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GC-MS ANALYSIS, QUALITATIVE AND QUANTITATIVE PHYTOCHEMICAL COMPOSITION OF *Boerhavia diffusa* (Linn.) LEAF EXTRACT CHARACTERIZING ITS MEDICINAL USE

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ABSTRACT

Boerhavia diffusa Linn., commonly known as punarnava, is a perennial herb of the Nyctaginaceae family, renowned for its medicinal significance in traditional medicine, particularly in India. The leaves of *Boerhavia diffusa* were extracted using a Soxhlet extractor, and the resulting ethanolic extract was subjected to qualitative and quantitative phytochemical analysis. Qualitative screening revealed the presence of phenols, tannins, alkaloids, saponins, flavonoids, terpenoids, steroids, and cardiac glycosides. Quantitative analysis demonstrated significant concentrations of phenols (344.97 mg/100g), flavonoids (67.72 mg/100g), and tannins (84.33 mg/100g). Advance GC-MS profiling identified 16 bioactive compounds, including benzofuran, stigmasterol, pentadecanoic acid, and phytol, which exhibit diverse pharmacological activities such as antioxidant, anti-inflammatory, and antimicrobial effects. The study highlights the potential of *Boerhavia diffusa* as a source of novel therapeutic agents, bridging traditional knowledge with modern scientific techniques. Further research is recommended to explore the synergistic effects, pharmacokinetics, and bioavailability of its phytochemicals to validate its clinical applications. This investigation substantiates the medicinal value of Boerhavia diffusa and advocates for its integration into contemporary medicine, offering a promising avenue for the advancement of herbal medicine.

Keywords: *Boerhavia diffusa*, GC-MS analysis, Qualitative, Quantitative, Phytochemical

INTRODUCTION

Boerhavia diffusa Linn, commonly known as punarnava, is a perennial herbaceous plant belonging to the family Nyctaginaceae (Gour, 2021). It grows in many countries throughout tropical and subtropical regions, specifically within India, where it has been used in traditional medicine for ages (Che et al., 2024). The leaves of *Boerhavia diffusa* contain therapeutic actions: anti-inflammatory, diuretic, hepatoprotective, and antioxidant (Gaur et al., 2022).

In addition to its therapeutic properties, *Boerhavia diffusa* is rich in bioactive compounds, including alkaloids, flavonoids, sterols, and phenolic acids, which contribute to its medicinal benefits (Kumar *et a*l., 2020). Traditional Ayurvedic practices have long utilized *Boerhavia diffusa* in formulations for treating ailments such as edema, jaundice, and inflammatory conditions (Muley *et al*., 2024). Recent pharmacological research further supports its use, demonstrating significant anti-cancer and immunomodulatory activities, indicating its potential role in modern integrative medicine (Das *et al.,* 2023).

Recent phytochemical screening and gas chromatography-mass spectrometry (GC-MS) analysis of its bioactive compounds have brought new insight into the scientific basis for its medicinal use (Willie *et al.,* 2021). Phytochemical screening of the leaf extract of *Boerhavia diffusa* showed the presence of various secondary metabolites, including alkaloids, flavonoids, tannins, saponins, and phenolic compounds. The phytochemicals are normally responsible for pharmacological activities attributed to the plant (Velu *et al.,* 2018). For instance, flavonoids have appreciable antioxidant activity, which is crucial in the reduction of oxidative stress-related diseases (Miraj *et al.*, 2017). Similarly, alkaloids have anti-inflammatory properties and thus could be vital in the treatment of arthritis and other inflammatory diseases (Peng *et al.,* 2019). The evaluation of the chemical composition in the leaf extract of *Boerhavia diffusa* has been further stated using GC-MS analysis (Sharma and Singh, 2021). This analytical technique allows one to identify and quantify the volatile and semi-volatile compounds within the extract (Acevedo *et al.,* 2017). Various bioactive compounds, such as essential oils and fatty acids, have been identified through studies employing GC-MS analysis; these compounds contribute to the medicinal values of the plant . For example, β-sitosterol and stigmasterol have been isolated from this plant, both of which are known for their anti-inflammatory and cholesterol-lowering activities (Bin *et al.,* 2016). Profiling such compounds in detail increases not only our knowledge regarding the therapeutic potential of the plant but also justifies its use in herbal medicine (Tiwari *et al.,* 2018). Research has proved the medicinal uses of *Boerhavia diffusa* to be more than folklore. Many pharmacological studies also proved its efficacy in the treatment of various diseases (Gaur *et al.,* 2022). For instance, the diuretic action of the plant has been proved, and thus it becomes useful in the treatment of retention states and disorders of the kidney (Aparecida *et al.,* 2017). In addition, its hepatoprotective action indicates another possible therapeutic function of this herb in the treatment of diseases related to the liver (Ali *et al.,* 2018).

The integration of traditional knowledge with modern scientific techniques, such as GC-MS analysis and phytochemical screening, has highlighted the significant medicinal potential of *Boerhavia diffusa* Linn. (Ahirwar *et al*., 2024). The identification of various bioactive compounds in its leaf extract validates its traditional uses and suggests promising avenues for the development of herbal medicines (Mukherjee *et al*., 2022). Despite the growing global interest in natural products, a significant literature gap exists in comprehensively characterizing the phytochemical

composition and understanding the full therapeutic potential of *Boerhavia diffusa* in contemporary medicine (Bhattarai *et al.,* 2024).

This study aims to bridge this gap by conducting a detailed GC-MS analysis, as well as qualitative and quantitative phytochemical assessments of *Boerhavia diffusa* Linn. leaf extract. By doing so, it seeks to characterize its bioactive compounds and further validate its medicinal properties, thereby contributing to the development of novel therapeutic agents and strengthening the scientific foundation of its traditional applications.

MATERIALS AND METHODS

Collection of Plant Materials

Boerhavia diffusa plant (Locally known as Tarvine) was collected from around 500 units housing area in Lokoja, Kogi State, Nigeria

Plant Identification

Boerhavia diffusa (Linn.) common name (Red spiderling) with voucher Number:UBH-B543 was identified in the department of plant Biology and Biotechnology Herbarium Unit Faculty of Life Sciences University of Benin City, Edo State by Prof. Akinniboun Henry Adewale(FL.MRSB:London.LMBOSON,MNES,MAEIAN: Nigeria)

Preparation of Plant Extract using Soxhlet Apparatus

20g of the dry leaves powder was placed in the body of soxhlet extractor. 250 ml of solvent-ethanol placed into round buttom flask of the soxhlet apparatus (Escorsim *et al.,* 2018). The process of filling and emptying of the extractor was repeated until the drug is exhausted. Then soxhlet solvent containing extract was poured into large petridish and allowed to evaporate . The final crude extract was stored in the refrigerator at 4ºC for further use during phytochemical analysis (Anusuba and Priya, 2018).(Ogbonnaya *et al., 2024)*

Phytochemical analysis

The preliminary qualitative and quantitative phytochemical screening of ethanol extract was carried out according to the standard laboratory procedure (Madike *et al.,* 2017).

GC-MS analysis

GC-MS analysis and characterization of ethanol leaves extract *Boerhavia diffusa* was done EI-MS spectrum (Afshari and Rahimmalek, 2018). GC-MS is a combination two analytical techniques to a single method of analyzing the mixtures of chemical compounds, Gas chromatography separates the components of the mixture and mass spectroscopy analyses each of the components separately.The spectrums of the unknown and known components were compared (Abdulsalami *et al*., 2022) Pharmaceutical databases were used to identify and characterize the spectra revealed molecules (Mohimani *et al.,* 2018)..

RESULTS AND DISCUSSION

The qualitative and quantitative phytochemical analysis of *Boerhavia diffusa* ethanolic extract of leaves was performed by using different test.

Table 1: Qualitative phytochemical analysis of leaves extract of *Boerhavia diffusa*

Phytochemical	*Boerhavia diifusa* ethanolic extract
Phenols	+
Flavonoids	+
Tannins	+
Saponins	+
Alkaloids	+
Terpenoids	+
Cardiac glycosides	+
Steroids	+

Table 2: Quantitative phytochemical analysis of leaves extract of *Boerhavia diffusa*

Phytochemical	*Boerhavia diffusa* ethanolic extract mg/100g
Phenols	344.97 ± 1.21^{a}
Flavonoids	67.72 ± 0.38^{b}
Tannins	84.33 ± 0.44^{c}
Saponins	69.11 ± 0.93^{b}
Alkaloids	38.97 ± 0.79^{d}

The GC-MS spectrum of ethanolic extract of *Boerhavia diffusa* leaves is depicted in Figure 1 and individual mass spectrum of phytocompound is illustrated in Figure 2. In GC-MS analysis, a total of 16 compounds were identified. The identification of phytocompounds is based on molecular formular and retention times (RT) being compared to the database of NIST and the further collected information on therapeutic activities of each compound was done from Pubchem. Findings are presented in Table 3

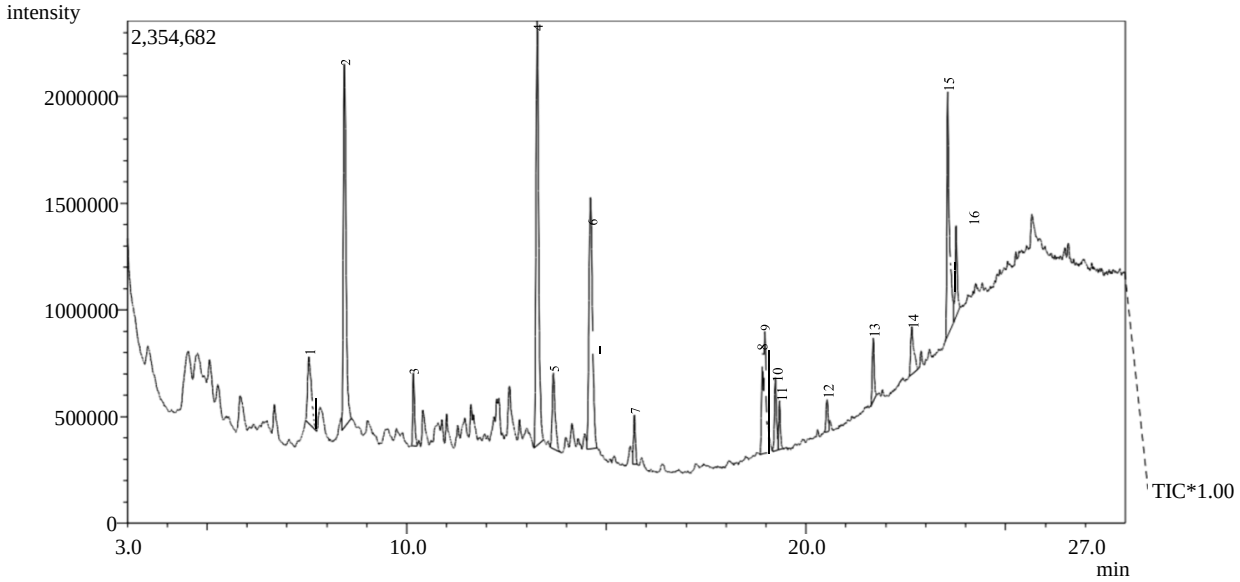
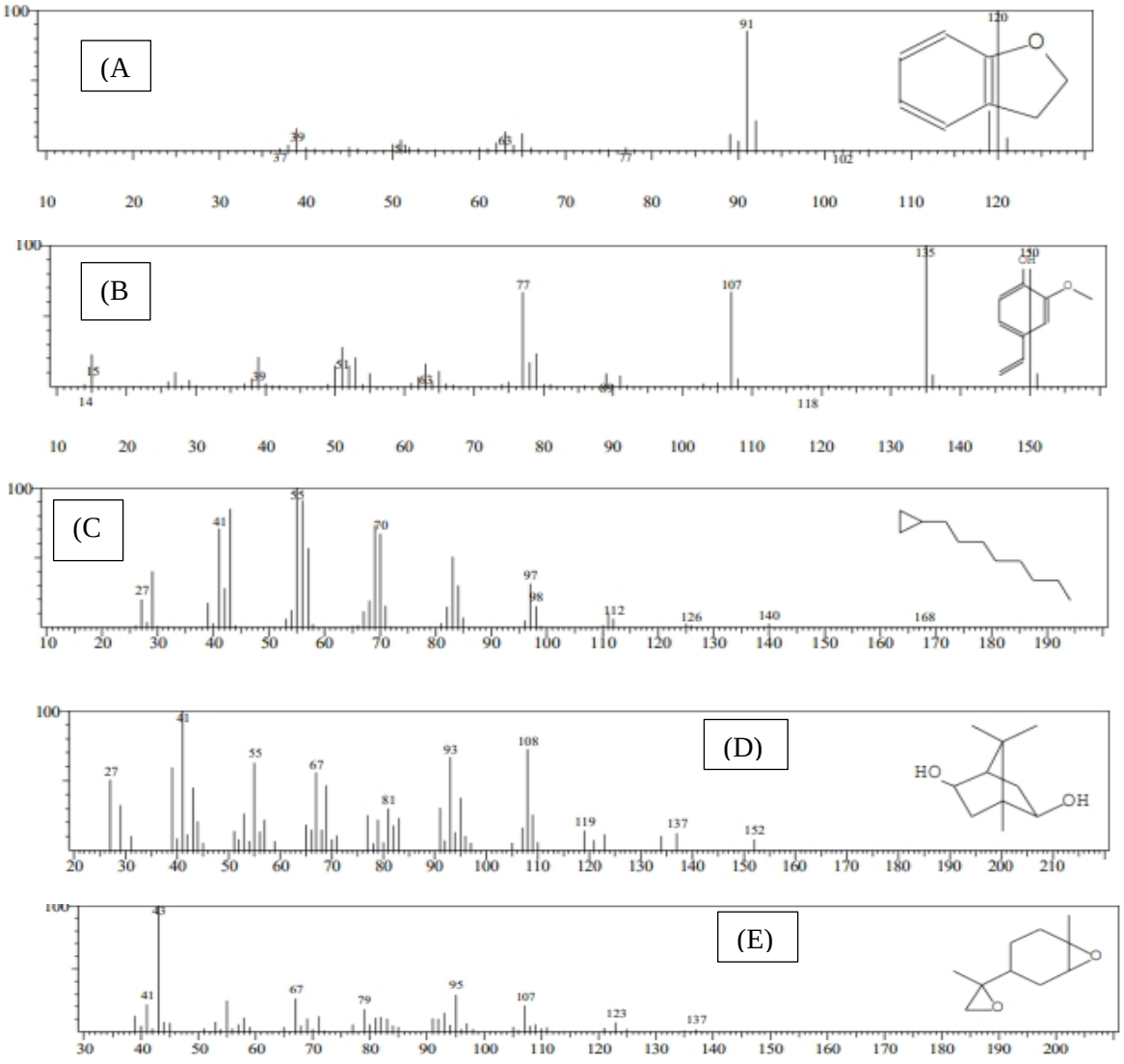
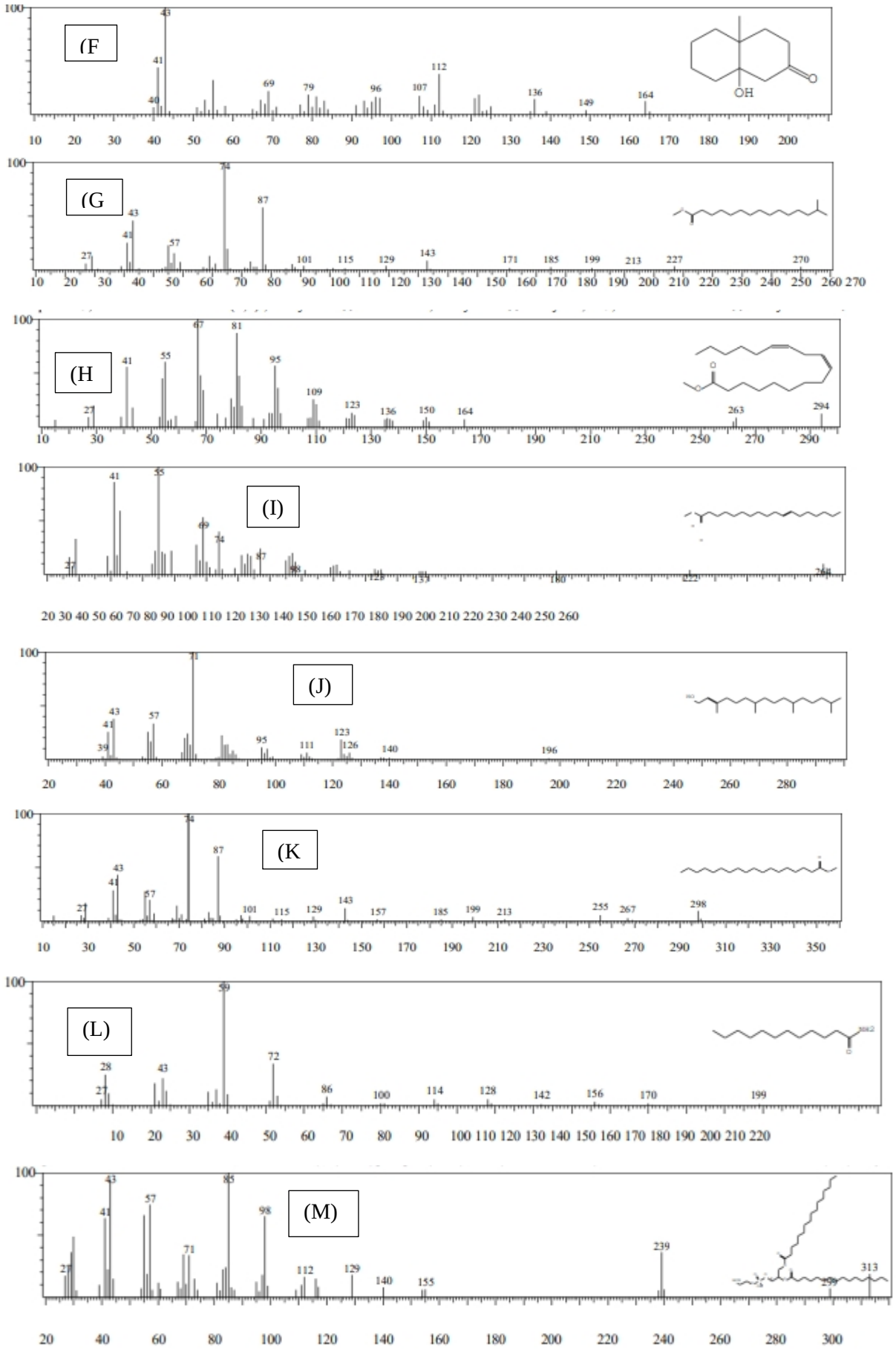


Figure 1: GC-MS chromatogram of ethanolic extract of *Boerhavia diffusa*

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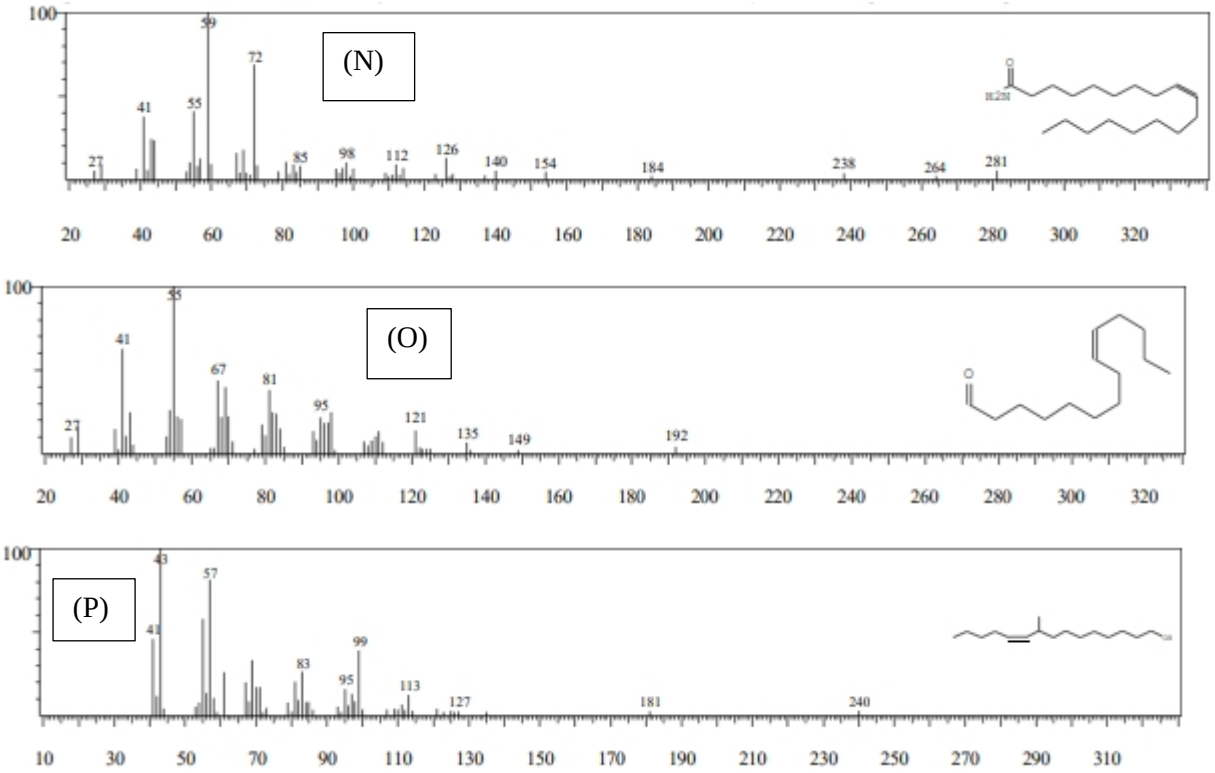

Figure 2: Mass spectrum of (A) Benzofuran 2,3-dihydro-phytocompound, (B) 2-methyl-4-vinylphenol phytocompound, (C) Cyclopropane, nonyl, (D) Bicyclo(2,2,1)heptane-2,5-diol, (E) 7-oxabicyclo(4,1), (F) 7-oxabicyclo(4,1), (G) 2(1H)-Naphthalenone, (H) 9,12-octadecadienoic acid, (I) 11-octadecenoic acid, (J) Phytol, (K) Octadecanoic acid, (L) Dodecanamide, (M) Hexadecanoic acid, (N) 9-octadecenamide, (O) 9-Tetradecenal, and (P) 9-methyl-z-10-pentadecen-1-ol

Table 3: Phytocompounds identified and the therapeutic activities of each compound

S. No.	Name of phytocompounds	RT (min)	Area %	Molecular Formular	Therapeutic activity
1	Benzofuran 2,3-dihydro-	7.548	4.76	C_8H_8O	Antioxidant, Anti-inflammatory, Antimicrobial, Neuroprotective, Analgesic, Anti-cancer, and Cardiovascular benefits.
2	2-methyl-4-vinylphenol	8.438	17.52	$C_9H_{10}O_2$	Antioxidants and Antimicrobial
3	Cyclopropane, nonyl	10.170	2.33	$C_{12}H_{24}$	Enzyme inhibitions to insecticidal, Antifungal, Herbicidal, Antimicrobial, Antibiotic, Antibacterial, Antitumor and Antiviral activities
4	Bicyclo(2,2,1)heptane-2,5-diol	13.268	20.75	$C_{10}H_{18}O_2$	Analgelsic and Anti-inflammatory
5	7-oxabicyclo(4,1)	13.674	4.15	$C_{10}H_{16}O_2$	Antimicrobial and Anti-inflammatory
6	2(1H)-Naphthalenone	14.604	16.13	$C_{11}H_{18}O2$	Antioxidant and Antimicrobial
7	Pentadecanoic acid	15.704	1.69	$C_{17}H_{34}O_2$	Anti-inflammatory and Antimicrobial
8	9,12-octadecadienoic acid	18.911	3.29	$C_{19}H_{34}O_2$	Anti-inflammatory and Anti-oxidant
9	11-octadecenoic acid	18.911	4.17	$C_{19}H_{36}O_2$	Anti-inflammatory and Antioxidant
10	Phytol	19.235	2.88	$C_{20}H_{40}O$	Antioxidant and Anti-inflammatory
11	Octadecanoic acid	19.336	1.49	$C_{19}H_{38}O_2$	Anti-inflammatory and Antimicrobial
12	Dodecanamide	20.527	1.13	$C_{12}H_{25}NO$	Anti-inflammatory and Analgesic
13	Hexadecanoic acid	21.682	2.22	$C_{37}H_74NO8P$	Anti-inflammatory and Antimicrobial
14	9-octadecenamide	22.650	2.72	$C_{18}H_{35}NO$	Anti-inflammatory and Analgesic
15	9-Tetradecenal	23.551	11.33	$C_{14}H_{26}O$	Antimicrobial and Anti-inflammatory
16	9-methyl-z-10-pentadecen-1-ol	23.763	3.49	$C_{16}H_{32}O$	Anti-inflammatory and Antimicrobial

Discussion

Investigation of *Boerhavia diffusa* Linn., commonly known as punarnava, has thrown open important medicinal properties through qualitative and quantitative phytochemical analysis and GC-MS characterization (Tanwar *et al.*, 2023). This perennial herb, being in the knowledge of many traditions,

especially the Indian subcontinent, depicts a rich profile of bioactive compounds that would provide evidence for therapeutic applications (Akbar, 2020). These compounds are reported to have different pharmacological activities (Karak, 2019). For example, numerous studies reported flavonoids as a group of compounds with very strong antioxidant activity, which may play a major role in counteracting oxidative stress and probably reduce the risk for chronic diseases such as cancer and cardiovascular diseases (Shen *et al.,* 2022). A higher quantity of the plant extract resulted in a greater inhibition of DPPH and lipid peroxidation percentage (Onyeukwu .*et al.,* 2024) The presence of tannins and saponins also goes in favor of the plant as a natural remedy, as these compounds possess antimicrobial and anti-inflammatory activity. Quantitatively, the analysis revealed huge concentrations of these phytochemicals. In particular, phenols were present at 344.97 mg/100g, which shows a strong antioxidant capacity Flavonoids and tannins, at 67.72 mg/100g and 84.33 mg/100g, respectively, further increase the therapeutic potential of the extract. Similarly, the presence of alkaloids at 38.97 mg/100g would tend to show additional anti-inflammatory benefits, reinforcing the traditional use of this plant in inflammatory processes.

GC-MS analysis has identified 16 bioactive compounds that give a clearer insight into the therapeutic value of *Boerhavia diffusa* (Sharma and Yadav, 2023). Compounds such as benzofuran and stigmasterol are most prominent because of the proven health benefits associated with them (Liu *et al.,* 2018). Benzofuran has been reported to exhibit a wide range of activities, including antioxidant, anti-inflammatory, and antimicrobial activities (Chand *et al.,* 2017). Stigmasterol is known for its activity in lowering cholesterol levels and would therefore be useful in the management of cardiovascular conditions (He *et al.,* 2018). The presence of fatty acids, such as pentadecanoic acid and octadecanoic acid, further provides evidence of the hepatoprotective and anti-inflammatory properties of the plant (Rasak *et al.,* 2020). These fatty acids are known to modulate inflammation and liver health; this is in agreement with traditional claims regarding the herb's efficacy in treating liver disorders . This research brought together traditional knowledge with modern techniques of science to emphasize the need for validation of herbal remedies through rigorous scientific inquiry (Reid *et al.,* 2021). Such findings not only authenticate the traditional uses of *Boerhavia diffusa* but also open more horizons for further exploration into its bioactive compounds (Kumar *et al.,* 2023). This type of research may open ways toward developing new therapeutic agents derived from natural products (Newman and Cragg, 2016). While the present study provides insights, further research is needed to explain how exactly the identified phytochemicals act (Wetzel and Whitehead, 2020). This would be especially instrumental in vivo, showing bioavailability and pharmacokinetics of the compounds and further increasing the understanding of their therapeutic efficacy in clinical settings (Estrela *et al.,* 2017). Moreover, possible synergistic effects among the identified compounds could result in a more effective therapeutic strategy (Hu *et al.,* 2023). With the rising global interest in natural products, further exploration of *Boerhavia diffusa* is warranted (Dutta *et al.,* 2024). Its multitargeted pharmacological activities make it apt for integration within modern health practice, more so in the management of chronic diseases and water parasitic infection (Thomford *et al.,* 2018).(Omowaye O. S *et al*,2014) Its diuretic and hepatoprotective actions, along with strong anti-inflammatory and antioxidant properties, make this plant a strong candidate in herbal medicine (Inganakal, 2021).

Thus, it strongly suggests evidence of the medicinal potential of *Boerhavia diffusa* through the combination of qualitative and quantitative phytochemical analysis with GC-MS profiling (Mathias *et al.,* 2020). Identification of various bioactive compounds supports its traditional use and further research that may bring out very important advancements in herbal medicine (Mustafa *et al.,* 2017). Such a bridging of traditional knowledge with modern scientific validation might lead to better therapeutic applications for this amazing plant and contribute to the development of new natural remedies in the healthcare sector (Mistry and Berardi, 2016).

CONCLUSION

The present study ascertains the medicinal potential of *Boerhavia diffusa* through an elaborate qualitative and quantitative phytochemical analysis and GC-MS profiling. Few bioactive compounds, including flavonoids, alkaloids, and fatty acids, are identified, which may be responsible for the traditional uses, and possess various therapeutic properties such as antioxidant, anti-inflammatory, and hepatoprotective activities. It further calls for more studies to discover its clinical applications, thereby initiating all-time value in the integration of traditional knowledge with modern scientific validation in herbal medicine..

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