IJBPAS, September, 2021, 10(9): 3321-3330

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

PHYTOCHEMICAL SCREENING OF ETHANOLIC EXTRACT OF *EICHHORNIA CRASSIPES* FLOWERS

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Received 29th Jan. 2021; Revised 27th Feb. 2021; Accepted 24th June 2021; Available online 1st Sept. 2021

https://doi.org/10.31032/IJBPAS/2021/10.9.6110

ABSTRACT

Traditionally many herbal medicines and medicinal plants have been used for treatment of diseases. Though plant and plant products are recognised as safe, a regular and wide spread use of herbs throughout the world has increased serious concern over their quality, safety and efficacy. Thus, a proper scientific evidence or assessment has become the criteria for acceptance of herbal health claims. In the present study, the phytochemical screening of *Eichhornia crassipes* flower extract was examined. Dried and powdered *Eichhornia crassipes* flowers were extracted with ethanol by hot continuous extraction in Soxhlet apparatus. The ethanolic extract of *Eichhornia crassipes* (EEEC) was subjected to phytochemical examination. The Preliminary phytochemical examination revealed the presence of alkaloids, phenolic compounds, tannins, flavonoids, sterols, terpenoids, glycosides, carbohydrates, proteins and amino acids. The phytochemical results obtained in this study clearly indicate that the bioactive compounds present in flowers of *Eichhornia crassipes* have effect in treating different diseases and could rationally justify the ethnobotanical uses in traditional medicine.

Keywords: *Eichhornia crassipes* flowers, Phytochemicals, Ethanolic extract, Flavonoids

INTRODUCTION

Traditionally, a variety of herbal medicines and medicinal plants have been utilised to treat complex diseases and ailments. Phytochemicals are bioactive

molecules found in nature. Alkaloids, tannins, flavonoids, steroids, terpenoids, carbohydrates, and phenolic chemicals are naturally occurring bioactive components of plants. Since prehistoric times, plants have been utilised as a substitute medicine to increase human health and endurance in many places of the world. The following bioactive compounds are found in plant parts: alkaloids, steroids, tannins, glycosides, volatile oils, fixed oils, resins, phenols, and flavonoids. Given its importance in the preceding context, such preliminary phytochemical screening of plants is critical for the discovery and development of novel medicinal medicines with increased efficacy. Numerous research organisations have also published similar studies on a global scale. There is an increasing awareness of the relationship between a medicinal plant's phytochemical elements and its pharmacological efficacy. The screening of active chemicals from plants has resulted in the development of new medicinal medications that provide effective protection and are used to treat a variety of ailments. Currently, natural compounds generated from plants are being screened for the presence of novel drugs with unique modes of pharmacological action **[1, 2]**.

Eichhornia crassipes (Pontederiaceae), commonly known as water hyacinth, is one of the aquatic plants that have attracted the most scientific interest in the last decade. Originally from South America, its ornamental appeal led to its introduction into Africa, Asia, the South Pacific, North America and Europe, where it has become invasive **[3, 4]**. Plants can grow up to 3 feet in height above the surface of the water. The leaves are oval to elliptical, thick, up to 6 inch wide and waxy with spongy petioles, curved inwards at the edges. Flowers are blue-purple on upright spikes. Each flower has six petals with the uppermost having a yellowish patch **[5]**. Its great capacity to multiply has become a real problem in the tropics, where high temperatures and lack of predators has led to its uncontrolled development. However, *Eichhornia crassipes* is not just an invasive and harmful plant; it is also a useful plant with remarkable metal pollutant phytoaccumulation capacities. *Eichhornia crassipes* is capable of bio-concentrating toxic metals such as Cr, Cu, Co, Ni, Pb, Cd and As in its root system **[6-8]**. It is also reported to possess valuable phytochemicals which are of medicinal importance **[9]**. The fresh juice of this weed is used by tribes to treat wounds, to ease swelling, burning and to stop bleeding **[10]**. *Eichhornia crassipes* leaves have been reported to possess a potential antibacterial, antifungal, antiparasitic, antiviral and anticancer activity **[11]**. Scientifically there are no valid reports available on

phytochemical and antioxidant properties of *Eichhornia crassipes* flowers. Hence, the present study was aimed to investigate the phytochemical activity of *Eichhornia crassipes* flower extract.

MATERIALS AND METHODS

Plant Material

The fresh flowers of *Eichhornia crassipes* were collected in the month of June from Bhavani River, Tamilnadu, India. The plant material was taxonomically identified, confirmed and authenticated by Dr. A. Balasubramanian, Executive Director and Former Siddha Research Consultant (AYUSH), ABS Herbal gardens, Salem and the authentication was retained in our laboratory for further reference. The collected flowers were shade dried and the dried materials were crushed to coarse powder with mechanical grinder. The powder was stored in an airtight container for extraction. The image of *Eichhornia crassipes* flower is shown in **Figure 1**.

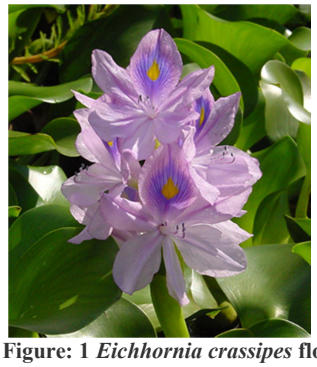

Figure: 1 *Eichhornia crassipes* flowers

Extraction

The powdered flowers of *Eichhornia crassipes* were subjected to hot continuous extraction using ethanol as a solvent material in Soxhlet apparatus for 72 hours. After extraction, the solvents were removed by distillation and evaporated under reduced pressure in a rotary evaporator to obtain crude extract of *Eichhornia crassipes* flowers. The collected extract was then transferred to a clean glass vessel and covered with a foil paper in which slits are made for evaporation of solvent traces. The dried extract thus obtained was stored in air tight glass container for further investigation.

Phytochemical screening

The extract obtained was subjected to preliminary phytochemical screening **[12]**.

Detection of alkaloids

Individually the extracts were dissolved in dilute hydrochloric acid and filtered. The filtered extract was subjected to detection of alkaloids.

Mayer's Test

Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). The formation of a yellow-coloured precipitate indicates the presence of alkaloids.

Wagner's Test

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

Dragendroff's Test

Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). The formation of red precipitate indicates the presence of alkaloids.

Hager's Test

Filtrates were treated with Hager's reagent (saturated picric acid solution). The formation of yellow coloured precipitate indicates the presence of alkaloids.

Detection of phytosterols

Libermann's Test

To 2 ml filtrate in hot alcohol, few drops of acetic anhydride were added. Formation of brown precipitate indicates the presence of sterols.

Libermann Burchard's test

Extract was treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

Salkowski's Test

Extract was treated with chloroform and filtered. The filtrates were treated with few drops of concentrated sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

Detection of flavonoids

Alkaline Reagent Test

Extract was 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.

Shinoda test

A few fragments of magnesium ribbon and concentrated hydrochloric acid were added to the ethanol extract. The appearance of red to pink colour after few minutes indicates the presence of flavonoids.

Detection of saponins

Froth Test

Extract was diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Foam Test

0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

Detection of proteins and amino acids

Millon's Test

2 ml of filtrate was treated with 2 ml of millon's reagent in a test tube and heated in a water bath for 5 minutes, cooled and few drops of sodium nitrite were added. Formation of white precipitate which turns to red upon heating indicates the presence of proteins and amino acids.

Ninhydrin Test

To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

Biuret Test

2 ml of filtrate was treated with 2ml of 10% sodium hydroxide in a test and heated for 10 minutes. A drop of 7% copper sulphate solution was added in the above mixture. Formation of purplish violent indicates the presence of Proteins.

Detection of fixed oils and fats

Oily spot test

One drop of extract was placed on filter paper and the solvent was evaporated. An oily stain of filter paper indicates the presence of fixed oil.

Detection of phenols

Detection of phenols and tannins

Ferric Chloride Test

Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Detection of tannins

Lead acetate Test

To 2ml of filtrate, few drops of lead acetate solution were added in a test tube. Formation of yellow precipitate indicates the presence of tannins.

Detection of carbohydrates

The extract was dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Molisch's Test

Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. The formation of the violet ring at the junction indicates the presence of carbohydrates.

Fehling's Test

Filtrates were hydrolysed with dilute hydrochloric acid, neutralized with alkali and heated with Fehling's A & B solutions. The formation of red precipitate indicates the presence of reducing sugars.

Benedict's Test

Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Detection of glycosides

The extract was hydrolysed with dilute hydrochloric acid and then subjected to test for glycosides.

Modified Borntrager's Test

The extract was treated with ferric chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides.

Legal's Test

The extract was treated with sodium nitropruside in pyridine and sodium hydroxide. The formation of pink to blood red colour indicates the presence of cardiac glycosides.

Keller Killiani Test

Small portion from the extract was shaken with 1ml of glacial acetic acid containing trace of ferric chloride. 1ml of concentrated sulphuric acid was added carefully by the sides of the test tube. A blue colour in the acetic acid layer and red color at the junction of two liquids indicate the presence of glycosides.

Test for Terpenoids

2 ml of the organic extract was dissolved in 2ml of CHCl3 and evaporated to dryness. 2ml of conc. H2SO4 was then added and heated for about 2 minutes. Development of a greyish colour indicates the presence of terpenoids.

RESULTS

Percentage yield

The percentage yield of extract obtained from powdered *Eichhornia crassipes* flowers using ethanol as solvent was 7.25 % w/w.

Preliminary phytochemical studies

Preliminary qualitative investigation performed in the ethanol extract of *Eichhornia crassipes* flowers revealed the presence of major phytoconstituents alkaloids, phenolic compounds, tannins, flavonoids, sterols, terpenoids, glycosides, carbohydrates, proteins and amino acids. Preliminary Phytochemical screening of *Eichhornia crassipes* revealed the presence of following phytoconstituents showed in **Table 1**.

Table 1: Phytochemical screening of *Eichhornia crassipes*

S. No.	Test	Ethanolic extract of *Eichhornia crassipes*
1.	Alkaloids	+
2.	Phytosterols	+
3.	Flavonoids	+
4.	Saponins	−
5.	Proteins & Amino acids	+
6.	Fixed oils and Fats	−
7.	Phenolic compounds and Tannins	+
8.	Carbohydrates	+
9.	Glycosides	+
10.	Terpenoids	+

(-) indicates the absence of compound; (+) indicates the presence of compound

DISCUSSION

Since time immemorial, man has been using plant extracts to protect himself against several diseases and also to improve his health and life-style. Over the past decade, herbal medicines have become a topic of global importance, making an impact on both world health and international trade. Herbal medicines continue to play a central role in health care system of large proportions of the world's population **[13]**. In recent years, there has

been considerable interest in natural products with antioxidant property. Traditionally, many herbal medicines and medicinal plants have been used for the treatment of complicated diseases. Various experimental models have confirmed the protective effect of plants and plant isolates against different type of diseases like cancer, liver disorders, diabetes, atherosclerosis and inflammatory diseases etc. The present study investigated the ethanolic extract of *Eichhornia crassipes* flowers showed the presence of major phytoconstituents such as alkaloids, phenolic compounds, tannins, flavonoids, sterols, terpenoids, glycosides, carbohydrates, proteins and amino acids. Recent research indicates that flavonoids, a class of polyphenolics, are free radical scavengers and super antioxidants with anti-inflammatory properties, the ability to prevent oxidative cell damage due to their water-soluble nature, and anti-cancer action **[14, 15]** have demonstrated tremendous anti-oxidant capabilities. Flavonoids found in the gastrointestinal system help reduce the risk of heart disease. Triterpenoids exhibit anti-inflammatory, analgesic, and hepatoprotective effects **[16]**. Tannins may be useful as cytotoxic and anti-cancer agents, as well as aiding in the healing of wounds and inflamed mucous membranes **[17, 18]** Phytosterols are cardiotonic and have antibacterial and insecticidal effects. Phenolic compounds have antimicrobial properties, antioxidant, anti-diabetic, anti-cancer, anti-mutagenic, and anti-inflammatory **[19]**, inhibition of angiogenesis and cell proliferation, as well as endothelial function improvement. Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds **[20, 21]**. Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity. Several workers have reported the analgesic **[22]**, antispasmodic and antibacterial **[23, 24]** properties of alkaloids. Glycosides are known to lower the blood pressure according to many reports **[25, 26]**.

CONCLUSION

India has a diverse flora that is used in traditional medicine. These plants are therapeutic due to their phytochemical constituents. These phytochemicals have been shown to have therapeutic and antioxidant properties in humans. In conclusion, the ethanolic extract of *Eichhornia crassipes* flowers showed the presence of major phytoconstituents such as alkaloids, phenolic compounds, tannins, flavonoids, sterols, terpenoids, glycosides, carbohydrates, proteins and amino acids. The presence of these phytochemicals in ethanolic extract of *Eichhornia crassipes* flowers have medicinal as well as

physiological properties in the treatment of different ailments.

ACKNOWLEDGEMENTS

The authors are thankful to the management of JKK Munirajah College of Pharmacy, Komarapalayam, Tamil Nadu, INDIA for providing necessary facilities to carry out the research work.

REFERENCES

[1] Roghini, R. and Vijayalakshmi, K. 2018. Phytochemical Screening, Quantitative Analysis of flavonoids and Minerals in ethanolic extract of Citrus paradise, International Journal of Pharmaceutical Sciences and Research, 9(11): 4859-4864.

[2] A. Poongothai. Qualitative and quantitative phytochemical analysis of *Lantana camara* leaf extract. Journal of Emerging Technologies and Innovative Research.2019; 6(12):603-607.

[3] Jones RW, Hill JM, Coetzee JA, Hill MP. The contributions of biological control to reduced plant size and biomass of water hyacinth populations. Hydro biologia 2018; 807(1):377-388.

[4] Kriticos DJ., Brunel S. Assessing and managing the current and future pest risk from water hyacinth, (*Eichhornia crassipes*), an invasive aquatic plant threatening the environment and water security. PLoS One 2016; 11(8):e0120054.

[5] Gopal B. Water hyacinth. Elsevier Science Publishers, Amsterdam, 1987, pp.477.

[6] Ali M, Bhat AK, Dolkar T, Malik MA. Phytoremediation: A Plant-Based Technology. International Journal of Current Microbiology and Applied Sciences 2018; 7(3):766-777.

[7] Saha P, Shinde O, Sarkar. Phytoremediation of industrial mines wastewater using water hyacinth. International Journal of Phytoremediation 2017; 19(1):87-96.

[8] Ugya AY, Imam TS, Tahir SM. The role of phytoremediation in remediation of industrial waste". Journal of Pharmaceutical Research 2016; 5(12):1403-1430.

[9] Lalitha P, Sripathi SK, Jayanthi. Acute toxicity study of extracts of Eichhornia crassipes (mart.) Solms. Asian Journal of Pharmaceutical and Clinical Research 2012; 5(4):59-61.

[10] Oudhia P. Traditional medicinal knowledge about a noxious weed, jalakumbhi (*Eichhornia crassipes*), in Chhattisgarh (India). Aquaphyte Online, 2001.

[11] Aboul-Enein AM, Al-Abd AM, Shalaby EA, Abul-Ela F, Nasr-Allah AA. Mahmoud AM., El-Shemy HA. *Eichhornia crassipes* (Mart) solms. from water parasite to potential medicinal remedy. Plant Signaling and Behavior 2011; 6(6):834-836.

[12] Khandelwal KR and Kokate CK. Pratical pharmacognosy, 4th ed, Vallabh Prakashan, New Delhi, 1995, pp.110.

[13] Akerela O. Medicinal plants and primary health care: An agenda for action. Fitotherapia 1988; 59:355-363.

[14] Gurib-Fakim A. Medicinal plants: traditions of yesterday and drugs of tomorrow. Mol. Aspects Med.2006; 27(1): 1-93.

[15] Salah N, Miller NJ, Paganga G, Tijburg L,Bolwell GP, Rice-Evans C. Polyphenolic flavonols as scavengers aqueous phase radicals and as chain branching antioxidants. Arch. Biochem. Biophys.1995; 322(2): 339-346.21.

[16] Savithramma N, Ankanna S Preliminary Phytochemical Analysis of Traditionally used Medicinal Plants. Res. J. Pharm. Bol.Chem. Sci. 2012; 3(1): 308-314.22.

[17] Kozioc M.J and Marcia M.J. Chemical composition, nutritional evaluation and economic prospects of *Spondias purpurea* (Anarcadiaceae).Economic Botany, 1998;52: 373 – 380.23.

[18] Okwu D. E. The phytochemicals and vitamins contents of indigenous spices of South Eastern, Nigeria. J. Sust. Agric. Environ. 2004; 6: 30 – 34.

[19] S. Sivakrishnan and A. KottaiMuthu. Phytochemical Evaluation of Ethanolic Extract of Aerial Parts of *Albizia procera*. British Biomedical Bulletin. 2014; 2(1):235-241.

[20] Brown, J.E., Rice-Evans, C.A. 1998. Luteolin rich artichoke extract protects low density lipoprotein from oxidation in vitro. Free Radical Res., 29: 247-255.

[21] Krings, U., Berger, R.G. 2001. Antioxidant activity of roasted foods. Food Chem., 72: 223-229.

[22] Antherden, L.M. 1969. Textbook Of Pharmaceutical Chemistry, 8th edn., Oxford University Press, London, pp. 813-814.

[23] Stray, F. 1998. The Natural Guide to Medicinal herbs And Plants. Tiger Books International, London, pp. 12-16.

[24] Okwu, D.E., Okwu, M.E. 2004. Chemical composition of Spondias

mombin linn. plant parts. J. Sustain. Agric. Environ., 6(2): 140-147.

[25] Nyarko, A.A., Addy, M.E. 1990. Effects of aqueous extract of *Adenia cissampeloides* on blood pressure and serum analyte of hypertensive patients. Phytotherapy Res., 4(1): 25-28.

[26] RNS Yadav and Munin Agarwal. Phytochemical analysis of some medicinal plants. Journal of Phytology. 2011, 3(12): 10-14.