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QUALITATIVE PHYSICOCHEMICAL, PHYTOCHEMICAL ANALYSIS AND QUANTITATIVE ESTIMATION OF TOTAL PHENOLS, FLAVONOIDS AND ALKALOIDS OF *BOERHAAVIA DIFFUSA*

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ABSTRACT

The aim of present study was to investigate the physicochemical, phyto-constituents present within the methanol extract of *Boerhaavia diffusa and* to estimate the total phenolic, flavonoid and alkaloid contents. *Boerhaavia diffusa L.* (Family: Nyctaginaceae) is an herbaceous plant, also known as punarnava widely distributed in the tropical and subtropical regions in the world. The plants are a rich source of vitamins, minerals, proteins and carbohydrates. In Punjab region, the drug is useful for the eye d`6isease and in Bombay used for dropsical swellings. The leaf juice is used in jaundice and the root whole plant is generally used in infusion in internal inflammation, laxative and also in urinary diseases. In the present studied methanol extract of whole plant of *Boerhaavia diffusa* various parameters like fluorescence analysis as well as extractive values and quantitative phytochemical screening of different extractives were studied. qualitative phytochemical analysis and quantitative estimation of total phenols, flavonoids and alkaloids were performed . This study revealved that *B.diffusa* as a source of phyto constituents such as alkaloids, glycosides, steroids, saponins, carbohydrates, flavonoids were performed. The characteristic of physicochemical parameters, such as the presence of total ash (4% w/w), acid insoluble ash (20.9% w/w) water soluble ash (14.8% w/w) and Loss on drying (10.32%) . It is within the guidelines of IP. This reveals the alcohol soluble extractive and water soluble extractive values were found to be 21.6% & 31.2% w/w were performed. Quantitative estimation of total phenols, flavonoids and alkaloid contents observed in clorimetry in this study ranges from total phenols (0.59), flavonoids (0.45) and alkaloid (0.29) content respectively. The characteristic of physicochemical, fluorescence analysis and quantitative chemical screening were performed in whole plant extract of the plant material as a mean of authentication.

INTRODUCTION:

Boerhaavia diffusa Linn. (Family: Nyctaginaceae) is an herbaceous plant, cultivated in fields [1, 2] spreading vine widely distributed in the tropical and subtropical regions in the world[3] . *Boerhaavia diffusa* (Nyctaginaceae) commonly known as Raktapunarnava, Shothaghni, Kathillaka, Kshudra,

Varshabhu, Raktapushpa, Varshaketu, Shilatika, an herbaceous plant species growing prostrate or ascending upward in habitats like grasslands, agricultural fields, fallow lands, wastelands and residential compounds. The plant was named in honor of Hermann Boerhaave, a famous Dutch physician of the 18th century. The plant is mentioned in the Atharvaveda with the name 'Punarnava', because the top of the plant dries up during the summer season and regenerates again during the rainy season. In Ayurveda, Punarnava has many medicinal properties.

It is called as Punarnava (Punar + nava). Punar means - once again, nava means - becoming new. This is also known as spiderlings as this plant grows low and spreads like spider. The plants is a rich source of vitamins, minerals, proteins and carbohydrates.[4] A number of constituents mainly as alkaloids, flavonoids, saponins and steroids [5] . The superabundance of reports has been published. In Punjab region, the drug is useful for the eye disease and in Bombay used for dropsical swellings. The leaves juice is used in jaundice and the root is generally used in infusion in internal inflammation, laxative, and also in urinary disease [6]. The whole plant extract is hepatoprotective in nature [7] . It is also used in treatment of diabetes [8] . It contains quinolizidine alkaloids, potassium salts [9] and boeravinones G and H alkaloids have been reported to inhibit breast cancer resistant proteins [10], the depletion of the germinal epithelial lining of the seminiferous tubules with enhance number of germinal cell, decreased sperm counts with increase percentage of tail and head abnormalities and increases in both pre and post-implantation tissue [11]. More recently, it has anti-proliferative activity against a variety of tumour cell lines [12], also showed the methanolic extracts of the plant were effective in reducing metastases formation in some melanoma cells [13]. Most investigations on the plant have centred on the root, whereas significant differences in the chemical composition of the root and the leaves have been reported [14]. As far as our literature survey could ascertain, one in-vitro anti oxidative activity of leaf extract *B. diffusa* in tissues of alloxan induced diabetic in rats [15]. The plant is also reported to have a Adaptogenic and anti stress activity [16] and roots have anti inflammatory, fibrinolytic and anticonvulsant activities [17]. Among currently available drugs, synthetic drugs do have potential adverse reactions and which can be minimized to greater extent through natural compounds [18-22]. In the present study, the methanolic extract of the whole plant of *B. diffusa* were screened for antioxidant properties using in-vitro standard procedures so as to assess the medicinal potential of the plant and thus justify their folklore use.

MATERIALS AND METHODS

Plant collection and Authentication

The whole plant of *Boerhaavia diffusa* was collected around Andhra university, Visakhapatnam district, India. During the month of feburary-march, 2016 and authenticated at department of botany, Prof. Padal. Andhra University, Visakhapatnam. The freshly collected whole plant (leaves, stem, roots) was washed with water and dried under shade at room temperature for 1 week. The dried *Boerhaavia diffusa* plant material was cut into small pieces and powdered in a blender. The powder material was stored in sterile air tight container for further use.

Soxhlet extraction: The air dried powdered material of (1000gms) was subjected to hot continuous extraction with soxhelt apparatus by using methanol. After complete extraction the solvent was removed using a buchi type solvent evaporator. Then the extract was obtained which is concentrated and dried completely, weighed and stored in desiccators. The extract was transferred into sterile container until for further use.

Physico-chemical studies

Ash values: Used to determine quality and purity of crude drug and to establish the identity of it.

Determination of total ash: Weigh about 2 g of powder into a porcelain dish. Heat with a burner using a flame about 2cm high and supporting the dish about 7cm above the flame. Heat till vapours almost cease to be evolved and tare the sample. Cool in desiccators. Weigh the ash and calculate the percentage of total ash with reference to the air dried sample of crude drug.

Determination of Acid insoluble ash: Using 25 ml of dil. hydrochloric acid wash the ash from dish for total ash into 1000 ml of beaker. Place a mere guaze over a Bunsen burner. Boil for 5 min. Filter through ash less filer paper, wash the residue twice with hot water. Ignite the crucible in the flame and Cool in a dessicator. Weigh and calculcate the percentage of acid insoluble ash with reference to the air dried sample of crude drug.

Determination of water soluble ash: This is determined in a similar way to acid insoluble ash, using 25 ml of water in place of dilute hydrochloric acid. Boil for 5 min. filter through ash less filter paper wash the residue twice with hot water. Cool in desiccators. Weigh and calculate the percentage of water soluble ash with reference to the air dried sample of crude drug.

Loss on drying: Weight about 5 g of powder into a porcelain dish. Dry in oven at 100 & 105^0c. Cool in desiccator. Weigh and the loss in weight recorded as moisture content. Calculate the percentage of loss of drying recorded as moisture of sample.

Swelling index: It gives an idea about the mucilage content of the crude drug. Hence it is useful in the evaluation of crude drugs containing mucilage.

Procedure: Weigh about 1 g of powder into a 50 ml measuring cylinder. Add water up to 40 ml making. Shaking occasionally during 24 hrs. Keep a side for one hour. Measure the volume occupied by swollen powder

Foaming index: It is evaluated by measuring the foaming ability in term of foaming index. If the height of the foam in every tube is less than 1 cm it means foaming index is less than 100. If the height of foam is more than 1 cm every test tube; the foaming index is over 1000. In this case, repeat the experiment using a new series of dilutions o the decoction in order to get a result. If the height of 1 cm in any tube, the volume of the plant material decoction in this tube(a) is used for determination of foaming index `6using formula follows:

Foaming index = 100/a

Extractive values: Useful in evaluation of crude drug. To determine give idea of nature of chemical constituents present in crude drug. Useful of estimation of constituents extracted with solvent used for extraction. Employed for material for which as yet no suitable chemical or biological assay exists.

Procedure: about 1 g of powder in add different solvents like (Hexane, Dichloromethane, chloroform, ethyl acetate, acetone, methanol, water, petroleum ether) into a boiling tubes. Cork the flask and set aside for 24 hrs. Shaking frequently occasionally during 6 hrs and allowed to stand for 24 hrs. Filter into a 50 ml of measuring cylinder. Transfer into filtrate in thin porcelain dish. The fitrate evaporated to dryness in a tarred flat bottom dish. Weighed and calculate the % w/w of extractive values of different solvents

Fluorescence analysis: Fluorescence analysis of the powder drug was carried out with different chemical reage6`6nts in day (254 nm) and UV light (365 nm). The dry powder drug was studied on glass slide where as the different extracts were studied by adsorbing the extracts.

Phytochemical Analysis: The prepared extract was tested for the type of chemical constituents present by known qualitative tests. *The following tests were carried out

on the extracts to detect various phytoconstituents present in them.

1. Test for Alkaloids: About 50mg of solvent-free extract was stirred with little quantity of dilute hydrochloric acid and filtered. The filtrate was tested carefully with various alkaloidal reagents as follows.

Mayer's test: To a few ml of filtrate, two drops of Mayer's reagent was added along with the sides of test tube. If the test is positive, it gives white or creamy precipitate.

Wagner's test: To a few ml of the filtrate, few drops of Wagner's reagent were added along with the sides of the test tube. Formation of reddish brown precipitate confirms the test as positive.

Hager's test: To a few ml of filtrate 1 or 2 ml of Hager's reagent was added. A prominent yellow precipitate indicates positive test.

Dragendroff's test: To a few ml of filtrate, 1 or 2 ml of Dragendroff's reagent was added. A prominent reddish brown precipitate indicates positive test.

2. Test for Carbohydrates: About 100mg of the extract was dissolved in 5ml of distilled water and filtered. The filtrate was subjected to the following tests.

Molisch test: To 2 ml of filtrate, two drops of alcoholic solution of α- napthol was added. The mixture was shaken well and 1 ml of concentrated sulphuric acid was added slowly along the sides of the test tube, the test tube was cooled in ice water and allowed to stand. A violet ring at the junction of two liquids indicates the presence of carbohydrates

Fehling's test: 1 ml of filtrate was boiled on a water bath with 1 ml each of Fehling's solution A and B. Formation of red precipitate indicates the presence of sugar.

Barfoed's test: To 1 ml of the filtrate, 1 ml of Barfoed's reagent was added and heated on a boiling water ba`th for 2 minutes. Red precipitate indicates the presence of sugars.

Benedict's test: To 0.5 ml of filtrate 0.5 ml of Benedict's reagent was added. The mixture was heated on a boiling water bath for 2 minutes. A characteristic brick red precipitate indicates the presence of sugar.

3. Test for Glycosides: For the detection of glycosides, about 50 mg of extract was hydrolyzed with concentrated hydrochloric acid for 2 hours on a water bath, filtered and filtrate was subjected to following tests.

Borntrager's test: To 2 ml of filtrate hydrolysate, 3 ml of chloroform was added and shaken, chloroform layer was separated and 10% ammonia solution was added to it. Formation of pink color indicates the presence of anthraquinone glycosides.

Legal's test: About 50 mg of the extract was dissolved in pyridine. Sodium nitroprusside solution was added and made alkaline using 10% sodium hydroxide solution. Presence of glycoside is indicated by a characteristic pink color.`6`6

4) Test for Saponins

a) Froth test: A small quantity of the extract was diluted with distilled water to 20 ml. The suspension was shaken in graduated cylinder for 15 minutes. A two centimeter layer of foam or froth which is stable for 10 minutes indicates the presence of saponins.

5) Test for Phytosterols and Triterpenoids

a) Liebermann- burchard 's test: The extract was dissolved in acetic anhydride, heated to boiling cooled and then 1 ml of concentrated sulphuric acid was added along the side of the test tube. Red, pink or violet color at the junction of the liquids indicates the presence of steroidal triterpenoids and their glycosides.

Salkowski test: Few drops of concentrated sulphuric acid was added the chloroform extract, shaken on standing, red color in the lower layer indicates the presence of steroids and golden yellow color indicates the presence of triterpenoids.

6) Test for Phenols and Tannins

a) Ferric chloride test: About 50 mg of extract was dissolved in distilled water and to this few drops of neutral 5% ferric chloride solution was added. Formation of blue, green and violet color indicates the presence of phenolic compounds.

b) Gelatin test: A little quantity of extract was dissolved in distilled water and 2 ml of 1% solution of gelatin containing 10% sodium chloride was added to it. Development of white precipitate indicates the presence of phenolic compounds

c) Lead acetate test: A small quantity of extract was dissolved in distilled water and to this; 3 ml of 10% lead acetate solution was added. A bulky white precipitate indicates the presence of phenolic compounds.

7) Test for flavonoids

a) Alkaline reagent test: An aqueous solution of extract was treated with 10% ammonium hydroxide solution- yellow fluorescence indicates the presence of flavonoids.

Shinoda test: A little quantity of extract was dissolved in alcohol and few fragments of magnesium turnings and conc. Hydrochloric acid (drop wise) were added. If any pink or crimson- red color develops, presence of flavonol glycoside is inferred.

Zinc- hydrochloric acid reduction test: The alcoholic solution is treated with pinch of zinc dust and few drops of conc. Hydrochloric acid- magenta color is produced after few minute

Quantitave estimation of total phenolic, alkaloidal and flavonoid content

Total phenolic content: Total phenolic content was determined by follin - ciocalteau reagent. Folin – ciocalteau colorimetry is based on a chemical reduction of the reagent, a mixture of tungsten and molybdenum oxides. The products of the metal oxide reduction have a blue absorption at the wave length is proportional to the concentration of the phenols. By using standard gallic acid calibration curve, measure the concentration of phenolic content in gallic acid total equivalents using units mg/gms (GAE). Gallic acid was used as standard 0.5mg/ml (250 mg of gallic acid was dissolved in 1 ml of extract solvent and diluted to 500 ml with distilled water. This stock solution was stored at 4^0C. working standards of 0.01 to 0.0`65 mg/ml was prepared by diluting the stock with distilled water.100uL of extract was transferred into a test tube and 0.75 ml of FC reagent was added.0.7 ml of 6% (w/v) sodium carbonate was also added. Stand at room temperature for 90 minutes, then absorbance was read at 725nm using UV-visible spectrophotometer. Results were reported in table no.7.

Total alkaloid content: The plant extract 1 (mg/ml) was dissolved in 2N Hcl and then filtered. The pH of phosphate buffer was adjusted to neutral with 0.1 N sodium hydroxide. 1 ml of this solution was transferred to a separating funnel and then 5 ml of BCG solution along with 5 ml of phosphate buffer was added. The mixture was shaken and the complex formed was extracted with chloroform by vigorous shaking. The extract was collected in 10 ml volumetric flask and diluted to volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm. All the experiment was performed thrice, the results were averaged and reported in the form of mean or SEM.

Table: 1: Extraction of plant material

Plant material	Solvent used	No of cycles	Yield of extract
Dried whole plant of B.diffusa	Methanol (1200ml)	4 Cycles	20 Gms

Fig no 1: Calibration curve of gallic acid

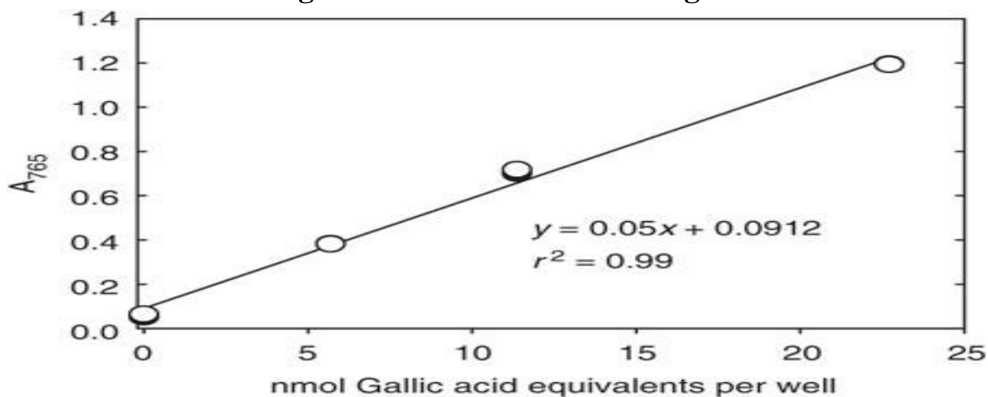

Fig 2. Calibration curve of Rutin

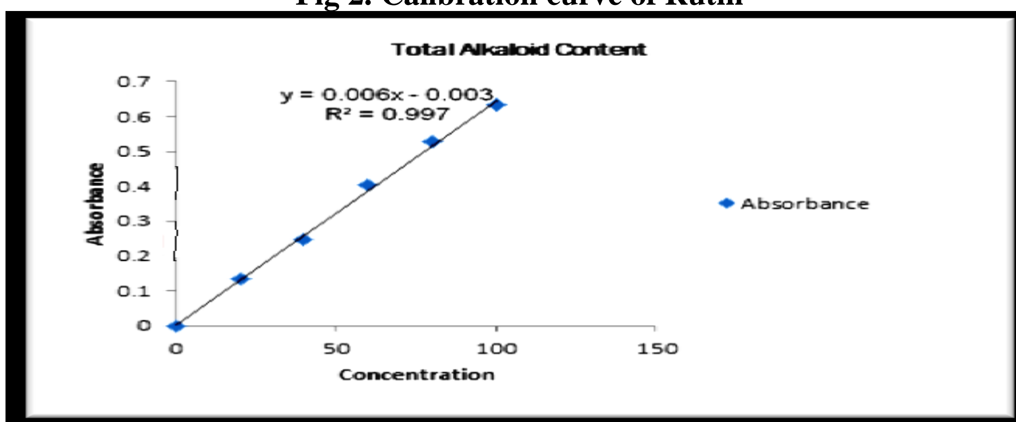

Fig no.3 Calibration curve of Quercetin

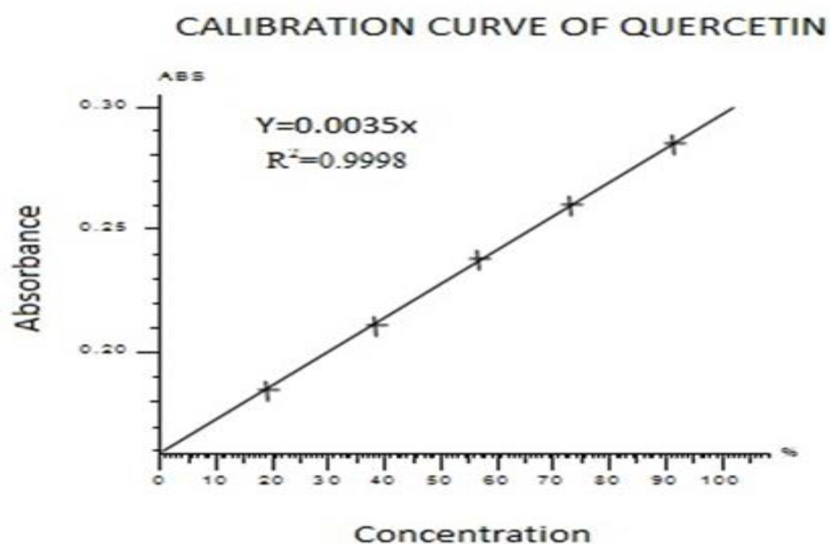

Table 2: Physico-chemical studies of *B.diffusa* whole plant

S.No	Parameters	% w/w
1	Total ash	4%
2	Acid insoluble ash	20.91%
3	Water soluble ash	14.95%
4	Loss on drying	10.32%
5	Swelling index	5 ml
6	Foaming index	Less than 100 in 1 cm

Table 3: Extractive values of different solvent *B.diffusa* whole plant

Solvent	Polarity	Extractive value(w/w)	%(w/v)Extractive value
Hexan	0	0.0117 g	1.17 %
Di chloro methane	3.4	0.09 g	9.11 %
Chloroform	3.4-4.4	0.03 g	3 %
Ethyl acetate	4.3	0.033 g	3.36%
Acetone	5.2	0.05 g	5.06%
Methanol	5.4	0.21 g	21%
Water	6.6	0.34 g	34%

Table : 4. Phyto chemical tests on *B. diffusa* whole plant

Phyto chemical tests	Hexane	Dichloro Methane	Chloroform	Ethyl acetate	aceto ne	methanol	Water	Pet. ether
(Mayer's test)	+	+	_	+	+	+	_	+
Glycosides (Alkaloids Keller- killani test)	+	+	–	–	–	+	–	–
Flavanoids (Lead acetate test)	+	+	++	+	+	+	—	+
Steroids (salkowski test)	+	–	++	–	+	+	–	+
Tri terpenoids (salkiwski test)	–	+	–	–	–	–	–	+
Tannins (ferric chloride test)	+	—	–	—	–	–	–	–
Carbohydrates (fehillings test)	+	–	++	–	–	–	–	–
Saponins (foam test)	++	++	_	_	_	+	_	+

Table No 5: Fluroscence studies on *B.diffusa* whole plant powder

Reagents	Day Light	UV Long Wave	UV Short Wave	Florescence
Picric Acid	Light green	Dark green	Dark green	Light green
Acetic acid	Dark green	Dark green	Pale Yellow	Dark green
Conc.HCL	Pale yellow	Pale yellow	Pale yellow	Pale yellow
Conc.H_2SO_4	Pale yellow	Pale yellow	Yellow	Pale yellow
1N NaOH	Pale yellow	Dark green	Dark green	Dark green
10% NaOH	Pale yellow	Dark green	Pale yellow	Pale yellow
5%NaOH	Pale yellow	Pale yellow	Pale yellow	Pale yellow
5%$Fecl_3$	Dark green	Dark green	Light green	Dark green
Dil.NH_3	Light green	Pale yellow	Pale yellow	Green

Table: 6 Quantitative estimation of total phenolic, alkaloid and flavonoid content

Test type	Inference
Total alkaloid content	0.29
Total flavonoid content	0.45
Total phenolic content	0.59

Total flavonoid Content:

The sample contained 1 ml of methanol solution of the extract in the concentration of 1 mg/ml 2% aluminum tri chloride was dissolved in methanol. The samples were incubated for an hour at room temperature. The absorbance was determines at 415 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. Same procedure was repeated for rutin (as standard) and the calibration curve.

DISCUSSION:

The whole plant powder of *Boerhaavia diffusa* L., was coarse, light brown in colour with a bitter taste and characteristic odour. TLC studies showed similar chromatogram as that of reference standard of *Boerhaavia diffusa* L . It showed the presence of total ash (4% w/w), acid insoluble ash (20.9% w/w) and water soluble ash (14.8% w/w), Loss on drying (10.32%) . It is within the guidelines of IP. This reveals the drug is pure. Alcohol soluble extractive and water soluble extractive values were found to be 21.6% & 31.2% w/w respectively. This shows the nature of the constituents present in crude drug. As the percent yield is good its use can be economical. The phytochemical investigation showed the presence of glycosides, alkaloid, steroids, saponins and flavanoid in methanolic extract. The presence of these constituents is responsible for the wide medicinal use of *Boerhaavia diffusa* L. whole plant. The Quantative estimation showed the presence of Total phenols (0.59), flavonoids (0.45) and alkaloid (0.29) content respectively.

CONCLUSION:

The present study powder indicate the presence of carbohydrate, glycoside, alkaloid, protein, tannin, saponin, flavonoid and terpinoid. In this study it was found that the *B. diffusa* extract has minerals, organic acids, flavonoids and phenolic compounds which has to found possesses antioxidant, mast cells stabilizing effects . *B. diffusa* root is utilized as food or parts of food may provide medical health benefits including

the prevention and or treatment of diseases like diabetes and liver diseases.

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Conflicts of interest: We declare that we have no conflict of interest.

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