



Studies on Microscopic, Physiochemical and Phytochemical profiling of Liver protective plant Gulf leaf-flower (*Phyllanthus fraternus*)

Zehra Husaini¹, Aaliya Fatima¹, Shayma Saifi¹, Saba Rana¹, Mohd Salman¹, Akansha Nirwal²

¹Department of Biosciences, Shri Ram College, Muzaffarnagar, UP, India

²Department of Pharmacy, Shri Ram Group of Colleges, Muzaffarnagar UP, India

Article info

Received: 11/04/2022

Revised: 27/05/2022

Accepted: 24/06/2022

© IJPLS

www.ijplsjournal.com

Abstract

The genus *Phyllanthus*, comprising numerous species within the Phyllanthaceae family, is recognized for its wide-ranging diversity in growth forms, floral structures, and chromosome numbers. Among its notable species, *Phyllanthus fraternus* L., commonly known as the Gulf leaf-flower, stands out for its indigenous presence in India and distinct morphological features. This research endeavors to explore the botanical, chemical, and medicinal properties of *Phyllanthus fraternus*, focusing on its potential therapeutic applications. Through an in-depth analysis of its chemical constituents, including hypo-phyllanthine and phyllanthine, the study aims to elucidate its medicinal properties, particularly in treating ailments like cough, bile disorders, itching, and inflammation. Extensive investigation into traditional remedies and modern pharmacological studies reveals *Phyllanthus fraternus*'s efficacy in addressing various conditions such as mastitis, colic, chronic diarrhea, diabetes, bronchitis, and liver diseases.

The research employs a multidisciplinary approach, integrating traditional knowledge with modern scientific inquiry, to bridge the gap between traditional uses and contemporary medical applications. By shedding light on the multifaceted properties of *Phyllanthus fraternus*, this study contributes to the broader understanding and utilization of this valuable botanical resource in healthcare and pharmaceutical industries.

Key-words: Phytochemical, Liver, *Phyllanthus* sp.

Introduction

Phyllanthus, the largest genus within the Phyllanthaceae family, is renowned for its vast array of species, ranging from 750 to 1200, showcasing remarkable diversity in growth forms, floral structures, and chromosome numbers. This genus encompasses various growth forms, including annual and perennial herbs, shrubs, climbers, floating aquatics, and succulents, some featuring flattened stem structures known as cladodes. Notably, *Phyllanthus* boasts one of the broadest ranges of pollen types among seed plant genera. (Chopade *et al.*, 2015).

Among its species, *Phyllanthus fraternus* L., commonly referred to as the Gulf leaf-flower, stands out. Indigenous to India, it exhibits distinct characteristics, including erect, annual growth reaching up to 45-60 cm in height, with alternate leaves and small, round fruits reminiscent of dhatriphal but smaller, known as bahudhatri. This research aims to explore the botanical, chemical, and medicinal properties of *Phyllanthus fraternus*, shedding light on its potential therapeutic applications.

***Corresponding Author**

Email: husainizehra6@gmail.com

Through a comprehensive examination of its chemical constituents, such as hypo-phyllanthine and phyllanthine found in its leaves, we aim to understand its medicinal properties, including its efficacy in treating various ailments like cough, bile disorders, itching, and inflammation. (Nikule *et.al.*, 2020).

The medicinal potential of *Phyllanthus fraternus* extends to diverse conditions such as mastitis, catarrhal diseases, ascites, colic, chronic diarrhea, diabetes, hemorrhage, gonorrhea, wounds, itching, bronchitis, malarial fever, and liver diseases. By investigating traditional remedies and modern pharmacological studies, this research seeks to elucidate the therapeutic mechanisms underlying its traditional uses and explore its potential in modern medicine. (Mangwani *et.al.*, 2020).

Understanding the multifaceted properties of *Phyllanthus fraternus* holds promise for the development of novel pharmaceuticals and therapeutic interventions. By bridging traditional knowledge with modern scientific inquiry, this research endeavors to contribute to the broader understanding and utilization of this valuable botanical resource in healthcare and pharmaceutical industries. (Thomford *et.al.*, 2015).

Materials and Methods

The selection and collection of plant material, followed by its identification, drying, grinding, and extraction, are crucial steps in phytochemical research. Here, we detail the methods employed in the study of *Phyllanthus fraternus*, encompassing plant selection, collection, identification, drying, grinding, extraction, preliminary phytochemical screening, qualitative physiochemical examination, thin-layer chromatography (TLC), standardization, and molecular genetic studies.

Plant Selection: Selection of *Phyllanthus fraternus* was based on its abundance in India and previous chemical investigations on its leaves, offering a foundation for this research.

Plant Collection: Leaves of *Phyllanthus fraternus* were collected from the grounds of the research institute.

Plant Identification: Field identification of the collected samples was attempted.

Drying and Grinding of Plant Sample: The collected leaves were dried below 30°C to

preserve thermolabile compounds. Sun drying was employed, followed by grinding to a coarse powder using a mechanical grinder.

Preparation of Plant Extracts: Plant extracts were prepared using a rotary shaker-based extraction method. Methanol was used as the solvent, and the extracts were filtered, evaporated, and diluted for further investigation.

Preliminary Phytochemical Screening: Qualitative chemical examination was conducted to detect the presence of various phytochemicals including saponins, carbohydrates, alkaloids, resins, phenols, tannins, diterpenes, and fixed oils and fats.

Qualitative Physiochemical Examination of Powdered Drug: Physiochemical tests were performed on powdered *Phyllanthus fraternus* leaves to ascertain the presence of secondary metabolites.

Thin Layer Chromatography (TLC): TLC was employed to confirm the presence of major groups like alkaloids, flavonoids, and saponins in the extract, using specific solvent systems and detection agents.

Standardization: Macroscopic and microscopic examinations were conducted to standardize the plant material. Macroscopic examination involved measurements of size, color, surface characteristics, odor, and taste, while microscopic examination focused on leaf and stem structures.

Qualitative Phytochemical Screening with UV-VIS Spectrophotometer: A UV-VIS spectrophotometer was utilized for qualitative analysis of phytochemicals present in the extracts.

Molecular Genetic Studies on *Phyllanthus fraternus*: Genomic DNA was extracted from leaf samples, purified, quantified, and checked for purity using spectrophotometric measurements and agarose gel analysis.

These methods collectively provided a comprehensive understanding of the chemical composition and genetic characteristics of *Phyllanthus fraternus*, laying the groundwork for further pharmacological and therapeutic investigations.

Results and Discussion

Phyllanthus fraternus exhibits considerable potential as a medicinal plant, as evidenced by its robust phytochemical profile and promising

pharmacological properties. Through methanolic extraction, a significant yield of extract rich in alkaloids, flavonoids, saponins, tannins, and carbohydrates was obtained, while anthraquinones were notably absent. (Fig.1) Macroscopic and microscopic examinations revealed distinct characteristics of the leaves and stems, further supporting its identification. Various qualitative phytochemical tests and thin layer chromatography highlighted the diversity of bioactive compounds present in *Phyllanthus fraternus*. (Table.1) Additionally, UV-VIS spectrophotometry provided further insight into its chemical composition. (Fig.2) Molecular genetic studies demonstrated successful DNA isolation, overcoming challenges posed by the presence of interfering plant chemicals, thus facilitating further genetic analysis.

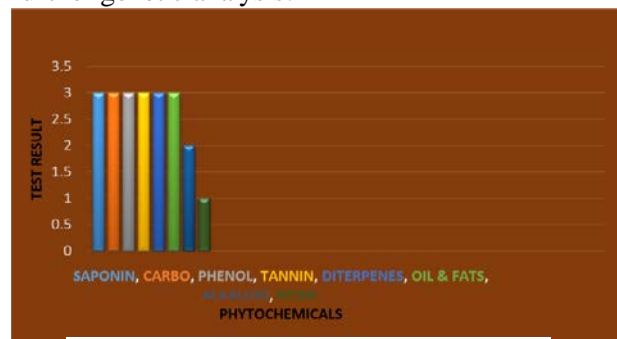


Fig.1: Qualitative Phytochemical Tests

These findings collectively underscore *Phyllanthus fraternus* as a promising candidate for medicinal and genetic research, warranting further investigation into its therapeutic potential and genetic variability.

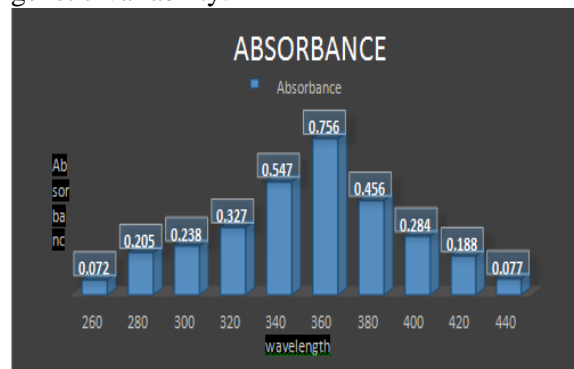


Fig. 2: Qualitative phytochemical screening of UV-VIS Spectrophotometer

Table.1: TLC with solvent system Chloroform: Methanol: n Butanol: Water (10:10:1:6)

Plant Species	Ext ract	Distance travelled by solute	Distance travelled by solvent	Rf value	Colour
<i>Phyllanthus fraternus</i>	Methanol	0.4	3	0.18	Dark orange
		1.2	3	0.44	Light green
		1.8	3	0.5	Light brown
		2.1	3	0.7	Light orange

Conclusion

In conclusion, the research conducted on *Phyllanthus fraternus* underscores its significant potential as a medicinal plant with diverse pharmacological properties. The comprehensive botanical, chemical, and genetic analysis revealed a rich phytochemical profile, highlighting the presence of bioactive compounds such as alkaloids, flavonoids, saponins, tannins, and carbohydrates. The absence of anthraquinones, along with the distinct macroscopic and microscopic characteristics, further validates its identification. Through qualitative phytochemical tests, thin-layer chromatography, UV-VIS spectrophotometry, and molecular genetic studies, a deeper understanding of *Phyllanthus fraternus*'s chemical composition and genetic characteristics was achieved. These findings pave the way for further pharmacological investigations, aiming to harness its therapeutic potential in modern medicine. The integration of traditional knowledge with scientific inquiry underscores the importance of preserving and studying traditional medicinal plants like *Phyllanthus fraternus*. Moving forward, continued research on this botanical resource holds promise for the development of novel pharmaceuticals and therapeutic interventions, contributing to the advancement of healthcare and pharmaceutical industries.

References

- Bapat, U. C., & Mhapsekar, D. (2012). OA01. 49. Antifungal activity study of the extracts of Homonoia riparia, Pedilanthus tithymaloides and Phyllanthus fraternus. *Ancient Science of Life*, 32(Suppl 1), S49.

2. Bhat, S. S., Hegde, K. S., Chandrashekhar, S., Rao, S. N., & Manikkoth, S. (2015). Preclinical screening of *Phyllanthus amarus* ethanolic extract for its analgesic and antimicrobial activity. *Pharmacognosy Research*, 7(4), 378.
3. Chopade, A. R., Sayyad, F. J., & Pore, Y. V. (2015). Molecular docking studies of phytocompounds from the *phyllanthus* species as potential chronic pain modulators. *Scientia pharmaceutica*, 83(2), 243-267.
4. Hossain, M. M., Ahamed, S. K., Dewan, S. M. R., Hassan, M. M., Istiaq, A., Islam, M. S., & Moghal, M. M. R. (2014). In vivo antipyretic, antiemetic, in vitro membrane stabilization, antimicrobial, and cytotoxic activities of different extracts from *Spilanthes paniculata* leaves. *Biological Research*, 47, 1-9.
5. Jantan, I., Haque, M. A., Ilangkovan, M., & Arshad, L. (2019). An insight into the modulatory effects and mechanisms of action of *phyllanthus* species and their bioactive metabolites on the immune system. *Frontiers in pharmacology*, 10, 425684.
6. Legba, B., Dougnon, V., Deguenon, E., Agbankpe, J., Senou, M., Aniambossou, A., ... & Dougnon, J. (2019). Toxicological characterization of six plants of the beninese pharmacopoeia used in the treatment of salmonellosis. *Journal of Toxicology*, 2019.
7. Kalyana Sundaram, I., Sarangi, D. D., Sundararajan, V., George, S., & Sheik Mohideen, S. (2018). Poly herbal formulation with anti-elastase and anti-oxidant properties for skin anti-aging. *BMC complementary and alternative medicine*, 18, 1-12.
8. Kathriarachchi, H., Hoffmann, P., Samuel, R., Wurdack, K. J., & Chase, M. W. (2005). Molecular phylogenetics of *Phyllanthaceae* inferred from five genes (plastid *atpB*, *matK*, 3' *ndhF*, *rbcL*, and nuclear *PHYC*). *Molecular phylogenetics and evolution*, 36(1), 112-134.
9. Kumar, S., Singh, A., & Kumar, B. (2017). Identification and characterization of phenolics and terpenoids from ethanolic extracts of *Phyllanthus* species by HPLC-ESI-QTOF-MS/MS. *Journal of pharmaceutical analysis*, 7(4), 214-222.
10. Mabberley, D. J. (2008). *Mabberley's Plant-book: a portable dictionary of plants, their classifications and uses* (No. Ed. 3). Cambridge university press.
11. Mangwani, N., Singh, P. K., & Kumar, V. (2020). Medicinal plants: adjunct treatment to tuberculosis chemotherapy to prevent hepatic damage. *Journal of Ayurveda and integrative medicine*, 11(4), 522-528.
12. Mao, X., Wu, L. F., Guo, H. L., Chen, W. J., Cui, Y. P., Qi, Q., ... & Zhang, L. Z. (2016). The genus *Phyllanthus*: an ethnopharmacological, phytochemical, and pharmacological review. *Evidence-Based Complementary and Alternative Medicine*, 2016.
13. Memon, A. H., Ismail, Z., Aisha, A. F., Al-Suede, F. S. R., Hamil, M. S. R., Hashim, S., ... & Abdul Majid, A. M. S. (2014). Isolation, characterization, crystal structure elucidation, and anticancer study of dimethyl cardamonin, isolated from *Syzygium campanulatum* Korth. *Evidence-Based Complementary and Alternative Medicine*, 2014.
14. Moukette Moukette, B., Pieme, C. A., Nya Biapa, P. C., Ama Moor, V. J., Berinyuy, E., & Yonkeu Ngogang, J. (2015). *Afrostryax lepidophyllus* extracts exhibit in vitro free radical scavenging, antioxidant potential and protective properties against liver enzymes ion mediated oxidative damage. *BMC Research Notes*, 8, 1-12.
15. Nikule, H. A., Nitaware, K. M., Chambhare, M. R., Kadam, N. S., Borde, M. Y., & Nikam, T. D. (2020). In-vitro propagation, callus culture and bioactive lignan production in *Phyllanthus tenellus* Roxb: a new source of phyllanthin,

- hypophyllanthin and phylltetralin. *Scientific Reports*, 10(1), 10668.
16. Nisar, M. F., He, J., Ahmed, A., Yang, Y., Li, M., & Wan, C. (2018). Chemical components and biological activities of the genus *Phyllanthus*: A review of the recent literature. *Molecules*, 23(10), 2567.
17. Oyewole, O., Oshin, T. A., & Atotuoma, B. O. (2021). *Corchorus olitorius* stem as corrosion inhibitor on mild steel in sulphuric acid. *Heliyon*, 7(4).
18. Raghuvanshi, D., Dhalaria, R., Sharma, A., Kumar, D., Kumar, H., Valis, M., ... & Puri, S. (2021). Ethnomedicinal plants traditionally used for the treatment of jaundice (icterus) in Himachal Pradesh in Western Himalaya—a review. *Plants*, 10(2), 232.
19. Ravi, S., Shanmugam, B., Subbaiah, G. V., Prasad, S. H., & Reddy, K. S. (2017). Identification of food preservative, stress relief compounds by GC–MS and HR–LC/Q–TOF/MS: evaluation of antioxidant activity of *Acalypha indica* leaves methanolic extract (in vitro) and polyphenolic fraction (in vivo). *Journal of Food Science and Technology*, 54, 1585–1596.
20. Salehi, B., Ata, A., V. Anil Kumar, N., Sharopov, F., Ramírez-Alarcón, K., Ruiz-Ortega, A., ... & Sharifi-Rad, J. (2019). Antidiabetic potential of medicinal plants and their active components. *Biomolecules*, 9(10), 551.
21. Samal, J. (2016). Ayurvedic management of pulmonary tuberculosis: A systematic review. *Journal of interventional ethnopharmacology*, 5(1), 86.
22. Sankar Narayan, K., Esack, E. R., Radhapriya, P., Gopal, V. B., Muthu, S., & Perumal, P. (2018). Impact of geography on adaptation of *Phyllanthus amarus* seeds. *3 Biotech*, 8, 1–10.
23. Singh, H., Singh, R., Arora, R., Mannan, R., Buttar, H. S., Arora, S., & Singh, B. (2020). Protective role of *Phyllanthus fraternus* in alloxan-induced diabetes in rats. *Journal of Ayurveda and integrative medicine*, 11(4), 391–398.
24. Tajuddeen, N., & Van Heerden, F. R. (2019). Antiplasmodial natural products: an update. *Malaria journal*, 18, 1–62.
25. Thomford, N. E., Dzobo, K., Chopera, D., Wonkam, A., Skelton, M., Blackhurst, D., ... & Dandara, C. (2015). Pharmacogenomics implications of using herbal medicinal plants on African populations in health transition. *Pharmaceuticals*, 8(3), 637–663.
26. Tsouh Fokou, P. V., Kissi-Twum, A. A., Yeboah-Manu, D., Appiah-Opong, R., Addo, P., Tchokouaha Yamthe, L. R., ... & Nvarko, A. K. (2016). In vitro activity of selected West African medicinal plants against *Mycobacterium ulcerans* disease. *Molecules*, 21(4), 445.