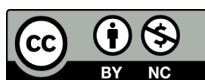


Phytol as a hepatoprotective compound in the leaves of *Eichhornia crassipes*Anuradha Shukla¹ and Rashmi Tripathi¹ ✉¹Department of Bioscience and Biotechnology, Banasthali Vidyapith, Banasthali (304022), Rajasthan, India.

Received June 27, 2022
 Revised September 26, 2022
 Accepted October 22, 2022
 Published February 06, 2023



Copyright: © 2022 Anuradha Shukla & Rashmi Tripathi. This is an open access article distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Abstract: Water hyacinth is undoubtedly an environmental problem due to its invasiveness, but it also has some promising futures. It might be preferable to consider the specific attributes of each environment where the plant grows. When there are facilities available to fully utilize this plant's potential, efforts should be focused on maximizing its growth. It has been shown that secondary metabolites, such as phenolic and anti-oxidant components, play a crucial role in the chemical defenses of plants against invaded microscopic organisms. This test was conducted to analyze whether *Eichhornia crassipes* leaves contained antioxidants or the hepatoprotectant. The ethyl acetate-fractionated hydroethanolic extract was used for the analysis. Using gas chromatography-mass spectrometer (GC-MS), and high-pressure liquid chromatography, the bioactive components of the ethyl acetate fraction of the hydroethanolic extract of the leaves of *E. crassipes* (Mart.) Solms were analyzed. The GC-MS analysis of the extract revealed the presence of phytol, a constituent that is essential for hepatoprotection. The human body needs phytol for its benefits. Phytol may also be considered a potential new drug and treatment for hepatic dysfunction. The numerous substances found in plants used in traditional medicine can be used to treat several ailments. The majority of people follow traditional medicine, which contains substances derived from medicinal plants, is practiced. Therefore, research into these plants is necessary to better understand their characteristics, safety, and effectiveness. Hence, the present work aimed to evaluate the protective efficacy of the leaf extract of *E. crassipes* against fluoride-induced toxicity.

Keywords: *Eichhornia crassipes*; ethyl acetate; GC-MS; leaves; phytol.

1. Introduction

Herbal remedies are risk-free, well-tolerated, and devoid of negative side effects [1]. The bioactive secondary metabolites of *Eichhornia crassipes* are abundant and include sterols, alkaloids, phenolics, flavonoids, tannins, and saponins. These secondary metabolites have a variety of well-known therapeutic benefits [2]. The plant is also abundant in different bioactive substances, which have a wide range of pharmacological properties. These include antioxidant, antimicrobial, anti-inflammatory as well as hepatoprotective, and wound healing [3-7]. The liver is a vital organ in the human body [8-10]. It performs crucial bodily tasks like eliminating toxins, neutralizing toxins, and eliminating harmful substances. Fluoride exposure may be a factor in complex alterations to parameters related to the

kidney and liver [11]. Allopathic drugs have a variety of harmful side effects, ranging from minor nausea and vomiting to potentially fatal conditions, and they don't offer enough hepatoprotection. Alternative drugs must therefore be created to manage and prevent hepatic disorders. So, the present study was taken up to determine the hepatoprotective constituents in the leaves of *E. crassipes* and the significance of this plant in the conception of drugs to counteract hepatotoxic stimulants.

2. Experimental

In this study, all analytical grade chemicals will be procured and used from Himedia, Sigma-Aldrich, and E-Merck Company (India).

2.1 Collection and extract preparation of plant leaves

E. crassipes an aquatic plant in the summer was collected from Chekhla, Sanand, Ahmedabad district, Gujarat, India because of their medicinal qualities. Fresh green leaves were gathered, cleaned with water, dried, grounded, and placed in airtight containers for storage. The plant was recognized and verified at the Patanjali Research Centre in Haridwar (Authentication no. 2626). The powder was macerated with hydroethanol for 72 h. With the help of an



Dr. Rashmi Tripathi
 Department of Bioscience and Biotechnology,
 Banasthali Vidyapith,
 Banasthali- 304022
 Rajasthan, India
 E-mail: tripathi.rashmi@gmail.com

Table 1. GC-MS investigation of ethyl acetate extract of *E. crassipes* leaves.

Phytochemical constituents	M.wt.	Retention time	Probability	Biological Activity
Phytol C ₂₀ H ₄₀ O	296.307	43.95	41.59	Anticancer, Anti-diuretic Anti-inflammatory Antimalaria Antimicrobial Antioxidant, Cancer-Preventive Hepatoprotective.

ethyl acetate solvent, the hydroethanolic crude extract was fractionated. The fractionated extracts were dried and kept at 4°C for later use.

2.2 Gas chromatography-mass spectrometry (GC-MS)

To count the number of chemicals in the ethyl acetate fraction, the ethyl acetate extract was suspended in acetone (HPLC grade). Impurities were removed using filtration using Varian Bond Elute C18 solid phase extraction at a concentration of 1 mg/ml. We used 1 µl of the aforementioned solution for the GC-MS analysis. A system with an auto-sampler was used to conduct the GC-MS (GC Clarus 500 Perkin Elmer) analysis (AOC-20i). The instrument operated under the following circumstances: Helium (99.999%) was used as the carrier gas at a steady flow of 1 ml/min, TG 5MS column (5% phenyl methyl

polysiloxane) was operated at 70 eV electron impact mode, and injector temperature and ion source temperature were both 200°C. The oven's temperature was set at 110°C (isothermal for 2 min), then increased by 10°C to 200°C/min, then by 5°C to 280°C/min, with a final isothermal at 280°C lasting for 9 min. The fragments used in the mass spectra ranged in size from 40 to 650 Da and were detected at 70 eV with a 0.5 s scan interval. The MS transfer line is 280°C in temperature. the spectrum obtained using NIST Ver. 2.1 MS data library was combined with the chemicals in the plant extract.

3. Results & Discussion

In phytoconstituent evaluation of medicinal plants containing biologically active components, GCMS is significant [12]. The ethyl acetate extract was studied by

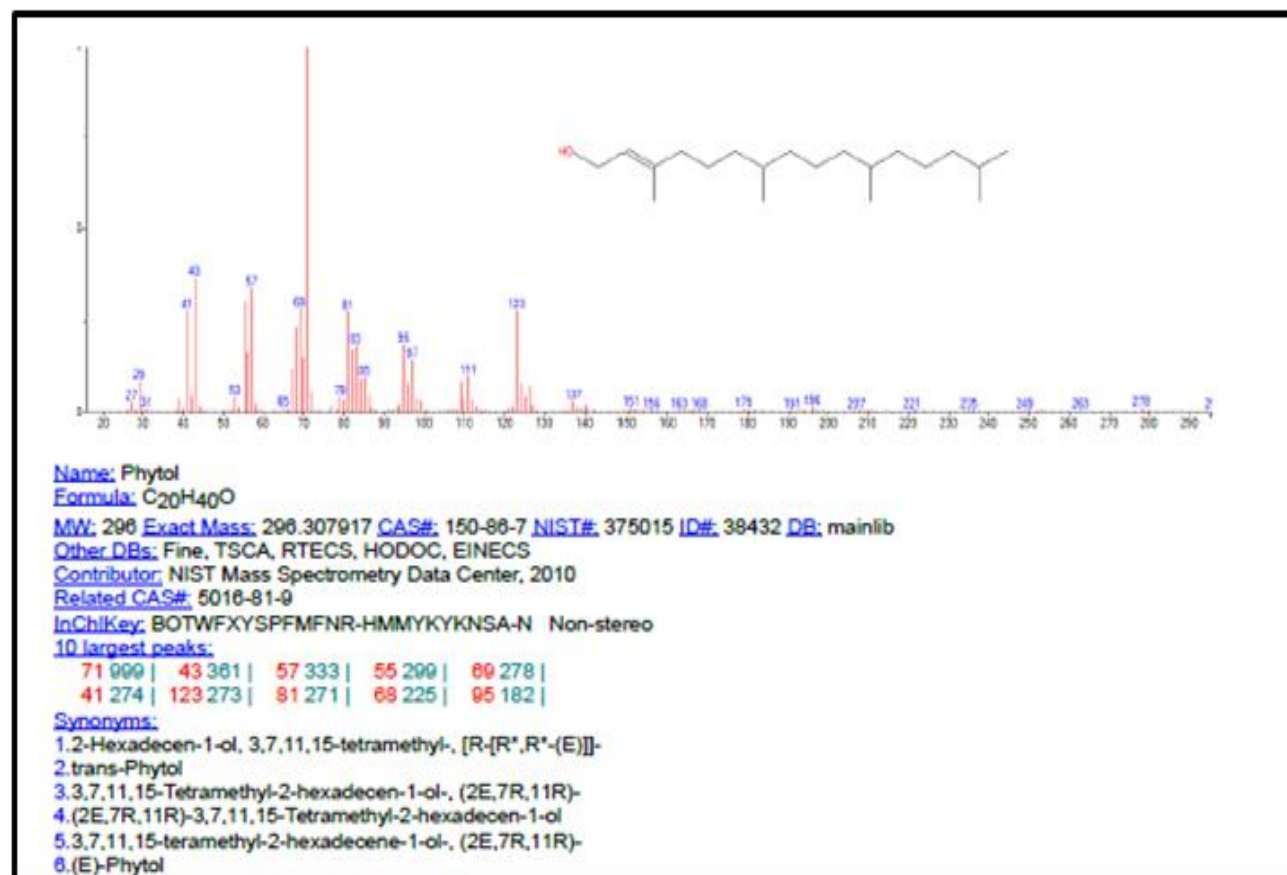


Figure 1: Phytocompound phytol identified from ethyl acetate fraction of leaves of *E. crassipes* by GC-MS analysis chromatogram.

GC-MS technique to identify the presence of the possible chemicals in the leaf extract. The ethyl acetate fraction of *E. crassipes* leaves showed the presence of various peaks which indicates the existence of various phytochemicals. The phytochemicals characterized were further recognized by their chemical properties. The results show the presence of various forms of phytol (Table 1; Figure 1) having a probability of 41.59. The chromatogram of the crude extract revealed not only the presence of phytol but also other compounds like 6-(3-hydroxy-but-1-enyl)-1,5,5-trimethyl-7-oxabicyclo[4.1.0]heptan-2-ol; ketone; 2,2-dimethyl cyclohexyl methyl, 1,18-nonadecadien-7,10-dione; n-propyl 9,12-octadecadienoate; 9,12-octadecadienoic acid; ethyl ester; ethyl 9,12-hexadecadienoate; methyl 2-hydroxy-octadeca-9,12,15-trienoate. Phytol was identified by GC-MS in the polar extract from the whole plant collected from India. This compound is considered a major bioactive compound present in the leaves of plants collected from India [13, 14]. Phytol is a linear diterpene alcohol occurring in many Essential oils as the degradation form of chlorophylls [15]. Recently, phytol was found effective as an anti-depressive agent, acting through serotonergic and adrenergic modulation [16]. Also, anti-inflammatory, antioxidant, immunomodulatory, cytotoxic, and antimicrobial effects were demonstrated [17]. Phytol is known as an antioxidant agent in mammals [18]. Hepatic data indicated that phytol stimulated antioxidant enzymes. Phytol suppressed plasma ALT and AST elevation after ammonia exposure, which may be interpreted as hepatoprotective effects of phytol [19]. Further, the *in vivo* hepatoprotective study would be conducted to confirm the hepatoprotective effect of the extract.

4. Conclusion

In conclusion, the phytol was isolated as the majority of components among other phytochemical compounds from the leaves of *E. crassipes*. Phytol is a phytochemical component found in plants. It is found in all types of plants in nature and cyclic diterpene. The amount of phytol is significantly more than any other constituents in the ethyl acetate extract which indicates that the leaf of *E. crassipes* could be a great herbal asset for future drug discovery. Phytol is a natural linear diterpene fatty alcohol present in an integral part of chlorophyll. The possible mechanism behind the hepatoprotection of phytol was might be due to its antioxidant activity, free radical scavenging activity, and synergistic effect [20].

Declarations

Author Contribution: AS conducted, wrote, and revised the manuscript and RT conceptualized, wrote, reviewed, and supervised the work.

Acknowledgments: The authors thank Hon'ble V.C. Prof. Ina Shastri Banasthali Vidyapith, Banasthali, Rajasthan, for the research infrastructure and facilities. The authors take this opportunity to thank Prof. Dipjyoti Chakraborty Head, Department of Bioscience and Biotechnology, and Dean of Research and Development, Banasthali Vidyapith, Banasthali, for establishing the environment and support. The authors would like to

acknowledge Bioinformatics Centre, Banasthali Vidyapith supported by DBT for providing computation support. The authors would also like to acknowledge DST for providing networking support through the FIST program at the Department of Bioscience and Biotechnology

Funding: Not Applicable

Conflict of Interest: The authors declare no conflict of interest.

References

- [1] Sam S (2019). Importance and effectiveness of herbal medicines. *J Pharmacog Phytochem*; 8(2):354-357.
- [2] Seca AML, Pinto DCGA (2019). Biological potential and medical use of secondary metabolites. *Medicines (Basel)*; 6(2):66. [[CrossRef](#)] [[PubMed](#)]
- [3] Bhavsar V, Vaghasiya J, Suhagia BN, Thaker P (2020). Protective effect of *Eichhornia crassipes* against cerebral ischemia reperfusion injury in normal and diabetic rats. *J Stroke Cerebrovasc Dis*; 29(12):105385. [[CrossRef](#)] [[PubMed](#)]
- [4] Khalid S, Shaheen S, Hussain K, Shahid MN, Sarwar S (2020). Pharmacological analysis of obnoxious water weed: *Eichhornia crassipes* (Mart.) Solms. *J Animal Plant Sci*; 30(6):1465-75. [[CrossRef](#)]
- [5] Verma VK, Prakash O, Kumar R, Rani KV, Seghal N (2021). Water hyacinth (*Eichhornia crassipes*) leaves enhances disease resistance in *Channa punctata* from *Vibrio harveyi* infection. *J Basic Appl Zool*; 82(1):1-11. [[CrossRef](#)]
- [6] Bakrim WB, Ezzariai A, Karouach F, Sobeh M, Kibret M, Hafidi M, Kouisni L, Yasri A (2022). *Eichhornia crassipes* (Mart.) Solms: A comprehensive review of its chemical composition, traditional use, and value-added products. *Front Pharmacol*; 18(13):842511. [[CrossRef](#)] [[PubMed](#)]
- [7] Gebrehiwot H, Dekebo A, Annisa ME (2022). Chemical composition, pharmacological activities and biofuel production of *Eichhornia crassipes* (water hyacinth): a review. *Turkish Chemical Society Section A: Chemistry*; 9(3): 849-866. [[CrossRef](#)]
- [8] Isangedighi IA, David GS (2019). Heavy metals contamination in fish: effects on human health. *J Aquat Sci Mar Biol*; 2(4):7-12.
- [9] Musleh MM, Alajrami E, Khalil AJ, Abu-Nasser BS, Barhoom AM, Abu-Naser SS (2019). Predicting liver patients using artificial neural network. *Int J Academ Inform System Res*; 3(10):1-11.
- [10] Rabbi MF, Hasan SM, Champa AI, Zaman AM, Hasan MK (2020). Prediction of liver disorders using machine learning algorithms: a comparative study. In *2nd International Conference on Advanced Information and Communication Technology (ICAICT)*, Dhaka, Bangladesh; 111-116. [[CrossRef](#)]
- [11] Malin AJ, Lesseur C, Busgang SA, Curtin P, Wright RO, Sanders AP (2019). Fluoride exposure and kidney

- and liver function among adolescents in the United States: NHANES, 2013-2016. *Environ Int*; 132:105012. [[CrossRef](#)] [[PubMed](#)]
- [12] Chak P, Chaudhary D, Jain P, Jain S, Sharma S, Dwivedi J (2021). Phytochemical and GC-MS analysis of *Chenopodium album* and *Stellaria media*. *Indian J Pharm Sci*; 83(6):1261-1272. [[CrossRef](#)]
- [13] Tyagi T, Agarwal M (2017). Phytochemical screening and GC-MS analysis of bioactive constituents in the ethanolic extract of *Pistia stratiotes* L. and *Eichhornia crassipes* (Mart.) Solms. *J Pharmacogn Phytochem*; 6(1):195-206.
- [14] Kumar D, Karthik M, Rajakumar R (2018). GC-MS analysis of bioactive compounds from ethanolic leaves extract of *Eichhornia crassipes* (Mart) Solms. and their pharmacological activities. *Pharma Innov J*; 7(8):459-462.
- [15] Venditti A, Frezza C, Bianco A, Serafini M, Cianfaglione K, Nagy DU, Iannarelli R, Caprioli G, Maggi F (2017). Polar constituents, essential oil and antioxidant activity of marsh woundwort (*Stachys palustris* L.). *Chem Biodivers*; 14(3):e1600401. [[CrossRef](#)] [[PubMed](#)]
- [16] Costa JP, Machado KC, Santos P, Islam MT, Machado KC, Amélia A, Freitas R (2019). Antidepressant-like action of phytol, possibly via reducing oxidative stress in the mice brain. *Pharmacology Online*; 1:376-384.
- [17] Islam MT, Ali ES, Uddin SJ, Shaw S, Islam MA, Ahmed MI, et al. (2018). Phytol: a review of biomedical activities. *Food Chem Toxicol*; 121:82-94. [[CrossRef](#)] [[PubMed](#)]
- [18] Sakthivel R, Sheeja Malar D, Archunan G, Pandima Devi K (2019). Phytol ameliorated benzo(a)pyrene induced lung carcinogenesis in Swiss albino mice via inhibition of oxidative stress and apoptosis. *Environ Toxicol*; 34(4):355-363. [[CrossRef](#)] [[PubMed](#)]
- [19] Saha M, Bandyopadhyay PK (2020). In vivo and in vitro antimicrobial activity of phytol, a diterpene molecule, isolated and characterized from *Adhatoda vasica* Nees. (Acanthaceae), to control severe bacterial disease of ornamental fish, *Carassius auratus*, caused by *Bacillus licheniformis* PKBMS16. *Microb Pathog*; 141:103977. [[CrossRef](#)] [[PubMed](#)]
- [20] Gupta K, Taj T, Thansiya B, Kamath JV (2019). Pre-clinical evaluation of hepatoprotective activity of phytol in Wistar albino rats. *Int J Compr Adv Pharmacol*; 4(1):17-20. [[CrossRef](#)]