Isolation, Chromatographic separation, and GC-MS analysis of bioactive constituents from *Achyranthes aspera root*

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Abstract

Background: The present study aimed to investigate the phytochemical constituents and chemical profile of the methanolic root extract of *Achyranthes aspera* Linn. using chromatographic and spectroscopic techniques to validate its traditional medicinal importance. **Methods:** Fresh roots of *Achyranthes aspera* were collected, authenticated, shade-dried, powdered, and extracted with methanol using Soxhlet extraction. Preliminary phytochemical screening was performed using standard qualitative methods. Thin Layer Chromatography (TLC) and silica gel column chromatography were used for the isolation of phytoconstituents with n-hexane: acetone (6:4 v/v) as the mobile phase. The isolated fractions were characterized using UV–visible spectroscopy and Gas Chromatography–Mass Spectrometry (GC–MS). **Results:** Phytochemical analysis revealed the presence of alkaloids, tannins, flavonoids, terpenoids, steroids, and coumarins, while saponins were absent. UV–visible spectroscopy showed significant absorption peaks between 210 and 270 nm, indicating the presence of conjugated and aromatic chromophoric compounds. GC–MS analysis identified several bioactive constituents, including hexadecyl acrylate, 1-dodecanol, trans-2-dodecen-1-ol, oxalic acid derivatives, benzenepropanoic acid derivatives, phenolic compounds, esters, ketones, siloxanes, and steroid derivatives. In sample-1, hexadecyl acrylate (14.60%) and 1-dodecanol (12.30%) were the major constituents, whereas sample-2 showed a high abundance of oxalic acid cyclohexylmethyl tridecyl ester (16.15%) and 2-pentanone, 4-hydroxy-4-methyl- (15.90%). **Conclusion:** The findings demonstrate that the methanolic root extract of *Achyranthes aspera* contains diverse bioactive phytoconstituents with potential pharmacological activity. This study scientifically supports the traditional medicinal use of the plant and provides a foundation for future isolation, structural elucidation, and pharmacological evaluation of its active compounds.

Keywords: Achyranthes aspera, Phytochemical analysis, GC–MS, Methanolic extract, Bioactive compounds, Medicinal plant

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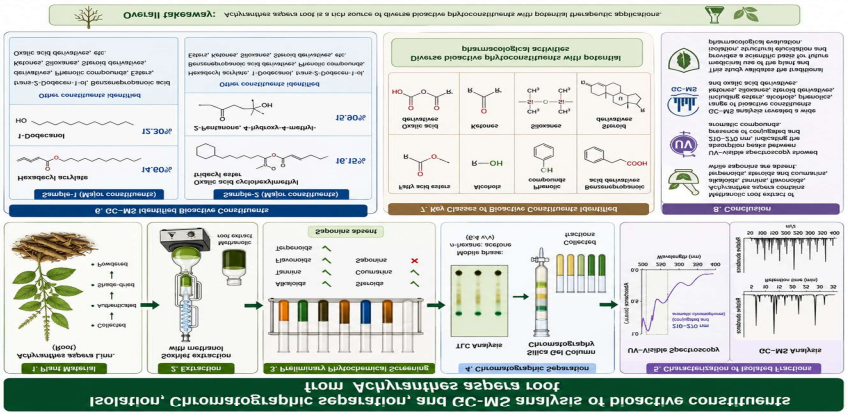
Graphical Abstract:



Background:

In the 21st century, herbal plants are regaining global importance as substitutes for modern drugs, largely

because of the irreversible damage caused by synthetic medications (Boyapati et al., 2017). Traditional medicine has gained widespread acceptance because of its affordability, therapeutic efficacy, and fewer side effects than conventional pharmaceuticals (Verma & Singh, 2008). It is estimated that nearly 80% of the world's population relies on traditional herbal medicine for primary healthcare, particularly in countries like India, where a significant proportion of treatments are derived from plant-based formulations used in systems such as Ayurveda, Unani, Siddha, and Homoeopathy (Balamurugan et al., 2018). Phytochemicals, including flavonoids, alkaloids, phenolics, terpenoids, saponins, and glycosides, exhibit diverse bioactive properties, including antioxidant, anti-inflammatory, neuroprotective, and anti-cholinesterase activities (Ekom Monday Etukudo et al., 2025), (Oviosun et al., 2025), (Piccirillo et al., 2025), (Roy et al., 2022), (Usman et al., 2025), (Kumar et al., 2025). The therapeutic advantage of herbal plants lies in the synergistic action of their multiple phytoconstituents.

Achyranthes aspera (Apamarga), a member of the family Amaranthaceae, is a widely recognized medicinal plant commonly known as Prickly Chaff Flower and is distributed across Asia, Africa, and South America (Devi et al., 2017). It is an erect annual herb of high ethnomedicinal value, traditionally used to treat fever, wounds, dysentery, hypertension, and various other ailments (Sharma et al., 2019). The plant is known for its broad spectrum of pharmacological activities, including antimicrobial, analgesic, antipyretic, anti-inflammatory, immunostimulant, antioxidant, hypoglycemic, diuretic, antihypertensive, and hepatoprotective effects (Boyapati et al., 2017). Its bioactivity is attributed to the presence of active constituents such as alkaloids, saponins, sterols, flavonoids, oleanolic acid, ecdysterone, and betaine (Sinan et al., 2020). Additionally, it exhibits antibacterial, antifungal, antiviral, antimalarial, antarthritic, and cardiotonic properties (Devi et al., 2017), and is traditionally used in the management of conditions such as piles, renal disorders, pneumonia, kidney stones, skin diseases, and snake bites, along with antiasthmatic, laxative, anti-allergic, and hepatoprotective effects (Sinan et al., 2020).

Furthermore, biological studies have demonstrated that A. aspera possesses a wide range of activities, including antibacterial, antifungal, thyroid-stimulating, antiperoxidative, anti-inflammatory, antiarthritic, immunomodulatory, wound healing, antiobesity, anticonvulsant, anticancer, and hepatoprotective properties, which are attributed to phytochemical classes such as saponins, phenolics, flavonoids, alkaloids, steroids, and terpenoids (Sharma et al., 2019). However, most existing studies have primarily focused on A. aspera growing in India, with limited scientific validation available for the species found in Africa, where investigations have mainly been restricted to antimicrobial, anthelmintic, and wound-healing activities (Sharma et al., 2019).

Result:

Result of Phytochemical screening

The phytochemical screening results presented in the table indicate the qualitative presence or absence of various bioactive constituents in the tested sample. The analysis revealed the presence of tannins, flavonoids, terpenoids, alkaloids, steroids, and coumarins, suggesting that these secondary metabolites are present in the extract. In contrast, saponins were not detected, as indicated by the negative results (Table 1). The presence of these phytochemicals highlights the potential biological and pharmacological significance of the sample, as flavonoids and alkaloids are often associated with antioxidant, antimicrobial, and therapeutic activities.

Table 1: Preliminary phytochemical screening of extracts of Achyranthes aspera root

Sr.no:	Phytochemical	Test	Test form
1.	Test for tannin	Ferric chloride test	+
2.	Test for flavonoid	Lead acetate test	+
3.	Test for terpenoid	H_2SO_4 test	+
4.	Test for alkaloid	Dragendorff's test	+
5.	Test for steroid	Salkowshi test	+
6.	Test for saponin	Foam test	-
7.	Test for coumarin	NaoH test	+

(+) : present, (-) : Absent

Result of UV-Vis spectroscopy analysis :

UV–visible spectroscopic analysis of the sample exhibited strong absorption in the lower wavelength region, with a maximum absorbance observed near 210–220 nm, followed by a gradual decline in absorbance with increasing wavelength. A noticeable shoulder peak was observed at approximately 268 nm with an absorbance value of 0.618, indicating the possible presence of conjugated systems or aromatic chromophores. Beyond 300 nm, the absorbance steadily decreased, approaching baseline levels near

400 nm, suggesting minimal absorption in the higher-wavelength region (Figure 1). UV–visible spectroscopic analysis of the sample revealed a prominent absorption peak at 234 nm with an absorbance of 0.407, indicating the presence of strong chromophoric groups. A secondary, less intense peak was observed at 270 nm with an absorbance of 0.190, (Table 2) suggesting possible π–π* transitions associated with the conjugated or aromatic systems. Beyond 300 nm, the absorbance rapidly decreased and approached near-baseline levels, with a negligible absorbance value of −0.005 at 412 nm, indicating minimal absorption in the visible region (Figure 2).

Table 2: UV-Vis spectroscopy of isolated compound

Sr.no:	Sample	λ max
1	Sample-1	Wavelength:268.0nm Absorbance: 0.618
2	Sample-2	Wavelength: 270.0nm and 234.0nm Absorbance: 0.190 and 0.407

Figure 1: UV-vis spectrum of isolated compound (Sample 1)

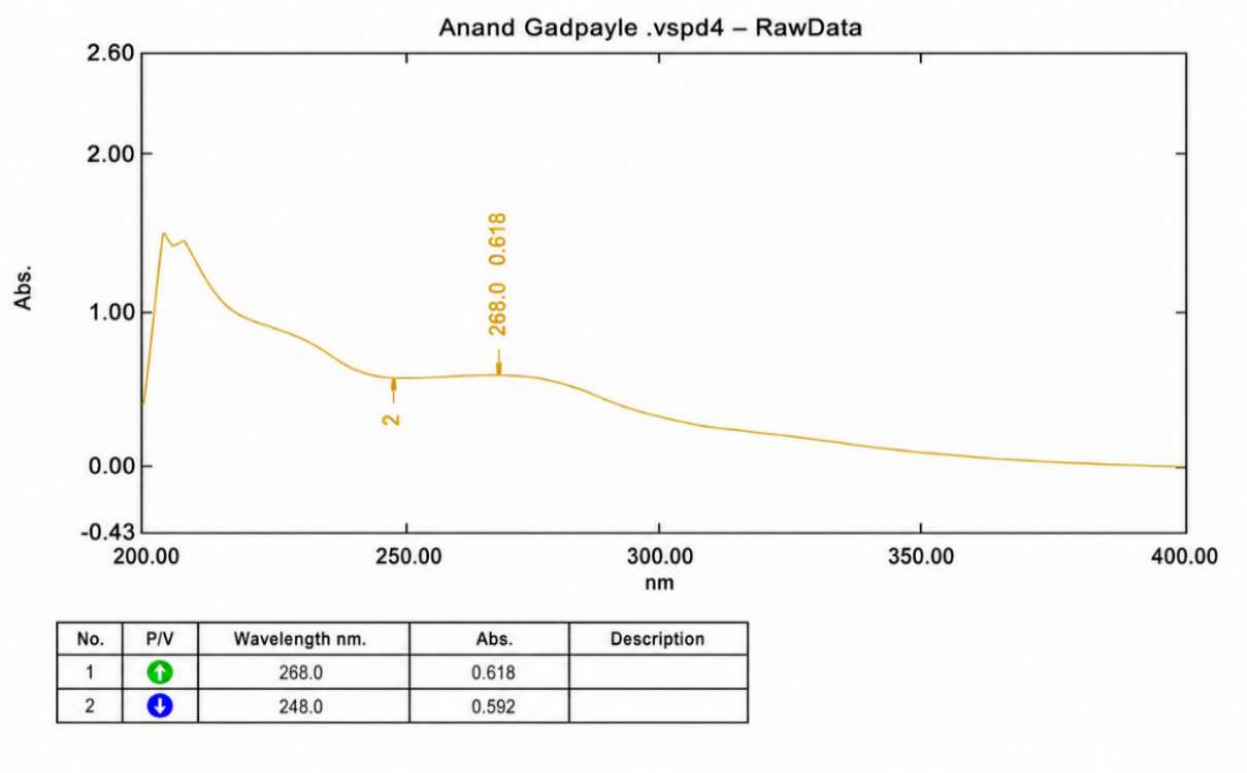
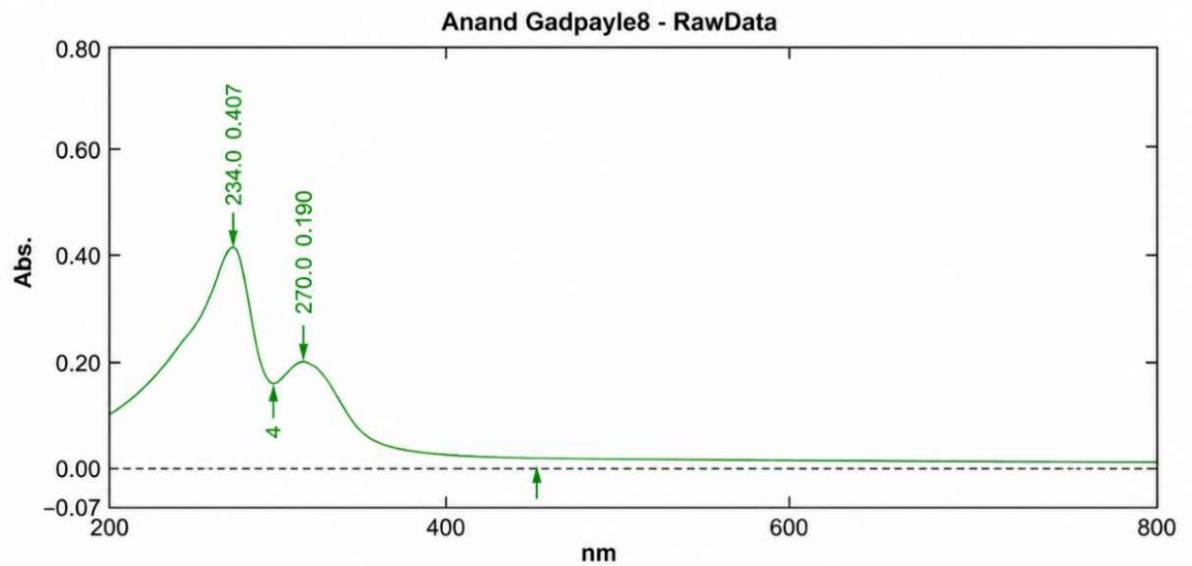


Figure 2: UV-vis spectrum of isolated compound (Sample 2)



No.	P/V	Wavelength (nm)	Abs.	Description
1	↑	270.0	0.190	–
2	↑	234.0	0.407	–
3	↓	412.0	−0.005	–
4	↓	256.0	0.167	–

Result of GC-MS analysis :

Chromatographic analysis of sample-1 revealed a total of fourteen distinct peaks, indicating the presence of multiple phytochemical constituents in the sample. Among these, the compound identified as hexadecyl acrylate exhibited the highest relative abundance, with an area percentage of 14.60% and a corresponding height percentage of 20.93% at a retention time of 19.146 min. This was followed by 1-Dodecanol (12.30% area, 14.54% height; RT = 16.556 min) and trisiloxane, 1,1,1,5,5,5-hexamethyl-3- (11.93% area; RT = 16.375 min). Other notable compounds included undecane (8.90% area; RT = 11.041 min), melezitose (8.32% area; RT = 23.959 min), and 2,4-Di-tert-butylphenol (7.17% area; RT = 17.006 min). Minor constituents such as 2 Propanol, 1,1'-oxybis- (6.65% area), 1,4-Dioxane, 2-ethyl-5-methyl- (6.17% area), Cyclooctasiloxane, hexadecamethyl- (5.60% area), Propanoic acid, 3-mercapto-, dodecyl ester (4.97% area), Cyclohexasiloxane, dodecamethyl- (4.20% area), 1H-Cyclonona difuran derivative (3.38% area), Glucopyranuronamide derivative (3.15% area), and Cyclononasiloxane, octadecamethyl- (2.65% area) were also detected in smaller proportions. The total chromatographic area was recorded as 4,062,761, with a cumulative area percentage of 100%, confirming the comprehensive profiling of the sample composition (Figure 3). Chromatographic analysis of sample-2 revealed a total of twenty-two peaks, indicating a complex mixture of phytochemical constituents. Among these, Oxalic acid and cyclohexylmethyl tridecyl ester exhibited the highest relative abundance, with an area percentage of 16.15% and a height percentage of 18.83% at a retention time of 19.249 min, followed closely by 2-Pentanone, 4-hydroxy-4-methyl- (15.90% area; RT = 5.880 min) and benzenepropanoic acid, 3,5 bis(1,1-dimethyl)-derivative (14.12% area; RT = 21.880 min). Trans-2-Dodecen-1-ol was also present in significant amounts, with an area percentage of 10.59% at a retention time of 12.334 min. Moderate concentrations of compounds such as dl-menthol (4.51% area), hexadecanoic acid methyl ester (4.01% area), decyl acrylate (3.98% area), undecane (3.07% area), and 1-Dodecanol (2.80% area) were observed. Additionally, several minor constituents, including 3,3-Dimethoxy-2-butanone (2.28% area), thiazolidine, 2-propyl- (2.53% area), phenol, 3,5-bis(1,1-dimethylethyl)- (2.52% area), 1-Hexadecanol (2.59% area), 15-Octadecenoic acid, methyl ester (2.70% area), Pregn-5-ene-3,11-dione derivative (2.14% area), 3,9-Epoxypregnane derivative (2.00% area), heptacosanoic acid, methyl ester (1.61% area), and other trace-level compounds

such as Tetradecane, 1-Pentadecene, and phthalic acid derivatives were identified in smaller proportions (Table 3). The total chromatographic area was recorded as 8,766,717 with a cumulative area percentage of 100%, confirming comprehensive profiling of the chemical composition of the sample (Figure 4)

Table 3: Phytoconstituents of A.aspera identified and quantified by GC-MS (Pubchem - https://pubchem.ncbi.nlm.nih.gov)

Sl. no.	R. time	Area %	Compound name	Molecular formula	Chemical class
1.	5.815	6.65	2-Propanol,1,1'-oxybis-	$C_{26}H_{26}O_5$	Ether
2.	5.970	6.17	1,4-Dioxane,2-ethyl-5-methyl-	$C_7H_{14}O_2$	Ether
3.	11.041	8.90	Undecane	$C_{11}H_{24}$	Alkane
4.	14.033	3.15	Glucopyranuronamide, 1-(4-amino-2-	$C_{16}H_{25}N_7O_8$	carbohydrate derivative
5.	14.144	4.20	Cyclohexasiloxane, dodecamethyl-	$C_{12}H_{36}O_6Si_6$	Siloxane
6.	16.375	11.93	Trisiloxane, 1,1,1,5,5,5-hexamethyl-3	$C_{17}H_{34}O_3Si_4$	Siloxane
7.	16.556	12.30	1-Dodecanol	$C_{12}H_{26}O$	Alcohol
8.	17.006	7.17	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	Phenol
9.	18.335	5.60	Cyclooctasiloxane, hexadecamethyl-	$C_{16}H_{48}O_8Si_8$	Siloxane
10.	19.146	14.60	Hexadecyl acrylate	$C_{19}H_{36}O_2$	Ester
11.	20.082	2.65	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	Siloxane
12.	23.317	3.38	1H-Cyclonona[1,2-c:5,6-c']difuran-1	$C_{18}H_{20}O_6$	Heterocyclic compound
13.	23.959	8.32	Melezitose	$C_{18}H_{32}O_{16}$	Carbohydrate
14.	5.818	2.28	3,3-Dimethoxy-2-butanone	$C_6H_{12}O_3$	Ketone
15.	5.880	15.90	2-Pentanone, 4-hydroxy-4-methyl-	$C_6H_{12}O_2$	Ketone
16.	11.042	3.07	Undecane	$C_{11}H_{24}$	Alkane
17.	12.334	10.59	trans-2-Dodecen-1-ol	$C_{12}H_{24}O$	Alcohol
18.	12.418	4.51	dl-Menthol	$C_{10}H_{20}O$	Alcohol
19.	12.547	2.53	Thiazolidine, 2-propyl-	$C_6H_{13}NS$	Heterocyclic compound
20.	15.448	0.91	1-Pentadecene	$C_{15}H_{30}$	Hydrocarbon
21.	15.554	0.94	Tetradecane	$C_{14}H_{30}$	Hydrocarbon

22.	16.375	1.43	Ethyl gallate, 3TMS derivative	$C_{18}H_{34}O_5Si_3$	Ester
23.	16.557	2.80	1-Dodecanol	$C_{12}H_{26}O$	Alcohol
24.	17.006	2.52	Phenol, 3,5-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	Phenol
25.	17.986	2.59	1-Hexadecanol	$C_{16}H_{34}O$	Alcohol
26.	19.147	3.98	Decyl acrylate	$C_{13}H_{24}O_2$	Ester
27.	21.786	4.01	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	Ester
28.	22.436	2.14	Pregn-5-ene-3,11-dione, 17,20:20,21-	$C_{25}H_{34}O_7$	Steroid
29.	22.597	2.00	3,9-Epoxypregnane-11.beta.,20-diol	$C_{25}H_{41}NO_5$	Steroid
30.	23.287	2.70	15-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	Ester
31.	23.517	1.61	Heptacosanoic acid, methyl ester	$C_{28}H_{56}O_2$	Ester

Figure 3: GC-MS analysis of an isolated compound from column chromatography (sample 1)

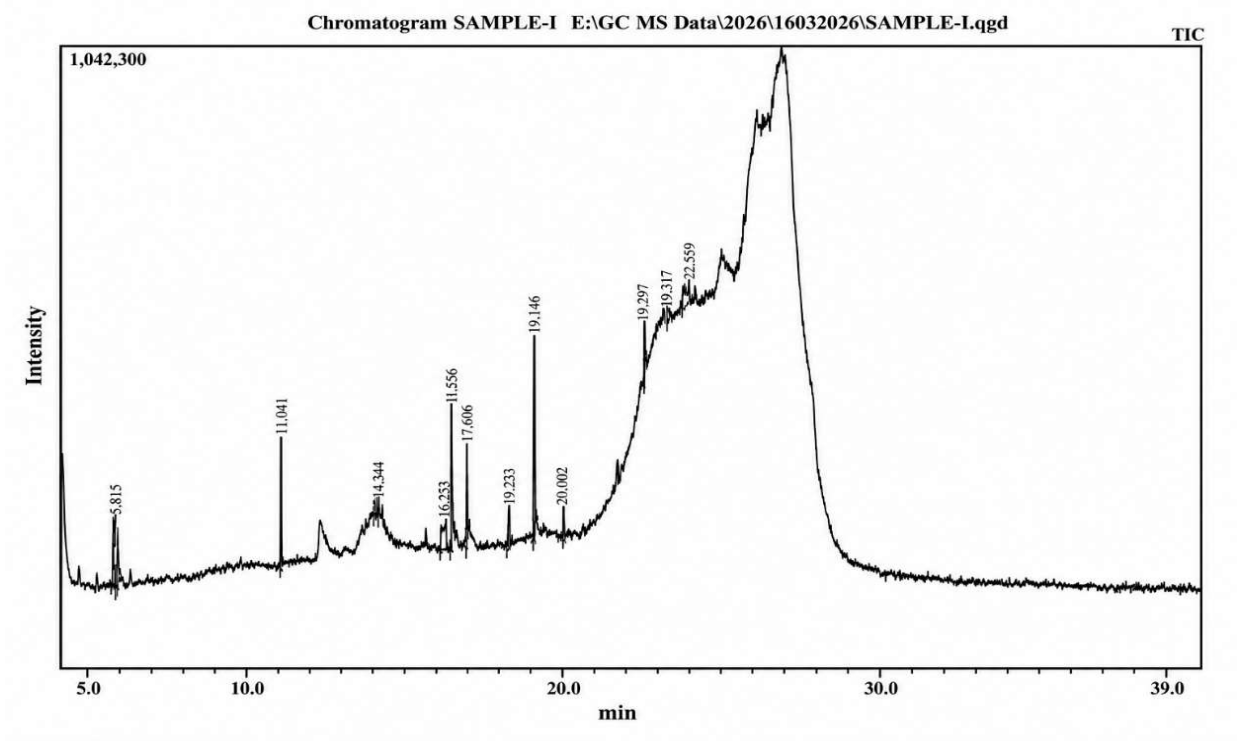
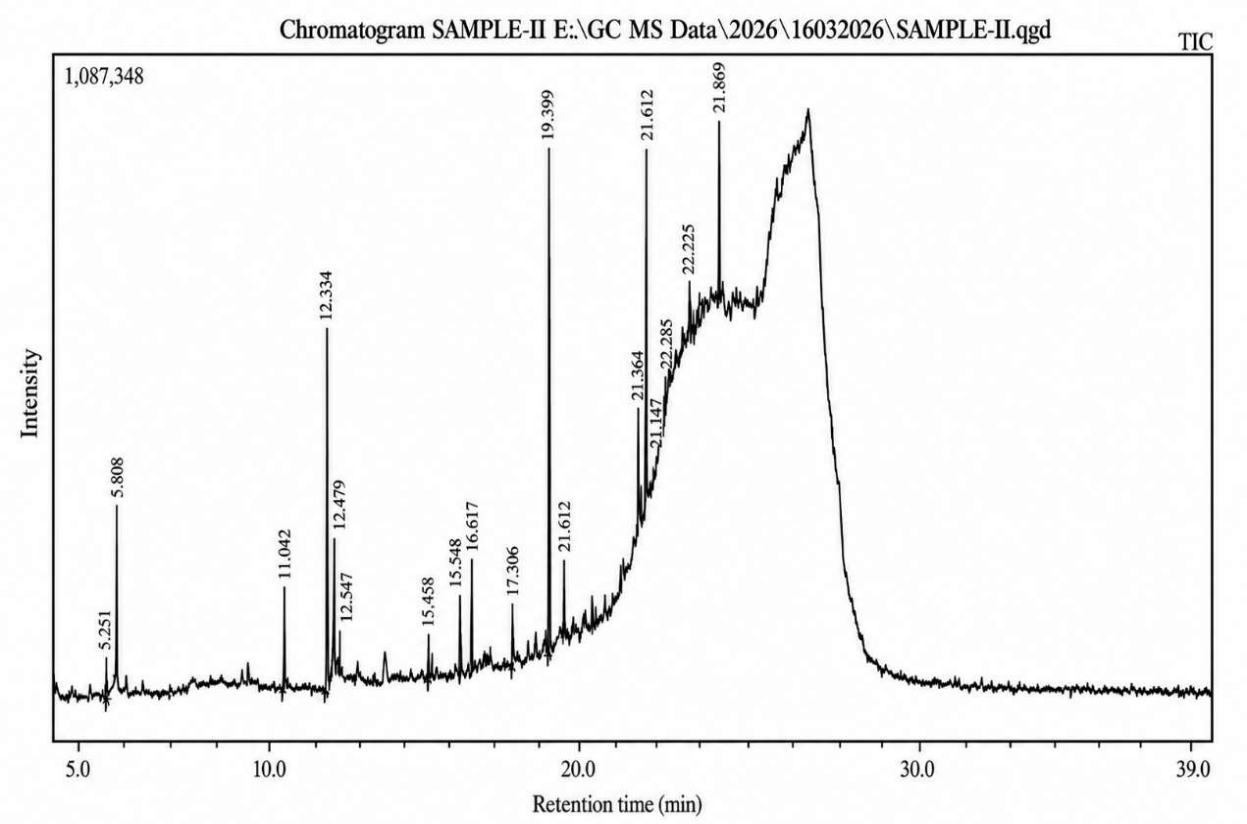


Figure 4: GC-MS analysis of an isolated compound from column chromatography (sample 2)



Conclusion:

The present investigation on the methanolic root extract of Achyranthes aspera demonstrated the presence of several important phytochemical constituents through preliminary phytochemical screening, UV–Visible spectroscopy, and GC–MS analysis. The phytochemical screening confirmed the presence of tannins, flavonoids, terpenoids, alkaloids, steroids, coumarins, phenolic compounds, and saponins, indicating that the plant root is rich in bioactive secondary metabolites known for their antioxidant, antimicrobial, anti-inflammatory, and therapeutic properties. UV–Visible spectroscopic analysis revealed characteristic absorption peaks at 268 nm in Sample 1 and at 234 nm and 270 nm in Sample 2, suggesting the presence of conjugated and aromatic chromophoric systems associated with $\pi \rightarrow \pi^*$ electronic transitions commonly observed in phenolic and unsaturated compounds. Furthermore, GC–MS analysis confirmed the chemical complexity of the extract by identifying numerous phytoconstituents belonging to different chemical classes such as alcohols, esters, phenols, ketones, siloxanes, steroids, hydrocarbons, and heterocyclic compounds. Sample 1 contained fourteen compounds, whereas Sample 2 exhibited twenty-two compounds, reflecting the rich phytochemical diversity of the plant extract. Several biologically important compounds were identified, including Hexadecyl acrylate, 1-Dodecanol, 2,4-Di-tert-butylphenol, Melezitose, trans-2-Dodecen-1-ol, dl-Menthol, Hexadecanoic acid methyl ester, pregnane derivatives, and phenolic derivatives, many of which are reported to possess antimicrobial, antioxidant, analgesic, anti-inflammatory, hepatoprotective, and wound-healing activities. The presence of flavonoids, phenolic compounds, alkaloids, terpenoids, and steroidal constituents highlights the significant therapeutic potential of the methanolic root extract. Additionally, the study scientifically validates the traditional medicinal uses of *A. aspera* and emphasizes the importance of chromatographic and spectroscopic techniques in the identification and characterization of plant-derived bioactive compounds. Overall, the

findings indicate that *Achyranthes aspera* root is a promising natural source of phytoconstituents with potential pharmaceutical and medicinal applications.

Materials and methods:

Plant material and preparation of the methanolic root extract of AA

Fresh roots of Achyranthes aspera were collected from the Saikheda village region of Pandurna, Madhya Pradesh, India. The botanical identity of the plant specimen was verified by a taxonomist at the Department of Botany, RTMNU, Nagpur, and the specimen was deposited at their institute under accession number 235 (Abhay et al.,2018). The plant was authenticated as Achyranthes aspera root, belonging to the family Amaranthaceae. The roots were washed with water two to three times to remove the soil. Subsequently, the roots were sun-dried for eight to ten days. After drying, the roots were cut into small pieces using a cutter and then powdered with a rotary mill, followed by sieving through a 10-mesh sieve. The powdered material was stored in an airtight container until further use. The powdered root was extracted using a Soxhlet apparatus with methanol as the solvent. The root powder to be extracted was placed inside the thimble and loaded into the main chamber of the Soxhlet extractor. The extraction solvent, methanol (500 ml), was placed in a distilled flask, and the flask and reflux condenser were placed on top of the extractor. Soxhlation was performed for 4–5 days. The Soxhlet extractor was then equipped with a condenser. The solvent was heated, and the solvent vapour travelled up a distillation arm and flooded the chamber housing the thimble of the solid. The chamber containing the solid material was slowly filled with a warm solvent. When the Soxhlet chamber was almost full, it was automatically emptied by a siphon side arm, with the solvent returning to the distillation flask. This cycle was repeated many times over the days. After extraction, the solvent was removed via distillation.

Preliminary phytochemical screening of extracts of Achyranthes aspera root

Qualitative tests were performed to identify the phytochemicals present in the methanol root extract of A. aspera. Alkaloids, tannins, flavonoids, terpenoids, steroids, saponins, and coumarins were determined using standard methods. The quantitative flavonoid methanol extract of A. aspera was analyzed using the lead acetate solution method (Venkadassalapathy et al., 2023), (Ogbole et al., 2020).

Isolation of chemical constituents

The extract was chemically analyzed using thin-layer chromatography (TLC). A modified method, previously described and employed by our group, was adopted for the TLC-based chemical detection. Pre-coated TLC plates were used, and the solvent system comprised n-hexane:acetone (6:4, v/v). After spraying, the plates were allowed to dry and subsequently examined under UV light at 245 and 365 nm. The TLC procedure was optimized to develop a stability-indicating assay method by evaluating several mobile phases. Ultimately, a mobile phase consisting of n-hexane: acetone (6:4, v/v) was selected because it provided well-resolved peaks for the sample constituents. Well-defined spots were observed when the development chamber was pre-saturated with the mobile phase for 20 min at room temperature (Hamidi et al., 2017), (ladda et al.,2018). The methanolic extract was subjected to column chromatography over silica gel (60-120 mesh). The column was eluted using a graded mixture of n-hexane and acetone (6:4 v/v). Different 2 fractions of elution were collected based on time. Similar fractions were based on their similarity in composition, deduced from TLC analyses, and labelled as Sample-1 and 2 (Arshad et al., 2021).

Characterization of isolated compounds

The synthesized compounds were characterized in detail using UV–Vis spectroscopy (Sharifi-Rad et al., 2020). The synthesis was monitored using UV–Vis absorption spectroscopy. The absorbance spectra of the reaction mixtures were acquired using a Shimadzu UV–Vis spectrophotometer (model UV-1900i, A12535981666) in the range of 200–800 nm at different time intervals. For the blank sample, methanol was considered (Walid Elsayed Abdallah et al., 2024).

GC-MS

GC–MS analysis of the methanolic extract of AA was performed using a capillary gas chromatography system, Trace GC ULTRA (Thermo Scientific), directly coupled to an ISQ Single Quadrupole mass spectrometer and equipped with an Rtx-5MS non-polar capillary column (30 m × 0.32 mm ID × 0.50 µm), following the operating conditions described by Swati (Reza et al., 2024). Furthermore, the AA root extract and isolated compound were analyzed using a GCMS-QP-2021 Plus instrument manufactured by Shimadzu Corporation, Japan. Chromatographic separation was achieved using a capillary column (30 m length × 0.32 mm × 0.50 µm) coated with 5% phenyl methylpolysiloxane (Kumari et al., 2022). The instrument was operated in the split injection mode with a split ratio of 25, and the injector temperature was maintained at 270 °C. Helium was used as the

carrier gas under a pressure of 11.6 kPa with a total flow rate of 41.0 mL/min (and a linear flow of 1.72 mL/min). The initial column temperature was set at 45 °C, followed by a programmed increase: held at 150 °C for 0 min, then raised to 200 °C with a 10 min hold, and finally increased to 270 °C with a 10 min hold. The ion source temperature was maintained at 200 °C, and the interface temperature was set at 260 °C to ensure efficient ionization and transfer of analytes for accurate mass spectral detection.

List of abbreviations:

A. aspera	Achyranthes aspera
AA	Achyranthes aspera
GC-MS	Gas Chromatography-Mass Spectrometry
UV-Vis	Ultraviolet-Visible Spectroscopy
TLC	Thin Layer Chromatography
RT	Retention Time
nm	Nanometer
mL	Milliliter
µm	Micrometre
v/v	Volume by Volume
°C	Degree Celsius
RTMNU	Rashtrasant Tukadoji Maharaj Nagpur University
H_2SO_4	Sulfuric Acid
NaOH	Sodium Hydroxide
TMS	Trimethylsilyl
UV	Ultraviolet
min	Minutes
m/z	Mass-to-Change Ratio
RT	Room Temperature
ISQ	Integrated Single Quadrupole

Declarations:

Author's declaration

The author utilized Chat GPT (https://openai.com/?utm_source=chatgpt.com) to assist with image editing and improve the readability of the manuscript.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare no competing interests

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Data availability

No datasets were generated or analyzed during the current study.

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