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Research Article

Chemical Profiling and *in silico* Pharmacokinetic Assessment of Bioactive Constituents from *Achyranthes aspera* Root Extract

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ABSTRACT

Background: *Achyranthes aspera* L. is a medicinal weed extensively utilized in traditional medicine and is known for its rich repertoire of bioactive phytochemicals. **Objectives:** The present study aimed to characterize the phytochemical constituents of the ethanolic root extract of *A. aspera* using Gas Chromatography-Mass Spectrometry (GC-MS) and to evaluate the drug-likeness, pharmacokinetic and toxicity profiles of the major identified compounds through *in silico* approaches. Fresh roots were collected, shade-dried, powdered and extracted with ethanol. **Materials and Methods:** The phytochemical constituents were identified by GC-MS analysis and major compounds were selected based on their relative peak area percentages. These compounds were further subjected to computational evaluation using SwissADME for drug-likeness and pharmacokinetic prediction and ProTox-3.0 for toxicity assessment. GC-MS analysis revealed the presence of diverse phytoconstituents in the extract. **Results:** Based on their relative peak area percentages, fifteen major compounds were selected for further investigation. The analysis demonstrated that several phytoconstituents exhibited favourable pharmacokinetic characteristics, acceptable oral bioavailability and compliance with major drug-likeness criteria. **Conclusion:** Toxicity prediction further indicated promising safety profiles for a number of compounds, suggesting their potential suitability for pharmaceutical applications. The findings highlight the value of integrating GC-MS profiling with computational ADME and toxicity assessment for the rapid screening and prioritization of bioactive phytochemicals from *A. aspera*. This study provides a scientific basis for future investigations aimed at exploring the therapeutic potential of these naturally occurring compounds.

Keywords: *Achyranthes aspera*, Bioactive constituents, GC-MS, Swiss ADME, ProTox-3.0

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INTRODUCTION

Achyranthes aspera L., a medicinal weed belonging to the *Amaranthaceae* family, is extensively distributed across tropical and subtropical regions of Asia, Africa and Europe. Its exceptional adaptability to diverse ecological conditions has facilitated its widespread occurrence, supporting its continued use in traditional medicinal practices for the management of various ailments (Barua *et al*., 2011; Gupta *et al*., 1972; Oliver-Bever, 1982; Shibeshi *et al*., 2006). It is a widely distributed medicinal weed that commonly inhabits roadsides, agricultural field margins and wastelands throughout India. Its adaptability to diverse environmental conditions allows it to occur across a broad altitudinal gradient, extending up to approximately 2100 m above sea level, and it is also found in the South Andaman Islands (Kumar and Soni, 2023). *Achyranthes aspera* has long been recognized in traditional medicine for its extensive therapeutic applications. The plant exhibits a variety of medicinal properties, including diuretic, purgative, laxative, antiperiodic, antiphlegmatic and pungent activities. Different parts of the plant have been employed in the management of numerous ailments such as asthma, cough, oedema, dropsy, piles, boils and skin disorders. In traditional practice, a decoction prepared from the whole plant is administered for the treatment of pneumonia, whereas root infusions are used as mild astringents to alleviate bowel-related complaints. The flowering spikes and seeds are commonly ground into a paste and applied externally for the treatment of snake and reptile bites, as well as certain cutaneous disorders and night blindness. Fresh roots are traditionally used for oral hygiene as natural toothbrushes, while root pastes are applied to manage ophthalmic conditions, including ophthalmia and corneal opacities. Furthermore, leaf paste preparations are

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utilized as topical remedies to reduce pain and inflammation associated with wasp stings (Gupta, 2010; Nadkarni, 2009).

The diverse ethnomedicinal applications and pharmacological significance of *Achyranthes aspera* have attracted considerable scientific interest in the identification and evaluation of its bioactive phytoconstituents. However, the therapeutic potential of a compound depends not only on its biological activity but also on its pharmacokinetic behaviour and safety profile. Consequently, naturally occurring phytochemicals are increasingly subjected to computational assessment of their Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) properties to determine their suitability as potential drug candidates. The rapid advancement of drug discovery and biological screening technologies has further emphasized the need for comprehensive ADMET evaluation. Unfavourable pharmacokinetic and toxicity profiles are among the major causes of candidate attrition during drug development and clinical evaluation, early prediction of ADMET parameters has become an integral component of the drug discovery process. Incorporation of ADME screening at the initial stages facilitates the identification of compounds with desirable pharmacokinetic characteristics, minimizes late-stage failures and reduces the time, cost and resources associated with pharmaceutical development (Jayasimha *et al*., 2013; Hay *et al*., 2014). Pharmacokinetics describes the fate of a compound within a biological system and encompasses the processes of absorption, distribution, metabolism and excretion. Among these parameters, bioavailability is of particular importance, as it determines the rate and extent to which an active compound reaches the systemic circulation and becomes available at its target site (Tripathi *et al*., 2019).

Drug-likeness assessment is a fundamental step in the early stages of drug discovery, as it helps predict whether a compound possesses physicochemical properties suitable for development as an orally active therapeutic agent. Among the various approaches available, the widely accepted Lipinski's Rule of Five provides a valuable framework for evaluating the oral bioavailability and drug-likeness of chemical compounds. According to this rule, compounds are more likely to exhibit favourable absorption and permeability when they possess a molecular weight below 500 Da, a partition coefficient (Log P) of less than 5, no more than five hydrogen bond donors and no more than ten hydrogen bond acceptors. In addition, compounds with fewer rotatable bonds generally demonstrate improved bioavailability. These physicochemical parameters are commonly employed to assess the suitability of bioactive molecules as potential drug candidates during the preliminary stages of pharmaceutical development (Lipinski *et al*., 1997; Veber *et al*., 2002).

Several factors may limit the bioavailability of bioactive compounds, including poor aqueous solubility, instability in the gastrointestinal environment, biotransformation by intestinal microflora, restricted absorption through the intestinal wall, active efflux mechanisms and extensive first-pass metabolism. Collectively, these factors influence the therapeutic effectiveness of orally administered compounds (Aqil *et al*., 2013).

To address these challenges, computational approaches have become increasingly important in the early stages of drug discovery. *In silico* prediction of pharmacokinetic properties provides a rapid, cost-effective and ethical alternative to experimental screening by enabling the evaluation of large numbers of compounds without extensive laboratory testing or animal experimentation. Among the available computational platforms, SwissADME has emerged as a reliable and widely used web-based tool for assessing the drug-likeness, physicochemical characteristics, pharmacokinetic behaviour and medicinal chemistry properties of both natural and synthetic compounds (Daina *et al*., 2014; Daina and Zoete, 2016; Daina *et al*., 2017a; Daina *et al*., 2017b). This platform facilitates the identification and prioritization of promising bioactive molecules for subsequent experimental investigations. Furthermore, key pharmacokinetic parameters, including gastrointestinal absorption, blood-brain barrier permeability, P-glycoprotein substrate status and bioavailability score, can be predicted to evaluate the absorption and distribution characteristics of compounds. The assessment of interactions with cytochrome P450 enzymes, such as CYP1A2, CYP2C19, CYP2C9, CYP2D6 and CYP3A4, provides valuable insights into metabolic stability and potential drug-drug interactions, while skin permeability predictions contribute to understanding compound permeation characteristics (Lipinski *et al*., 1997; Daina *et al*., 2017a; Daina *et al*., 2017b).

The present study aimed to evaluate the drug-likeness, pharmacokinetic, and toxicity profiles of major phytoconstituents identified from the ethanolic root extract of *Achyranthes aspera* through GC-MS analysis. The selected compounds were subjected to *in silico* assessment using the SwissADME web server to predict their physicochemical properties, Absorption, Distribution, Metabolism, Excretion (ADME) characteristics and medicinal chemistry parameters, while toxicity profiles were evaluated using ProTox-3.0. This approach was employed to identify phytochemicals with favourable pharmacokinetic behaviour and safety profiles for potential therapeutic applications.

MATERIALS AND METHODS

Plant Material Collection and Authentication

Fresh roots of *Achyranthes aspera* L. were collected from North Vijayanarayanam, Tirunelveli District, Tamil Nadu, India. The collected plant material was taxonomically identified and authenticated by the Botanical Survey of India (BSI), Southern Regional Centre, Coimbatore, Tamil Nadu, India. A representative specimen of the collected plant material.

Preparation of Plant material

The collected roots of *Achyranthes aspera* were cleaned thoroughly with distilled water to remove adhering dirt and extraneous matter. The cleaned roots were subsequently shade-dried at room temperature (25-30°C) for several days until complete drying was achieved. Following drying, the plant material was fragmented into smaller pieces and ground into a coarse powder using a mechanical grinder. The powdered material was preserved in sterile, air-tight containers and stored under suitable conditions until further extraction and analysis.

Preparation of Ethanolic root extract

Approximately 5 g of the powdered material was extracted with 50 mL of ethanol (1:10 w/v) using the cold maceration method. The extraction was carried out in a stoppered conical flask at room temperature for 48 hr with intermittent shaking to facilitate efficient extraction of phytoconstituents. Following extraction, the mixture was filtered through Whatman No. 1 filter paper, and the filtrate was collected. The solvent was subsequently evaporated to obtain the crude ethanolic extract, which was stored under appropriate conditions until further analysis (Handa *et al*., 2008). The dried ethanolic extract obtained after solvent evaporation is presented in Figure 1.

GC-MS analysis and identification of phytocompounds

Gas Chromatography-Mass Spectrometry (GC-MS) analysis of the ethanolic root extract of *Achyranthes aspera* was carried out to identify the bioactive compounds present in the extract. The analysis was performed using an Agilent GC-MS system (GC 8890 coupled with MS 5977°C) equipped with an autosampler (7693A). Separation was achieved using a DB-5MS capillary column (30 m length × 0.25 mm internal diameter × 0.25 µm film thickness). Helium (99.999% purity) was used as the carrier gas at a constant flow rate of 1 mL/min. The oven temperature was initially set at 50°C and held for 1 min, then increased at a rate of 10°C/min to 300°C and held for 1 min. The injector temperature was maintained at 250°C. The mass spectrometer was operated in scan mode with a mass range of 0.6-1091 m/z. The identification of compounds was carried out by comparing the obtained mass spectra with those available in the NIST20 spectral library using Mass Hunter software.

ADME analysis of phytocompounds

The selected phytocompounds identified through GC-MS analysis of the ethanolic root extract of *Achyranthes aspera* were subjected to ADME evaluation using the SwissADME online tool. The chemical structures of the compounds were retrieved from the PubChem database and their SMILES representations were used for the prediction of physicochemical properties, pharmacokinetic behaviour and drug-likeness characteristics. The SwissADME platform provides a bioavailability radar that assesses the drug-likeness of compounds based on six important physicochemical characteristics: lipophilicity, size, polarity, solubility, flexibility and saturation. These properties are used to evaluate the drug-likeness and oral bioavailability potential of the selected compounds. Various physicochemical parameters including molecular weight, Lipophilicity (Log P), Topological Polar Surface Area (TPSA), water solubility (Log S), fraction of sp^3 Carbons (Csp^3) and number of rotatable bonds were determined. The drug-likeness of the compounds was assessed based on Lipinski's Rule of Five, describes the key molecular properties influencing pharmacokinetics and oral bioavailability. According to this rule, compounds with a molecular weight less than 500 Da, fewer than 5 hydrogen bond donors, fewer than 10 hydrogen bond acceptors and a Log P value less than 5 are considered favourable for drug development. The number of rule violations was recorded. Pharmacokinetic parameters such as Gastrointestinal Absorption (GIA), Blood-Brain Barrier (BBB) permeability, P-glycoprotein (P-gp) substrate status and bioavailability score were predicted. The interaction of the compounds with cytochrome P450 enzymes, including CYP1A2, CYP2C19, CYP2C9, CYP2D6 and CYP3A4, was evaluated to assess metabolic stability. Skin permeability (Log Kp) was also predicted to understand the permeability characteristics of the compounds (Lipinski *et al*., 1997; Daina *et al*., 2017a).

Pro Tox Analysis

The toxicity profile of the selected phytocompounds was predicted using the ProTox-3.0 online server. Canonical SMILES obtained from the PubChem database were submitted to the server for toxicity prediction. The parameters evaluated included oral LD_{50}, toxicity class, hepatotoxicity, carcinogenicity, mutagenicity, immunotoxicity, cardiotoxicity and cytotoxicity.

RESULTS AND DISCUSSION

GC-MS analysis

The ethanolic root extract of *Achyranthes aspera* subjected to Gas Chromatography-Mass Spectrometry (GC-MS) analysis revealed the presence of several bioactive phytoconstituents, as shown in Figure 2. The phytoconstituents identified through GC-MS analysis was chosen according to their relative peak area percentages (T). The major compounds selected for further *in silico* analysis are presented in Table 1.

ADME analysis

The predictive ADME profile, bioavailability, drug-likeness, and medicinal chemistry friendliness of the fifteen Phytocompounds such as ϒ- Sitosterol, Isopropyl myristate, β- Amyrin, 3-(Octanoyloxy) propane-1,2-diyl bis(decanoate), Hexadecanoic acid methyl ester, Alpha- amyrin, Dodecanoic acid, 1,2,3-propanetriyl ester, Methyl stearate, 3-Hexadecanol, 9-Octadecenoic acid (Z)-, methyl ester, 1,3-Propanediol,

2-ethyl-2-(hydroxymethyl), 9,11-Octadecadienoic acid, methyl ester, (E,E), Campesterol, Stigmasterol and Lupeol are shown in Tables 2-5.

In Table 2, physicochemical and lipophilicity properties including molecular weight, Log p, TPSA, Log S (ESOL), Fraction csp3 and number of rotatable bonds of the selected fifteen compounds are given. Molecular weight values ranged from 134.17 g/mol for 1,3-Propanediol, 2-ethyl-2-(hydroxymethyl)- to 639.00 g/mol for Dodecanoic acid, 1,2,3-propanetriyl ester. Most compounds exhibited molecular weights below 500 g/mol, a characteristic generally associated with favourable drug absorption and permeability. The lipophilicity (Log P) values ranged from -0.27 to 6.92, indicating varying degrees of hydrophobicity among the Phyto ligands. Sterol and triterpenoid compounds such as γ-sitosterol, α-amyrin, β-amyrin and lupeol displayed high Log P values (>6), suggesting strong lipid affinity and potential membrane permeability, although excessive lipophilicity may adversely affect aqueous solubility. Topological Polar Surface Area (TPSA) values ranged from 20.23 to 78.90 Å². Most compounds exhibited TPSA values below 140 Å², indicating a favourable capacity for passive membrane transport and oral absorption. The lowest TPSA value (20.23 Å²) was observed in several sterol and triterpenoid compounds, whereas triglyceride derivatives exhibited the highest TPSA value (78.90 Å²). The predicted aqueous solubility (Log S) values varied considerably, ranging from 0.11 to -11.02. The compound 1,3-Propanediol, 2-ethyl-2-(hydroxymethyl)- demonstrated the highest solubility, while Dodecanoic acid, 1,2,3-propanetriyl ester showed the lowest solubility. The reduced solubility of highly lipophilic compounds may limit their bioavailability despite favourable membrane permeability. The fraction of sp^3-hybridized carbon atoms (Fraction Csp^3) ranged from 0.74 to 1.00, indicating a high degree of molecular saturation among the compounds. The number of rotatable bonds varied substantially from 0 to 38, reflecting differences in molecular flexibility. Triterpenoids such as α-amyrin and β-amyrin exhibited rigid structures with no rotatable bonds, whereas triglyceride derivatives possessed a large number of rotatable bonds, indicating greater

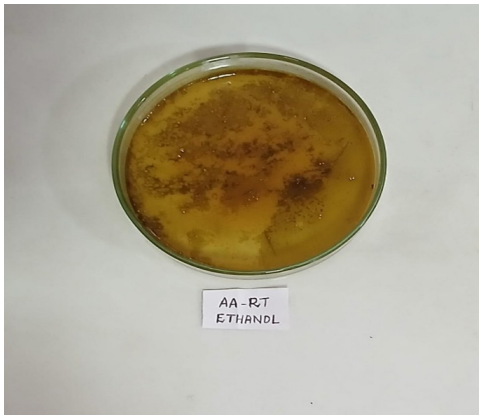


Figure 1: Dried ethanolic extract of *A. aspera*.

Table 1: Major phytocompounds identified in the ethanolic root extract of *Achyranthes aspera* by GC-MS analysis.

Sl. No.	Retention Time (RT)	Name of the compound	Molecular formula	Peak area %-T	Match Score
1.	25.7163	γ-Sitosterol	$C_{29}H_{50}O$	14.81	94.1
2.	16.8633	Isopropyl myristate	$C_{17}H_{34}O_2$	12.75	97.9
3.	26.1788	β-amyrin	$C_{30}H_{50}O$	8.77	88.5
4.	24.9479	3-(Octanoyloxy)propane-1,2-diyl bis(decanoate)	$C_{31}H_{58}O_6$	6.65	71.4
5.	17.8944	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	5.77	95.6
6.	26.8306	alpha-Amyrin	$C_{30}H_{50}O$	5.43	81.5
7.	25.1446	Dodecanoic acid, 1,2,3-propanetriyl ester	$C_{39}H_{74}O_6$	4.78	65.1
8.	19.7989	Methyl stearate	$C_{19}H_{38}O_2$	4.01	94.6
9.	16.6526	3-Hexadecanol	$C_{16}H_{34}O$	2.79	91.8
10.	19.5804	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	2.41	90.2
11.	12.8655	1,3-Propanediol,2-ethyl-2-(hydroxymethyl)-	$C_6H_{14}O_3$	2.29	75.5
12.	19.5331	9,11-Octadecadienoic acid, methyl ester, (E,E)-	$C_{19}H_{34}O_2$	2.14	92.9
13.	24.2451	Campesterol	$C_{28}H_{48}O$	1.33	68.8
14.	24.7986	Stigmasterol	$C_{29}H_{48}O$	0.36	68.1
15.	26.0695	Lupeol	$C_{30}H_{50}O$	0.72	60.1

Table 2: Physicochemical parameters and molecular properties of selected compounds.

Sl. No.	Compound Name	Molecular weight	Log p	TPSA (Å)	Log S (ESOL)	Fraction Csp3	No of rotatable bonds
1.	ϒ- Sitosterol	414.71	6.73	20.23	-7.90	0.93	6
2.	Isopropyl myristate	270.45	4.44	26.30	-5.14	0.94	14
3.	β- Amyrin	426.72	6.92	20.23	-8.25	0.93	0
4.	3-(Octanoyloxy) propane-1,2-diyl bis(decanoate)	526.79	5.44	78.90	-8.13	0.90	30
5.	Hexadecanoic acid methyl ester	270.45	4.44	26.30	-5.18	0.94	15
6.	Alpha- amyrin	426.72	6.92	20.23	-8.16	0.93	0
7.	Dodecanoic acid, 1,2,3-propanetriyl ester	639.00	6.88	78.90	-11.02	0.92	38
8.	Methyl stearate	298.50	4.91	26.30	-5.83	0.95	17
9.	3-Hexadecanol	242.44	4.44	20.23	-4.96	1.00	13
10.	9-Octadecenoic acid (Z)-, methyl ester	296.49	4.80	26.30	-5.32	0.84	16
11.	1,3-Propanediol, 2-ethyl-2-(hydroxymethyl)- 1,3-Propanediol, 2-ethyl-2-(hydroxymethyl)-	134.17	-0.27	60.69	0.11	1.00	4
12.	9,11-Octadecadienoic acid, methyl ester, (E,E)-	294.47	4.70	26.30	-5.09	0.74	15
13.	Campesterol	400.68	6.54	20.23	-7.54	0.93	5
14.	Stigmasterol	412.69	6.62	20.23	-7.46	0.86	5
15.	Lupeol	426.72	6.92	20.23	-8.64	0.93	1

Molecular weight (g/mol); Log P indicates lipophilicity; TPSA ($Å^2$) reflects polarity and permeability; Log S (ESOL) denotes solubility; Fraction Csp^3 indicates molecular complexity; number of rotatable bonds reflects molecular flexibility.

conformational flexibility that may influence binding interactions and pharmacokinetic behaviour.

Table 3 shows the drug-likeness and pharmacokinetic evaluation of the fifteen selected Phyto constituents revealed varying degrees of compliance with established criteria for oral drug candidates. Among the compounds analyzed, thirteen phytoligands satisfied Lipinski's Rule of Five, indicating favourable physicochemical characteristics for oral bioavailability. Only 3-(Octanoyloxy) propane-1,2-diyl bis(decanoate) and Dodecanoic acid, 1,2,3-propanetriyl ester exhibited two Lipinski violations, suggesting potential limitations in oral drug development due to their larger molecular size and increased lipophilicity. The remaining compounds showed either no violation or a single violation, which is generally considered acceptable for natural products. Blood-Brain Barrier (BBB) permeability predictions indicated that only Isopropyl myristate, Hexadecanoic acid methyl ester and 3-Hexadecanol were capable of crossing the BBB. The remaining Phyto ligands were predicted to be BBB-impermeant, suggesting a lower likelihood of central nervous system exposure and associated neurological side effects. Gastrointestinal Absorption (GIA) analysis demonstrated that Isopropyl myristate, Hexadecanoic acid methyl ester, Methyl stearate, 3-Hexadecanol, 9-Octadecenoic acid (Z)- methyl ester, 1,3-Propanediol, 2-ethyl-2-(hydroxymethyl)- and 9,11-Octadecadienoic acid methyl Ester (E,E) possessed high gastrointestinal absorption, indicating their potential suitability for oral administration. In contrast, sterols, triterpenoids, and triglyceride derivatives exhibited low gastrointestinal absorption, likely due to their higher molecular weights and lipophilic nature. P-glycoprotein (P-gp) substrate prediction revealed that only Dodecanoic acid, 1,2,3-propanetriyl ester was identified as a P-gp substrate. As P-gp can actively transport compounds out of cells, this characteristic may reduce intestinal absorption and bioavailability. All other phytoligands were predicted to be non-substrates, which may favour improved intracellular retention and absorption. The bioavailability score further supported these findings. Most phytoligands exhibited a bioavailability score of 0.55, indicating moderate oral bioavailability potential. In contrast, 3-(Octanoyloxy) propane-1,2-diyl bis(decanoate) and Dodecanoic acid,

1,2,3-propanetriyl ester showed a lower score of 0.17, suggesting reduced prospects for effective oral bioavailability.

The cytochrome P450 (CYP450) enzyme inhibition and skin permeation profile of the fifteen selected phytoligands was evaluated to assess their potential for drug-drug interactions and metabolic stability (Table 4). The results revealed that the majority of the compounds did not inhibit the major CYP450 isoforms investigated, indicating a generally favourable metabolic profile with minimal drug-drug interactions. None of the compounds inhibited CYP2C19 or CYP2D6, while only a few compounds showed inhibitory activity against CYP1A2, CYP2C9 or CYP3A4. Notably, γ-sitosterol, β-amyrin, α-amyrin, Dodecanoic acid, 1,2,3-propanetriyl ester, 1,3-Propanediol, 2-ethyl-2-(hydroxymethyl)-, campesterol and lupeol exhibited no inhibitory activity against any of the CYP450 isoforms investigated, indicating favourable metabolic compatibility. Skin permeability (Log Kp) values varied among the compounds, with Dodecanoic acid, 1,2,3-propanetriyl ester showing the highest permeability and 1,3-Propanediol, 2-ethyl-2-(hydroxymethyl)- exhibiting the lowest. Overall, the majority of the phytoligands demonstrated acceptable CYP450 inhibition profiles and moderate skin permeation potential, supporting their suitability for further pharmaceutical development.

The drug-likeness of the selected phytocompounds was assessed using five widely accepted filters, namely Lipinski, Ghose, Veber, Egan and Muegge. The analysis revealed considerable variation among the compounds with respect to their compliance with established drug-likeness criteria (Table 5). Among the fifteen compounds evaluated, 1,3-Propanediol, 2-ethyl-2-(hydroxymethyl)- exhibited complete compliance with all five filters, showing no violations and indicating highly favourable drug-like characteristics. 3-Hexadecanol also demonstrated good drug-likeness, with no violations in the Ghose and Egan filters and only minor deviations in the remaining criteria. Compounds such as isopropyl myristate, hexadecanoic acid methyl ester, 9,11-octadecadienoic acid methyl ester and 9-octadecenoic acid methyl ester showed relatively few violations. Phytosterols and triterpenoids including γ-sitosterol, campesterol, stigmasterol, lupeol, α-amyrin and β-amyrin exhibited multiple violations, particularly in the Ghose and Muegge filters. The highest number of violations was observed for 3-(Octanoyloxy) propane-1,2-diyl bis(decanoate) and dodecanoic acid, 1,2,3-propanetriyl ester, which failed several filters. Overall, the result revealed that some

Table 3: ADME-based drug-likeness and pharmacokinetic properties of selected compounds.

Sl. No	Compound Name	Lipinski rule	Violations	BBB	GIA	P-GP substrate	Bioavailability score
1.	ϒ- Sitosterol	Yes	1	No	Low	No	0.55
2.	Isopropyl myristate	Yes	1	Yes	High	No	0.55
3.	β- Amyrin	Yes	1	No	Low	No	0.55
4.	3-(Octanoyloxy) propane-1,2-diyl bis(decanoate)	No	2	No	Low	No	0.17
5.	Hexadecanoic acid methyl ester	Yes	1	Yes	High	No	0.55
6.	Alpha- amyrin	Yes	1	No	Low	No	0.55
7.	Dodecanoic acid, 1,2,3-propanetriyl ester	No	2	No	Low	Yes	0.17
8.	Methyl stearate	Yes	1	No	High	No	0.55
9.	3-Hexadecanol	Yes	1	Yes	High	No	0.55
10.	9-Octadecenoic acid (Z)-, methyl ester	Yes	1	No	High	No	0.55
11.	1,3-Propanediol, 2-ethyl-2-(hydroxymethyl)-	Yes	0	No	High	No	0.55
12.	9,11-Octadecadienoic acid, methyl ester, (E,E)-	Yes	1	No	High	No	0.55
13.	Campesterol	Yes	1	No	Low	No	0.55
14.	Stigmasterol	Yes	1	No	Low	No	0.55
15.	Lupeol	Yes	1	No	Low	No	0.55

Lipinski rule (Drug-Likeness Based on rule of five); Violations (number of deviations from Lipinski rule); BBB (Blood-Brain Barrier Permeability); GIA (Gastrointestinal Absorption Level); P-gp substrate (P-glycoprotein-mediated drug efflux); Bioavailability score (predicted oral bioavailability).

Table 4: The cytochrome P450 (CYP450) enzyme inhibition and skin permeation profile.

Sl. No.	Compound Name	CYPIA2 Inhibitor	CYP2C19 Inhibitor	CYP2C9 Inhibitor	CYP2D6 Inhibitor	CYP3A4 Inhibitor	Log Kp (cm/s)
1.	ϒ- Sitosterol	No	No	No	No	No	-2.20
2.	Isopropyl myristate	Yes	No	No	No	No	-2.83
3.	β- Amyrin	No	No	No	No	No	-2.41
4.	3-(Octanoyloxy) propane-1,2-diyl bis(decanoate)	No	No	No	No	Yes	-1.63
5.	Hexadecanoic acid methyl ester	yes	No	No	No	No	-2.71
6.	Alpha- amyrin	No	No	No	No	No	-2.51
7.	Dodecanoic acid, 1,2,3-propanetriyl ester	No	No	No	No	No	0.76
8.	Methyl stearate	Yes	No	No	No	No	-2.19
9.	3-Hexadecanol	No	No	Yes	No	No	-2.73
10.	9-Octadecenoic acid (Z)-, methyl ester	Yes	No	No	No	No	-2.82
11.	1,3-Propanediol, 2-ethyl-2-(hydroxymethyl)-	No	No	No	No	No	-7.70
12.	9,11-Octadecadienoic acid, methyl ester, (E,E)-	Yes	No	Yes	No	No	-3.12
13.	Campesterol	No	No	No	No	No	-2.50
14.	Stigmasterol	No	No	Yes	No	No	-2.74
15.	Lupeol	No	No	No	No	No	-1.90

CYP (cytochrome P450); CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4 (major CYP isoforms involved in drug metabolism; inhibitor status); Log Kp (skin permeability coefficient, cm/s).

of the compounds exhibit drug likeliness, while others required some structural modifications and suitable drug delivery system to enhance its drug likeliness an pharmacokinetic properties.

Bioavailability Radar Analysis

The oral bioavailability potential of the selected phytocompounds was further assessed using the SwissADME bioavailability radar. The radar plot evaluates six key physicochemical parameters, namely lipophilicity, size, polarity, solubility, flexibility and saturation, which collectively influence the oral bioavailability of a compound. Compounds whose radar profiles fall predominantly within the optimal pink region are considered to possess favourable drug-like characteristics and improved oral bioavailability (Figure 3).

Among the evaluated compounds, 1,3-Propanediol, 2-ethyl-2-(hydroxymethyl)-, 3-Hexadecanol, isopropyl myristate, hexadecanoic acid methyl ester, 9-octadecenoic acid methyl ester and 9,11-octadecadienoic acid methyl ester exhibited radar profiles that largely satisfied the recommended physicochemical criteria, indicating favourable oral bioavailability and drug-likeness properties. Phytosterols and triterpenoids such as γ-sitosterol, campesterol, stigmasterol, lupeol, α-amyrin and β-amyrin showed deviations from the optimal region, particularly with respect to lipophilicity and solubility. Nevertheless, despite these limitations, these compounds are recognized for their diverse pharmacological activities and remain promising candidates for further therapeutic investigation. The least favorable radar profiles were observed for 3-(Octanoyloxy) propane-1,2-diyl bis(decanoate) and dodecanoic acid, 1,2,3-propanetriyl ester, which deviated from multiple optimal physicochemical parameters. Such deviations may negatively influence their absorption and oral bioavailability. Overall, the bioavailability radar analysis supported the drug-likeness assessment and facilitated the identification of compounds with favourable pharmacokinetic characteristics for further study.

Pro Tox Analysis

The toxicity characteristics of the selected phytoconstituents were assessed using the ProTox-3.0 online platform. The predicted toxicity endpoints and toxicity classes are presented in Table 6. The predicted LD_{50} values ranged from 890 to 70,000 mg/kg body weight, corresponding to toxicity classes 4, 5 and 6. None of

Table 5: Drug-likeness of selected phytocompounds using different filters.

Sl. No	Phytocompounds	Lipinski rule	Ghose filter	Veber filter	Egan filter	Muegge filter
1.	ϒ- Sitosterol	1	3	0	1	2
2.	Isopropyl myristate	1	1	1	0	1
3.	β- Amyrin	1	3	0	1	2
4.	3-(Octanoyloxy) propane-1,2-diyl bis(decanoate)	2	4	1	1	2
5.	Hexadecanoic acid methyl ester	1	1	1	0	1
6.	Alpha- amyrin	1	3	0	1	2
7.	Dodecanoic acid, 1,2,3-propanetriyl ester	2	4	1	1	3
8.	Methyl stearate	1	1	1	1	2
9.	3-Hexadecanol	1	0	1	0	2
10.	9-Octadecenoic acid (Z)-, methyl ester	1	1	1	1	2
11.	1,3-Propanediol,2-ethyl-2-(hydroxymethyl)-	0	0	0	0	0
12.	9,11-Octadecadienoic acid, methyl ester, (E,E)-	1	1	1	1	1
13.	Campesterol	1	2	0	1	2
14.	Stigmasterol	1	3	0	1	2
15.	Lupeol	1	3	0	1	2

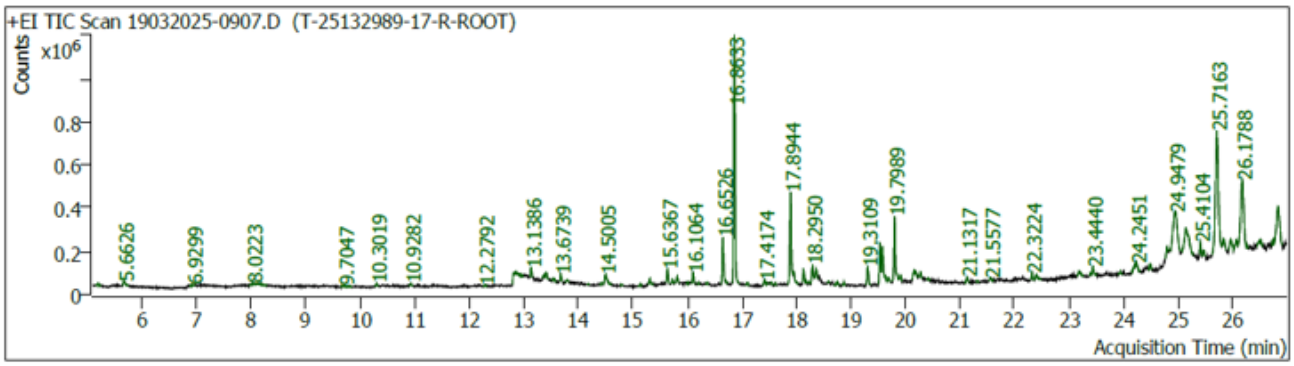


Figure 2: GC-MS chromatogram of the ethanolic root extract of *Achyranthes aspera* showing the phytochemical constituents identified at different retention times.

the compounds belonged to toxicity classes 1, 2 or 3, indicating the absence of highly toxic phytoconstituents among the fifteen compounds selected from GC-MS analysis. Among the evaluated compounds, β-amyrin and α-amyrin exhibited the highest LD_{50} values (70,000 mg/kg) and were classified under toxicity class 6, indicating a very low level of acute toxicity and suggesting a favourable safety profile. Several compounds, including isopropyl myristate, hexadecanoic acid methyl ester, methyl stearate, 9-octadecenoic acid methyl ester, 9,11-octadecadienoic acid methyl ester, and 3-(octanoyloxy) propane-1,2-diyl bis(decanoate), exhibited LD_{50} values of 3,000-5,000 mg/kg and belonged to toxicity class 5, indicating that they may be harmful only at relatively high doses. Gamma-sitosterol, campesterol, stigmasterol, lupeol and 3-hexadecanol were categorized under toxicity class 4, suggesting moderate acute toxicity but remaining within the acceptable range for further pharmacological investigation. Toxicity endpoint prediction revealed that most compounds were inactive for carcinogenicity, mutagenicity, cardiotoxicity and cytotoxicity, indicating a generally favourable toxicological profile.

DISCUSSION

The GC-MS analysis of the ethanolic root extract of *Achyranthes aspera* revealed a complex mixture of bioactive phytoconstituents belonging to various chemical classes such as fatty acid esters, terpenoids, sterols, alcohols and phenolic compounds. A total of numerous compounds was detected in the chromatogram, out of which the major constituents were selected based on peak area percentage for further interpretation. These were identified as predominant compounds in the root extract.

Phytosterols such as sitosterol, campesterol and stigmasterol are known to possess diverse pharmacological properties, including antioxidant, anti-inflammatory, and anticancer activities. These compounds have been reported to suppress tumor progression through modulation of cellular signaling pathways, inhibition

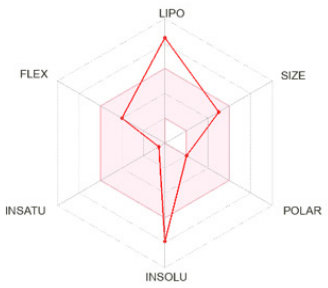
of cancer cell growth, and induction of programmed cell death (Awad *et al*., 2000; Woyengo *et al*., 2009). The results obtained in the present investigation show similarities with earlier GC-MS studies conducted on Achyranthes aspera, particularly those involving ethanolic leaf extracts. A study identified 25 phytochemical constituents in the leaf extract, among which 9-octadecenamide (21.15%), squalene (19.04%), phytol (7.06%) and α-amyrin (4.33%) were reported as the predominant compounds. These metabolites have been widely recognized for their diverse biological activities, including antioxidant, antimicrobial and anticancer properties. The occurrence of α-amyrin in both studies further supports the presence of pharmacologically important triterpenoids in *A. aspera* and highlights the therapeutic significance of this medicinal plant (Mary and Giri, 2017).

Beg and Athar (2020) performed pharmacokinetic and molecular docking studies on nineteen phytocompounds identified from *Achyranthes aspera* through GC-MS analysis. The authors used *in silico* pharmacokinetic evaluation to assess drug-likeness and ADME properties before molecular docking against tuberculosis targets. They concluded that several phytocompounds possessed favourable pharmacokinetic characteristics and could serve as potential lead molecules for anti-tubercular drug development. Likewise, Israr *et al*. (2024) demonstrated that phytoconstituents identified from *A. aspera*, including phenolic and flavonoid compounds, complied with Lipinski's rule of five and exhibited favourable pharmacokinetic profiles. The present findings therefore agree with earlier reports indicating that *A. aspera* contains a diverse spectrum of bioactive molecules with promising pharmacokinetic attributes. Although some compounds showed violations of conventional drug-likeness rules, such deviations are frequently observed among naturally occurring phytosterols and triterpenoids, which may still exert significant biological activities. Therefore, the ADME results support the potential of *A. aspera* phytocompounds as promising candidates for further pharmacological and drug development studies. A recent investigation by Sujay and Balaji (2026) identified γ-sitosterol as a major bioactive constituent of *Nyctanthes arbor-tristis* flowers and demonstrated its favourable ADMET profile along with notable anticancer potential against cervical cancer-related molecular targets. These findings highlight the therapeutic promise of γ-sitosterol; however, strategies such as nano formulations and lipid-based delivery systems may be necessary to overcome its limited bioavailability and enhance clinical effectiveness.

Overall, the ADME analysis revealed that several phytocompounds identified from *Achyranthes aspera* exhibited favourable

Table 6: Predicted toxicity profile of selected compounds using ProTox-3.0.

Sl. No.	Compounds	LD_{50} (mg/KG)	Class	Hepatotoxicity	Carcinogenicity	Mutagenicity	Immune toxicity	Cardiotoxicity	Cytotoxicity
1.	Gamma Sitosterol	890	4	Active	Inactive	Inactive	Active	Inactive	Inactive
2.	Isopropyl myristate	5000	5	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
3.	β- Amyrin	70000	6	Inactive	Inactive	Inactive	Active	Inactive	Inactive
4.	3-(Octanoyloxy) propane-1,2-diyl bis(decanoate)	5000	5	Inactive	Active	Active	Inactive	Inactive	Inactive
5.	Hexadecanoic acid methyl ester	5000	5	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
6.	Alpha- amyrin	70000	6	Inactive	Inactive	Inactive	Active	Inactive	Inactive
7.	Dodecanoic acid, 1,2,3-propanetriyl ester	5000	5	Inactive	Active	Active	Inactive	Inactive	Inactive
8.	Methyl stearate	5000	5	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
9.	3-Hexadecanol	1000	4	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
10.	9-Octadecenoic acid (Z)-, methyl ester	3000	5	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
11.	1,3-Propanediol,2-ethyl-2-(hydroxymethyl)-	12980	6	Inactive	Inactive	Inactive	Inactive	Active	Inactive
12.	9,11-Octadecadienoic acid, methyl ester, (E,E)-	5000	5	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
13.	Campesterol	890	4	Inactive	Inactive	Inactive	Active	Inactive	Inactive
14.	Stigmasterol	890	4	Inactive	Inactive	Inactive	Active	Inactive	Inactive
15.	Lupeol	2000	4	Inactive	Inactive	Inactive	Active	Inactive	Inactive



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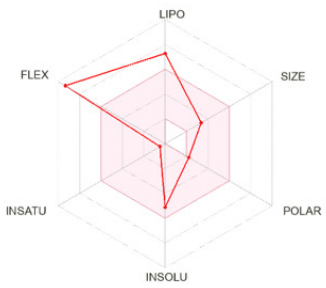

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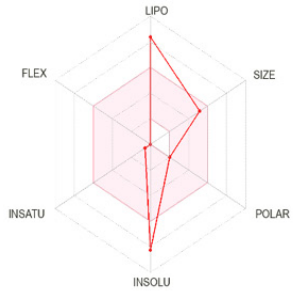

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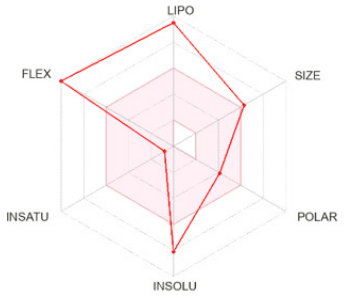

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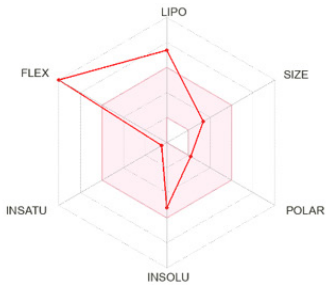

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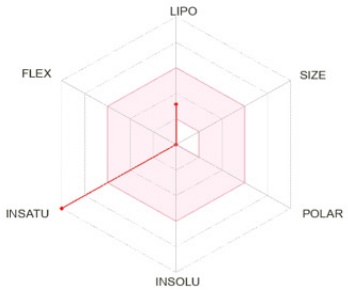

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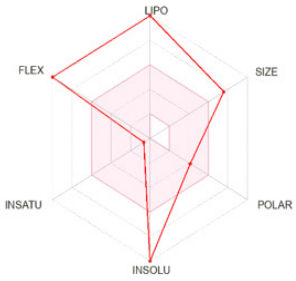

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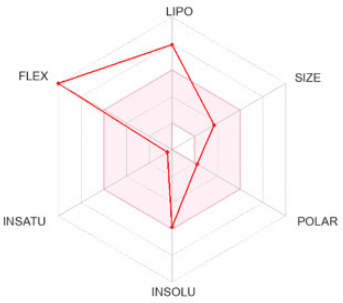

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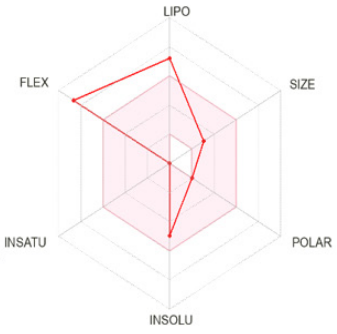

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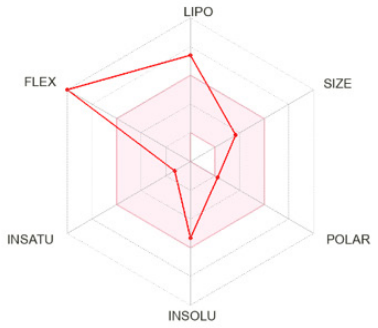

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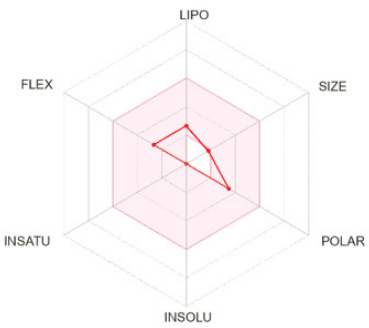

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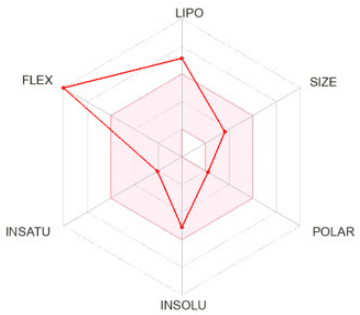

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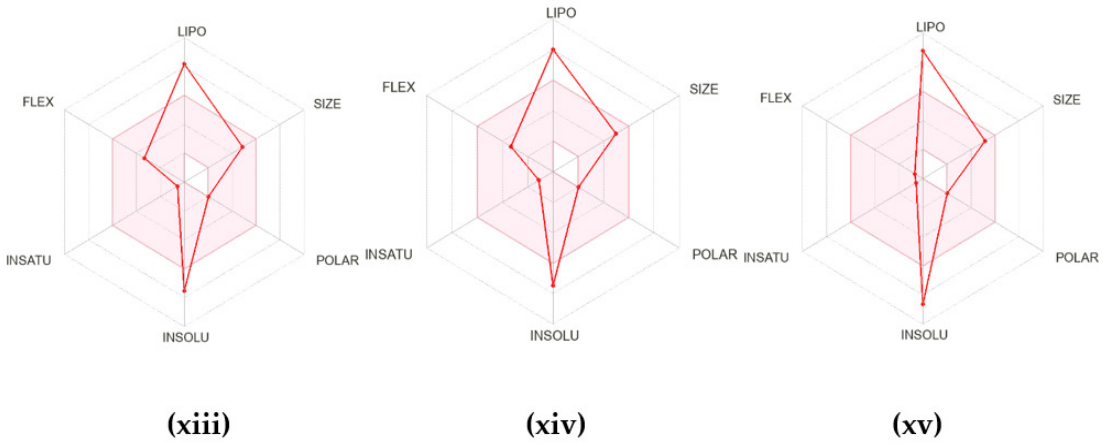

Figure 3: Radar plot representing the physicochemical properties of selected compound (i) Gamma Sitosterol (ii) Isopropyl myristate (iii) β-Amyrin (iv) 3-(Octanoyloxy) propane-1,2-diyl bis(decanoate) (v) Hexadecanoic acid methyl ester (vi) Alpha- amyrin (vii) Dodecanoic acid, 1,2,3-propanetriyl ester (viii) Methyl stearate (ix) 3-Hexadecanol (x) 9-Octadecenoic acid (Z)-, methyl ester (xi) 1,3-Propanediol,2-ethyl-2-(hydroxymethyl)- (xii) 9,11-Octadecadienoic acid, methyl ester, (E,E)- (xiii) Campesterol (xiv) Stigmasterol (xv) Lupeol.

drug-likeness and pharmacokinetic properties, suggesting their potential as orally active therapeutic candidates. Although some compounds did not fully satisfy all the conventional drug-likeness criteria and showed violations in one or more filters, such deviations are commonly observed among naturally occurring phytoconstituents, particularly phytosterols and triterpenoids. Importantly, numerous studies have demonstrated that these compounds possess significant pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial and anticancer effects, despite their limited compliance with established drug-likeness rules. Therefore, failure to meet all ADME criteria should not necessarily exclude these molecules from consideration as potential drug candidates. Instead, these bioactive compounds may serve as valuable lead molecules for further optimization and formulation approaches aimed at improving their pharmacokinetic performance and therapeutic efficacy.

Assessment of toxicity endpoints revealed that the majority of compounds were predicted to be inactive for carcinogenicity, mutagenicity, cardiotoxicity and cytotoxicity, suggesting a generally favourable safety profile. However, a few compounds showed predicted activity toward specific toxicity endpoints. γ-Sitosterol exhibited predicted hepatotoxicity and immunotoxicity, while campesterol, stigmasterol, lupeol, α-amyrin and β-amyrin demonstrated immunotoxic potential. Furthermore, 3-(octanoyloxy) propane-1,2-diyl bis(decanoate) and dodecanoic acid, 1,2,3-propanetriyl ester were predicted to possess carcinogenic and mutagenic activities. Nevertheless, these predictions represent computational estimations and require experimental validation through *in vitro* and *in vivo* toxicological studies. Overall, the ProTox-III analysis suggests that the majority of phytocompounds identified from *Achyranthes aspera* possess relatively low toxicity and favourable safety characteristics. Consequently, the observed toxicity predictions should not preclude their therapeutic potential but rather highlight the need for further toxicological evaluation and optimization before pharmaceutical development.

CONCLUSION

GC-MS analysis of the ethanolic root extract of *Achyranthes aspera* identified bioactive phytocompounds with potential therapeutic importance. ADME evaluation revealed that several compounds exhibited favourable drug-likeness and pharmacokinetic properties. Although some compounds showed violations of drug-likeness rules, they are known to possess significant biological activities. ProTox-III analysis indicated low to moderate toxicity for most compounds with acceptable safety profiles. Overall, the findings suggest that *Achyranthes aspera* root is a promising source of bioactive molecules for future drug discovery and pharmacological studies.

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ABBREVIATIONS

ADMET: Absorption, Distribution, Metabolism, Excretion and Toxicity; **BBB:** Blood-Brain Barrier; **BSI:** Botanical Survey of India; **Csp3:** Fraction of sp3 hybridized carbons; **CYP:** Cytochrome P450; **GC-MS:** Gas Chromatography-Mass Spectrometry; **GIA:** Gastrointestinal Absorption; **LD_{50}:** Lethal Dose, 50%; **m/z:** Mass-to-charge ratio; **P-gp:** P-glycoprotein;

SMILES: Simplified Molecular Input Line Entry System; **TPSA:** Topological Polar Surface Area.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR'S CONTRIBUTION

Both the authors made substantial contribution to the study conception, data acquisition and manuscript preparation. Each author has read and approved the final version of the manuscript and affirms its originality and integrity.

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