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Comparative Studies on Physicochemical parameters and phytochemical analysis of whole plant and root of Boerhavia erecta L.

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ABSTRACT

Boerhaavia erecta L. is a weedy herb of family Nyctaginaceae. It has similar properties with B. diffusa L. The whole plant of Boerhaavia. erecta i.e. root, leaves and stem have rich medicinal value. Roots of Boerhaavia. erecta used as diuretic, stomachic and cardio tonic. Whole plant is taken to treat gastro- intestinal, liver and infertility problems. Boerhaavia. erecta leaf extracts showed anti-malarial effects. Stem bark used as a natural acid –base indicator. So, to comparative physicochemical and phytochemical study has been carried out between whole plant and root of Boerhaavia erecta with different extracts. The results

revealed that the presence of most of the phytoconstitutents present in acetone extract and then in hydroalchoholic and only few i.e. Tannin, protein, lipid and carbohydrate was found in per ether extract.

Keywords: Boerhavia erecta L., African medicinal plant, Nyctaginaceae, Physicochemical characters, Phytochemical constituents.

Introduction

Boerhavia erecta L. is a weedy herb of family Nyctaginaceae. Boerhavia erecta L. is also called as shweta punarnava. B. erecta is also a high medicinal value plant. It shows similar medicinal uses to B. diffusa because of the same alkaloid compounds viz. Punarnavine. (Wajid Muhammad et al., 2017). It is native to United States, Mexico, Central America and Western South America.

Boerhavia erecta entire plant (leaves, stem and root) have medicinal value. Root is applied in India especially as diuretic to treat in case of strangury, Jaundice, enlargement of spleen, gonorrhea, and other internal inflammations. It is also used as stomachic, cardiotonic, hepatoprotective, laxative, anthelmintic (expels parasitic worms), febrifuge (reduces fever) and expectorant. In higher dosages it is used as an emetic and purgative; I moderate dosage it is used as successful treatment of asthma. A paste of root is rubbed on the skin to ripen abscesses and ulcers.

The phytochemical substances are responsible for the medicinal values of the plants. Phenol and flavonoid are major important substances which are responsible for medicinal values including anti-oxidant, anticancer, antimicrobial activities etc. The present study was comparative account of phytochemical and physicochemical parameters of whole plant and root.

Material and Methods

Collection of plant material- Mature, healthy and disease free plant of Boerhavia erecta L. were collected in the month of July 2016 to September 2016 from the Agriculture College campus, Pune.

Preparation of extract for qualitative analysis- 5 g powder of whole plant and root were extracted successively with 100 ml solvents viz. acetone, Pet ether(60-80^{0}C) and hydroalcohol (ethanol 60ml : water40ml) kept it overnight and filter through whatmann filter paper no. 1 and extract was used to performed all the qualitative test.

Sr. No	Test name	*B. erecta* whole plant	*B. erecta* root
1.	Conc. H_2SO_4	1) Powder floats on surface & it turns in to brown. 2) On shaking 2 – 2.5 cm amber color band formed, most of the powder present at the top.	1) Powder floats on surface & turns into brown. 2) On shaking 1 – 1.5 cm light brown Color Band will formed & remaining powder present at the top.
2.	Conc. HNO_3	1) Powder floats on surface & immediately turns in to brown & layer of 0.2 cm. layer was formed. 2) On shaking effervesces at the top & foam will be formed 0.5 – 0.8 cm layer. A dark amber color pulpy band 3 – 3.5 cm was formed.	1) Powder floats on surface & immediately turns in to brown. 2) On shaking effervesces were formed & foam layer of 0.5 cm. was formed. 1 – 2 cm. Yellowish brown band was formed & remaining powder float at the top.
3.	Conc. HCl	1) Power floats on surface. 2) On shaking some particles slowly settled down & some particles suspended in the solution & remaining powder float on the top.	1) Power floats on surface. 2) On shaking effervesces was formed. Very few partides suspended in the solution & remaining powder present at the top.
4.	Glacial Acetic Acid	1) Powder immediately goes down, few particles remain suspended & very small amount of powder remains at the top.	1) Powder floats on surface, few particles start moving downward and settled & remaining powder float at the top. 2) On shaking a milky pulpy band formed & most of the particles settled down, few suspended & remaining powder float at the top.
5.	5% I_2	1) Powder starts moving down after 1–2 seconds & settled down & remaining powder float at the top. 2) On shaking effervesces were formed ; powder settle at the bottom. And very few remains suspended in a solution and some of the present at the top.	1) Powder slowly starts moving downward; very few particles settle at the bottom and remaining powder present at the top. 2) On shaking most of the powder settle at the bottom and few particles remains suspended in a solution. At the top foam layer of 0.5-1cm was formed.
6.	5% $FeCl_3$	1) After one second very few partides moves downward and remaining powder remains at the top. 2) On shaking most of the particles settle down and very few suspended in a solution. Remaining powder present at the top.	1) Powder floats on surface of the solution. 2) On shaking very few partides settle down and most of the powder remains at the top and few particles suspended in a solution.
7.	5% NaOH	1) Powder floats on surface 2) On shaking effervesces was observed which is 0.5 cm layer. Most of the powder settles at the bottom. Some of the particles remain suspended in a solution and remaining powder present at the top.	1) Powder floats on surface 2) On shaking effervesces was formed. Most of the powder settles at the bottom. Some of the partides remain suspended in a solution and remaining powder present at the top.

Phytochemical analysis (Qualitative tests)

a) Carbohydrate: Molish's test: 1ml of sample is treated with two drops of alcoholic á-napthanol solution. Two ml Conc. H_2SO_4 is added on the side of the test tube. Formation of the violet ring at the junction indicates the presence of carbohydrate.

b) Non- Reducing Polysaccharide: Take three ml of sample mixed with few drops of 5%I_2 solution. The blue color of the solution confirms the presence of non- reducing polysaccharide.

c) Protein: Biuret test: Three ml sample mixed with 4% NaOH and few drops of 1% $CuSO_4$ solution was added. The appearance of violet or pink color would indicate the presence of proteins.

d) Amino acids: Ninhydrin test: To the sample 0.25 % W/V ninhydrin reagent is added and boiled for few minutes. Formation of blue violet color indicates the presence of amino acids.

e) Glycoside: Libermann- Burchard test: Hydrolysate treated with chloroform then add acetic anhydride solution and treated with Conc. H_2SO_4. Formation of blue or blue- green color would indicate the presence of steroidal saponins. Whereas, pink or violet color would indicate the presence of triterpenoid saponins.

f) Flavonoids: Shinoda test: A small piece of magnesium ribbon was added to the sample and add drop wise Conc. HCl. The green blue color would indicate the presence of flavonoids.

g) Alkaloids: Dragendroff reagent: Two-three ml sample were taken and treated with few drops of dragendroff's reagent. Orange brown precipitate would indicate the presence of alkaloid

h) Phenol and Tannin: A small quantity of extract was diluted with water and then used for following test:**$FeCl_3$:** Small quantities of extract were taken and add dilute $FeCl_3$ 5%. Intense blue, green, red or purple color would indicate the presence of phenol compound. An appearance of violet color would indicate the presence of tannins.

Quantitative Estimation:

1) Carbohydrate Content :

Take 0.6ml extract and add 3ml Conc. H_2SO_4 incubate it for 40 min and then add 0.6ml liquid phenol. The blank was prepared by adding 3ml Conc. H_2SO_4 incubated for 40 min and then add 0.6ml liquid phenol. Measured the absorbance at 490 nm.

2) Total Protein Content:

0.6ml extract was taken and add 3ml Reagent 'C' incubated for 10 min. Then add 0.3ml (1:1) FCR (Folin Cio-calteu reagent). (The blank sample- 3ml Reagent 'C' incubated for 10 min. Then add 0.3ml (1:1) FCR). Incubate it for 30min. Read absorbance at 660nm.

3) Glycoside Content :

Take 2ml extract incubated it for 1hr 2ml Balject reagent in dark then add 1.6ml distilled. The blank was prepared by adding 2ml Balject reagent and after 1hr incubation in dark add 1.6ml distilled water. Take absorbance at 495nm.

4) Total Flavonoid Content:

1mlextract was taken in that 0.1ml (10%) $AlCl_3$ 0.1ml (1M) Na-K- tartarate and 1.5ml distilled water were added respectively. The blank was taken 0.1ml (10%) $AlCl_3$ and 0.1ml 1M Na-K- tartarate and 1.5ml distilled water added one by one. Incubate it for 30 min. and absorbance were taken at 415nm.

5) Total Alkaloid Content:

2ml of extract,2ml of 1X 10^{-4} Bromo Cresol Green (BCG) and 2ml of Phosphate buffer (PBS) taken and 2ml of chloroform was added. Take 2ml of BCG and 2ml of Phosphate buffer as a blank and add 2ml of chloroform. Incubated for 5min and allowed to evaporate chloroform and absorbance was measured at 470nm.

6) Total Phenolic Content :

Take 1ml extract and add 0.2ml (1:4) Folin Cio-calteu regent. After 10 min incubation 0.8ml (20%) Na_2Co_3 were added. The blank sample was taken as 0.2ml (1:4) Folin Cio-calteu regent. After 10 min incubation 0.8ml (20%) Na_2Co_3 were added. Incubate this for 2hr. in dark and absorbance was measured at 735nm.

7) Lipid Content:

Take 0.4ml extract in that 0.8 Conc. H_2SO_4 and 2ml Phospho-vaniline reagent were added. The blank was prepared by adding 0.8ml Conc. H_2SO_4 and 2ml Phospho-vaniline reagent and absorbance were measured at 540nm.

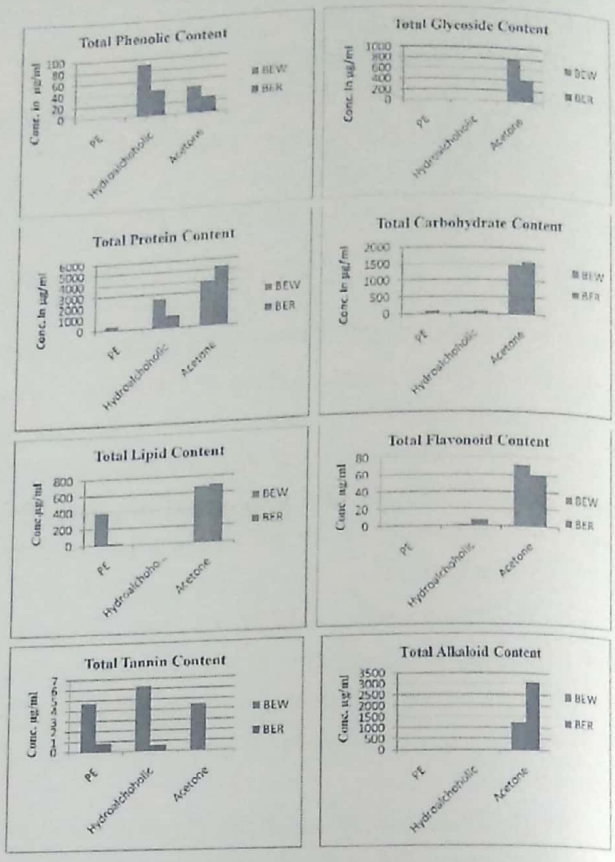
8) Total Tannin Content:

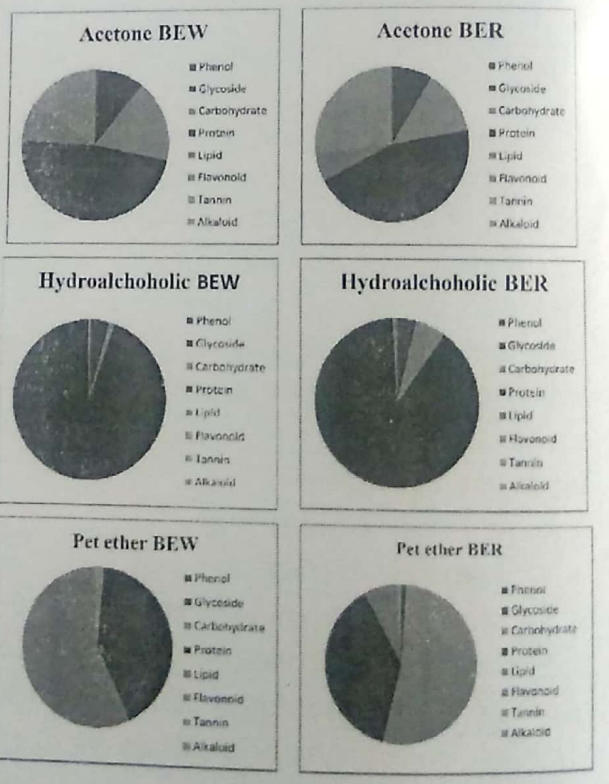
Take 1ml extract and 0.3ml (0.016M) $K_3Fe(CN)_6$ and 0.3ml $FeCl_3$ and stabilizer (0.5ml O- phosphoric acid and 1ml distilled water) were added. The Blank sample was prepared by adding 0.3ml (0.016M) $K_3Fe(CN)_6$ and 0.3ml $FeCl_3$ and stabilizer (0.5ml O- phosphoric acid and 1ml distilled water) and incubate it for 15 min. Absorbance was measured at 700nm.

Results :

Phytochemical Screening results

Sr. No.	Test name	*B. erecta* whole plant			*B. erecta* root		
		Pet ether	Hydro alcoholic	Acetone	Pet ether	Hydro alcoholic	Acetone
1.	Carbohydrate						
	Molish's Test	-	+	++	+	+	++
2.	Non reducing polysaccharide	-	-	-	-	-	-
3.	Protein						
	Biuret test	+	+	++	-	+	++
4.	Amino acids						
	Ninhydrin test	-	-	-	-	-	-
5.	Glycosides						
	Libermann Burchard	-	-	-	-	-	++
6.	Flavonoides						
	Shinoda test	-	+	++	-	+	++
7.	Alkaloids						
	Dragendroff test	-	-	++	-	-	++
8.	Phenol						
	$FeCl_3$	-	+	+	-	+	+





Quantitative estimation results: After measuring absorbance of all content the reading of respective samples were noted and comparative graphs were plotted by using values obtained from the standards and samples in different extracts in µl/ml.

Preparation of extract for quantitative estimation

Take 10mg powder of whole plant and root were extracted successively with 10 ml solvents viz. acetone, Pet ether(60-80^{0}C) and hydroalcohol (ethanol 60ml : water 40ml) kept it overnight and filter through whatmann filter paper and extract was used to performed all the

quantitative parameters with respective standard methods.

Discussion

The preliminary comparative phytochemical screening was carried out on *B. erecta* Pet ether, Hydro-alcohol and Acetone extracts of whole plant and root. The study was related to the presence of phytoconstitutents such as Carbohydrate, Protein, Glycoside, Flavonoids, alkaloids, Phenol, lipid and Tannin and in how much quantity.

In preliminary phytochemical study of Pet Ether (60-80°C) extract of whole plant showed positive results for only tannin, lipid and protein. pet ether (60-80°C) extract of root showed positive results for carbohydrate and tannin only. In quantitative analysis of pet ether (60-80°C) extracts of whole plant and root showed tannin content is more in whole plant than root.

Hydroalchoholic extract of whole plant and root showed positive results for carbohydrate, protein, tannin, flavonoid and phenol. Quantitative estimation it was found that, total phenol, total protein and total tannin content is more in whole plant than root but flavonoid is more in root than whole plant.

In extract of Acetone whole plant and root showed positive result for carbohydrate, protein, glycoside, alkaloid, phenol and lipid. Tannin content was present only in whole plant. Quantitative estimation showed that carbohydrate, protein, lipid and alkaloid is less in whole plant than root. Root showed alkaloid in a large quantity than whole plant. Total glycoside, Total flavonoid and Total phenol content was more in whole plant than root.

So quantitative estimation showed that root has rich with total carbohydrate, protein, lipid and alkaloid content, while whole plant rich with glycoside, flavonoid and phenolic content.

Conclusions

The phytoconstitutents are the important compounds which are responsible for the medicinal properties of the herbs. Medicinal plants contains many of the phytoconstitutents such as phenolic compounds, could be used for therapeutic purposes as they exhibit a huge amount of medicinal properties.

Our research concludes that in Hydroalchoholic extract of whole plant showed more phenolic content than root. So instead of root whole plant can be used for therapeutic purposes. Therefore plant did not damage and we save the medicinally important plants to become rare and endangered.

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