5.46±0.39 10.41±0.68 15.29±0.29 20.11±0.19 30.20±0.22	909.09
3.	Super oxide radical scavenging activity	100 200 300 400 500	1.73±0.33 5.42±0.33 7.55±0.20 10.40±0.26 15.64±0.27	1785.71
4.	Hydroxyl radical scavenging activity	100 200 300 400 500	5.35±0.59 10.11±0.59 14.48±0.34 19.84±0.34 30.75±0.34	833.33

TABLE-3. Ferric reducing / antioxident power(FRAP)assay of *Boerhaavia diffusa* leaf.

FRAP assay of Boerhaavia diffusa leaf	**755.47±3.64**

RESULTS AND DISSCUSSION:

The leaves would be useful as an antioxidant and free radical scavenging agent and it helps in treatment of many diseases that was mediated by reactive oxygen species. Accordingly in this study, a signigicant and linear relationship was found between the antioxidant activity and phenolic content, indicating that phenolic compounds could be major contributors to antioxidant activity. The total phenol, flavonoid and tannin content results are presented in Table-1.

These free radicals contribute to several hundred human disorders like atherosclerosis, ischmia, gastritis, cnacer, Hids, reperfusion injury, diabetes mellitus and aging procerses. Boerhaavia diffusa leaf extracts for free fadicals scanvenging activity by DPPH, nitric oxide super oxide, hydroxyl and FRAP assay (Table-2,3).

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