

PHYTOCHEMICAL SCREENING OF *Boerhavia diffusa* PLANTS

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Abstract:-

It is estimated that more than 25% of modern medicines are directly or indirectly derived from plants. In this context, it is worth mentioning that Indian medicinal plants are considered a vast source of several pharmaceutically active principles and compounds that are commonly used in home remedies against multiple ailments. The objective of this research is to conduct the preliminary phytochemical screening, of *Boerhavia diffusa*. *Boerhavia diffusa* leaves and stem were collected in march. Extracts of various plants parts were prepared using solvents like water (cold and hot) and organic solvents (methanol, ethanol, ethyl acetate, acetone). It refers to the extraction, screening and identification of the medicinally active substances found in plants. Some of the bioactive substances that can be derived from plants are carbohydrate, protein, flavonoids, alkaloids, tannin, and phenolic compounds.

I. INTRODUCTION

Boerhavia diffusa is a herbaceous perennial plant with vigorous, many-branched stems growing from a taproot; up to 2 metres long the stems can be erect or procumbent. The stems branch mainly from the base, they are prostrate when young, becoming ascending to erect when flowering. A very popular medicinal plant, especially in India, where it is widely used in Ayurveda. Medicines containing this plant are sold worldwide. The plant is gathered from the wild and is also sometimes used as food. It is taken in herbal medicine for pain relief and other uses. The leaves of *Boerhavia diffusa* are often used as a green vegetable in many parts of India.



Fig 1:- *Boerhavia diffusa* plant

II Materials and Methods

A. SAMPLE COLLECTION

The entire plant samples were collected in March 2018.

B. PREPARATION OF PLANT EXTRACTS USING AQUEOUS AND ORGANIC SOLVENTS

Extracts of various plants parts (leaves and stem) of *Boerhavia diffusa* were prepared using solvents like water (cold and hot) and organic solvents (methanol, ethanol, ethyl acetate, acetone). Fresh plant parts collected were surface sterilized with 0.1% HgCl₂ and washed repeatedly with sterile phosphate buffer saline (pH 7.2) followed by distilled water. Plant parts were than dried at 50⁰C using electric drier and crushed with the aid of a

mechanical grinder to powdered form. These powdered plant parts were used to prepare different extracts as described below.

1) Aqueous extract

Fifty grams of dried coarse powdered plant parts were soaked in autoclaved triple distilled water under constant stirring. The filtrate was collected three times at 24 h intervals during a total extraction period of 72 h. The aqueous dry extracts were obtained by concentrating the extract liquid under reduced pressure at 40°C using a vacuum rotary evaporator. The dry extracts were stored at -20 °C until use.

2) Organic solvent extracts

The dried samples were ground to coarse powder form and phyto-constituents were extracted by Soxhlet extractor at 60°C using various solvents like methanol, ethanol, ethyl acetate and acetone. The extracts were evaporated to dryness on the rotary evaporator and stored in a refrigerator at 4°C until required for use. Dry weight of powder before and after extraction was taken to calculate expected total amount of phyto-constituents extracted with given solvent.

C. QUALITATIVE ESTIMATION OF PHYTOCONSTITUENTS

These extract were subjected to chemical test for different phytoconstituents viz. alkaloids, carbohydrates, phenolics, flavonoids, proteins, amino acids, saponins using the standard procedures described (Gupta and Sharma, 2011; Tease and Evans, 1989).

Test for *Proteins & Amino acids*

a) Ninhydrin test: To the 2 ml extract 2 ml on ninhydrin reagent was added & boil for few minutes, formation of bluish purple colour indicates the presence of amino acid.

b) Biuret's Test: To 1 ml of test extract, 4% of sodium hydroxide solution and few drops of 1% copper sulphate solution were added. Formation of a violet red colour indicated the presence of proteins.

Test for Carbohydrates

a) Molisch's Test: Filtrates were treated with 2 drops of alcoholic alpha-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of carbohydrates.

b) Fehling's Test: Filtrates were hydrolyzed with dil. HCl, neutralized with alkali and heated with Fehling's A&B solutions. Formation of red precipitate indicates the presence of reducing sugars.

Test for Coumarin

3 ml of 10% NaOH was added to 2 ml of aqueous extract formation of yellow colour indicates coumarins.

Test for Diterpenes

Copper acetate Test: Extracts were dissolved in water and treated with 3-4 drops copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes. (Roopashree, et al., 2008 and Audu, et al., 2007).

Test for saponins

One mL of the tepal extract was diluted with distilled water to 20 ml and shaken in a graduated cylinder for 15 minutes. The formation of one centimeter layer of foam indicates the presence of saponins.

Test for Alkaloids

a) Mayer's Test: Filtrates were treated with Mayer's reagent (potassium Mercuric Iodide). Formation of a yellow colored precipitate indicates the presence of alkaloids.

b) Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Test for Flavonoids

a) Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Test for Tannins

a) Lead acetate Test Few drops of 1% lead acetate were added to 2 ml of extract. The formation of yellowish precipitate indicated the presence of tannins.

b) Ferric Chloride Test

Extract solutions were treated with 5% ferric chloride solution. As per Culet et al., (2010) formation of blue colour indicated the presence of hydrolysable tannins and formation of green colour indicated the presence of condensed tannins

II. RESULT AND DISCUSSION:**Table 1****Preliminary phytoconstituents analysis of *Boerhavia diffusa* leaves**

		<i>Boerhavia diffusa</i> leaves Extracts					
S.No.		Methanol	Ethanol	Ethyl acetate	Acetone	Aqueous (Cold)	Aqueous(Hot)
1.	Carbohydrate test						
a.	Molish's test	+	+	+	+	+	+
b.	Fehling's test	-	+	+	+	+	+
2.	Protein test						
a.	Ninhydrin test	-	+	+	+	+	+
b.	Biuret test	-	+	-	-	-	-
3.	Tannins						
a	Lead acetate Test	+	+	+	+	+	+
b	Ferric Chloride Test	+	+	+	-	-	-
4.	Saponin	+	-	+	+	+	+
5.	Flavanoid	+	+	-	+	-	-
6.	Alkaloid test						
a.	Mayer's test	-	+	-	-	+	-
b.	Wegner's test	-	-	-	-	-	-
7.	Coumarin	+	-	-	-	-	+
8.	Diterpenes	+	+	+	+	+	+

Table 2
Preliminary phytoconstituents analysis of *Boerhavia diffusa* stem

		<i>Boerhavia diffusa</i> Stem Extracts					
S.No.		Methanol	Ethanol	Ethyl acetate	Acetone	Aqueous (Cold)	Aqueous(Hot)
1.	Carbohydrate test						
a.	Molish's test	-	+	+	+	+	+
b.	Fehling's test	-	-	-	-	+	+
2.	Protein test						
a.	Ninhydrin test	+	+	+	+	+	+
b.	Biuret test	-	-	-	-	-	-
3.	Tannins						
a	Lead acetate Test	-	+	+	+	+	+
b	Ferric Chloride Test	-	-	-	-	-	-
4.	Saponin	+	-	+	+	+	+
5.	Flavanoid	+	-	-	-	-	+
6.	Alkaloid test						
a.	Mayer's test	-	+	-	+	+	-
b.	Wegner's test	-	-	-	-	+	-
7.	Coumarin	+	-	-	-	-	-
8.	Diterpenes	-	+	-	-	-	-

REFERENCES

- [1] Agrawal S. & Mishra K. (1979) Phytochemical study of the fruit pulp of *Grewia asiatica* Linn., journal of Indian Chemical Society, 56 (6): 649.
- [2] Ali S. I., Khan N. A., & Husain I. (1982) Flavonoid constituents of *Grewia asiatica*, Journal of Scientometric Research (Bhopal) 4(1):55 – 56.
- [3] Anonymous (1999). The Ayurvedic Pharmacopoeia of India, Ministry of Health and Family Welfare, Department of I.S.M. & H., Government of India, New Delhi, Part I, I : 140-143,190 – 196.
- [4] Brand-Williams W., Cuvelier M.E., Berset C. (1995) Use of a free radical method to evaluate antioxidant activity. *Lebenswiss Technol*, 28:25-30.
- [5] Chaisawadi, S., Thongbute, D., (1990), *Plants Bioprospecting and Ethnopharmacology*.
- [6] Chatterjee et al , (2011) Evaluation of antitumor activity of *Cuscuta Reflexa* Roxb (Cuscutaceae) Against Ehrlich Ascites Carcinoma in Swiss Albino Mice, *Tropical Journal of Pharmaceutical Research* August 10 (4): 447-454
- [7] Daisy Pitchai, (2007), A database for medicinal plants used in the treatment of diabetes and its secondary complications.
- [8] Das NP, Pereira TA (1990). effects of flavonoids on thermal autooxidation of palm oil: structure- activity relationship. *Journal of American Oil Chemists Society*, 67: 255- 258
- [9] Daswani P, Tetali P, Noshir A, Birdi T (2009) Studies on the antidiarrheal activity of *Aegle marmelos* unripe fruit: Validating its traditional usage. *BioMed Center Complementary and Alternative Medicine*; 9 (47):1-8.
- [10] Edeoga H.O., Okwu D.E., Mbaebie B.O., (2005) Phytochemical constituents of some Nigerian medicinal plants *African Journal of Biotechnology* 4 (7):685-688.
- [11] Fang,J (2002), "Structural Features of an Immunostimulating and Antioxidant Acidic Polysaccharide from the Seeds of *Cuscuta chinensis*." *Planta Medica* 68.

- [12] Government of India, Ministry of Health and Family Welfare. (1999) The Ayurvedic Pharmacopoeia of India, Department of Ayush, India, 35-36.
- [13] Gupta V., Sharma M. (2011) Screening of three Indian medicinal plants extracts for antioxidant activity. *International Journal of Institutional Pharmacy and Life Sciences*, 1(1): 118-137.
- [14] Harborne J.B. Williams C.A. (2000). Advances in flavonoid research. *Phytochemistry*, 55: 481.
- [15] Hibberd J.M, (1998), Localization of photosynthetic metabolism in the parasitic angiosperm *Cuscuta reflexa*, *Planta* ; 205: 506-513.
- [16] Jain R., Katare N., Kumar V., Samanta A.K., Goswami S., Shrotri C K. (2012) invitro anti bacterial potential of different extracts of *Tagetes Erecta* and *Tagetes Patula*. *Journal of Natural Sciences Research*, 2(5):8490
- [17] Katewa S.S. (April 2008). "Poisonous plants of the southern aravalli hills of Rajasthan. *Indian Journal of Traditional Knowledge*.5(9).
- [18] Kaur A. (2013). *Cuscuta reflexa* Roxb. A parasitic plant in Ayurved. *International Journal of Pharmaceutical research and BioScience*, 2(2): 180-190.
- [19] Lawal I. O. (2010) , Ethno medicinal information on collation and identification of some medicinal plants in Research Institutes of South-west Nigeria, *African Journal of Pharmacy and Pharmacology* 4(1):001-007.
- [20] Machado M.A. & Zetsche K. (1990) A structural, functional and molecular analysis of plastids of the holoparasites *Cuscuta reflexa* and *Cuscuta europaea*. *Planta* 181: 91-96.
- [21] Pal D.K, Mandal M. Senthilkumar G.P. Padhiari A. (2006). Antibacterial activity of *Cuscuta reflexa* stem and *Corchorus olitorius* seed. *Fitoter.*, 77: 589-591.
- [22] Pankaj Kumar. Keshav Kumar Choudhary, Swati Goswami , Reena Jain (2018) *International Journal for Scientific Research & Development/ Vol. 7, Issue 02, 2019*
- [23] Vijikumar S. (2010) , *Cuscuta reflexa* Roxb. – A Miracle Plant in Ethno Medicine" *Abstracts and Scientific papers of SCORE 1*:66-71.
- [24] Woisky R., Salatino A., (1998) Analysis of Propolis: some parameters and procedures for chemical quality control. *Journal of Apicultural Research*, 37: 99-105.
- [25] Xiao J.B. (2007), *Chromatographia*, 65:185-190.
- [26] Zia-Ul-Haq M., Stankovic M. S., Rizwan K., Feo V.D (2013). *Molecules* ISSN 1420-3049, *Molecules*, 18, 2663-2682; doi: 10.3390 / molecules 18032663, Review: *Grewia asiatica* L., a Food Plant with Multiple Uses.