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Comprehensive Phytochemical Profiling of *Andrographis paniculata* (Burm. f.) Wallex Nees using Integrated GC–MS and HPTLC Approaches

[1]Shruti M. Parmar, [2]R. N. Nakar and [3]K. Ishnava

[1]Department of Microbiology, [2]Department of Botany,
[1]Email: shrutiparmar348@gmail.com
Mo. 8488026959
Sheth P.T. Arts and Science Collage, Godhra,
Affiliated with Shri Govind Guru University, Godhra - 389001 (India)
[2]Email: drrupeshresearch@gmail.com
Mo. 8141431462
[3]Department of Biosciences, Sardar Patel University,
Satellite Campus, Vadtal Road, Bakrol - 388315 (India)
Phone: (02692) 234412/234402 (O) 9824918606 (M)
https://ishnavakalpesh.wordpress.com
[3]Email - kalpeshishnava@spuvvn.edu**.,**
ishnavakb203@yahoo.com
Corresponding author: [1]Shruti M. Parmar
shrutiparmar348@gmail.com

Abstract

Andrographis paniculata (Burm. f.) Wall ex Nees is a widely recognized medicinal plant known for its extensive use in traditional systems of medicine. The present study aimed to investigate the phytochemical profile of the ethanolic extracts of its root, stem, and leaves using High-Performance Thin Layer Chromatography for mobile phase used for development was a mixture of toluene:ethyl acetate:formic acid in the ratio 5:3.5:0.5 (v/v/v). (HPTLC) and Gas Chromatography–Mass Spectrometry (GC–MS) analysis. HPTLC profiling revealed disti patterns for each plant part, confirming the presence of diverse bioactive constituents and enabling comparative phytochemical evaluation. GC–MS analysis further identified several phytoconstituents, including diterpenoids, fatty acids, alcohols, ester derivatives, and other aliphatic compounds. Notably, six novel compounds were detected across the root, stem, and leaf extracts, indicating previously underexplored chemical

[1]Ph.D Research Scholar, [2]Assistant Professor & Ph.D. Research Supervisor & Guide,
[3]Associate Professor in Botany

diversity. the traditional use of *Andrographis paniculata* and highlight its potential as a promising source of bioactive molecules. The study emphasizes the importance of advanced phytochemical characterization for future pharmacokinetic, mechanistic, and drug development research.

Key words : GC–MS, High-Performance Thin Layer Chromatography, secondary metabolites, hepatoprotective.

Andrographis paniculata (Burm. f.) Wall. ex Nees (fam. Acanthaceae), commonly known as "king of bitters," is a medicinal plant traditionally utilized across Asia, particularly in Indian (Ayurvedic) and Chinese herbal medicine systems. Its wide therapeutic applications include treatment of respiratory infections, fever, gastrointestinal disorders, and inflammation. These pharmacological effects have been attri-buted to a diverse array of secondary metabolites, including diterpenoid lactones (*e.g.*, andrograph- olide), flavonoids, and phenolic compounds, which exhibit antimicrobial, antiviral, anti-inflammatory, hepatoprotective, and anticancer activities[9]. Phytochemical investigations have demonstrated that *Andrographis paniculata* contains a diverse array of secondary metabolites, including labdane diterpenoid lactones, flavonoids, and various other phytoconstituents. These compounds collectively contribute to its broad spectrum of pharmacological activi-ties[6,8]. Various ailments, including infectious diseases, febrile conditions, colic pain, loss of appetite, irregular bowel movements, and diarrhea[12]. Since its emergence in late 2019, coronavirus disease (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has posed a major global health challenge. The rapid surge in critically ill patients has significantly contributed to increased morbidity and mortality rates. As of May 2022, more than 500 million confirmed cases and over 6 million deaths have been reported worldwide. (WHO)[13].

The chemical and biological attributes of *Andrographis paniculata*, particularly its demonstrated antiviral activity against SARS-CoV-2, evidence from clinical trials on upper respiratory tract infections (URTI), and established safety profile, collectively highlight its potential as a promising natural candidate for post-infectious COVID-19 treatment, rather than for preventive (prophylactic) use[5].

A total of 344 compounds have been identified from *Andrographis paniculata*, including terpenoid lactones, flavonoids, phenolic acids, triterpenes, and various volatile constituents[7]. Notably, more than half of these compounds have not yet been studied for their pharmacological activities, highlighting significant untapped therapeutic potential. This research article aims to provide comprehensive, accurate, and evidence-based information on the phytochemistry, pharmacological properties, and phytoconstituents of *Andrographis paniculata* in its ethanolic extract. phytochemical screening provides critical insights into the bioactive constituents of medicinal plants, thereby uncovering their pharmacological potential and facilitating the discovery of novel therapeutic agents[3,11]. Phytochemical screening

extends beyond cataloging the diversity of plant-derived compounds to emphasizing their pharmaceutical relevance and therapeutic promise[1,3]. The application of advanced analytical methods has greatly enhanced the ability to elucidate chemical structures, determine functional groups, and investigate the mechanisms by which these bioactive molecules interact with biological systems.

HPTLC is one of the most robust, sensitive, and accurate chromatographic techniques used for the quality-based standardization of medicinal plants. The HPTLC analysis of *Andrographis paniculata* generated distinct chromatographic profiles, reflecting the complexity of their phytochemical compositions. These profiles serve as unique fingerprints and provide a reliable basis for plant authentication and standardization. *Andrographis paniculata* has been reported to contain numerous secondary metabolites with a wide range of therapeutic applications. In this study, an attempt was made to identify the bioactive constituents present in the ethanolic extracts of the separate plant powder.

Gas Chromatography–Mass Spectrometry (GC–MS) is a powerful analytical technique that combines the separation capability of gas chromatography with the identification potential of mass spectrometry. It enables the detection, identification, and quantification of volatile and semi-volatile compounds with high sensitivity and specificity, making it an essential tool in phytochemical analysis and natural product research. In the present study, GC–MS analysis was employed to investigate the phytochemical profile of *Andrographis paniculata*

This research article is focused on its medicinal properties, phytochemistry, chemical profiling of compounds of *Andrographis paniculata* of ethanolic extract using GC–MS and HPTLC analysis.

Plant collection :

A. paniculata was also collected at Ajod Vruksh Mandir, Vadodara, during both winter and summer. Following collection, the plants, ensured to be mature, fresh, healthy, and free from disease—were thoroughly washed first with tap water and then with distilled water. The plants should be dried in a shady spot with good air circulation so that later on, completely dried leaves, stems, and roots were separated. It was dried and homogenized into a fine powder by using a grinder and stored in an airtight dry container because it preserves the plant material for a long period for further use.

Extraction :

Maceration is a simple extraction technique used to isolate bioactive compounds from plant materials by soaking them in a suitable solvent at room temperature. During the process, the solvent penetrates the cell wall and dissolves the soluble phytoconstituents, which then diffuse into the solvent. The extract is subsequently separated by filtration, and the solvent (ethanol) may be evaporated to obtain the concentrated crude extract.

The methodologies described by Harborne[4] were followed for preparing ethanolic, and methanolic, extracts from *Swertia chirata* and *Andrographis paniculata*. Leaves, stems, and roots of *Swertia chirata* were subjected to cold maceration techniques to

extract the active constituents. Air-dried and finely ground plant materials (leaves, stems, and roots) were separately extracted using methanol and ethanol through cold maceration. Approximately 50g of powdered with a grinder (Maharaja Mixer-Grinder, India). Plant material was mixed with 250ml of the respective solvent in a conical flask and incubated at 37°C for 72 hours. The flasks were kept on a shaker throughout the extraction period to ensure continuous agitation. After 72 hours, the mixtures were filtered using Whatman No. 1 filter paper. The obtained filtrates were then concentrated using a rotary evaporator at 40°C to remove the solvents. The resulting dried extracts were carefully scraped off, transferred into sterile containers, and stored in a refrigerator until further use. The yields were calculated using the formula. Diagram of samples preparation to samples analysis of various biological assay mentioned in Fig. a.

% Yield = (Weight of crude extract / Weight of plant powder) × 100

HPTLC (High-Performance Thin Layer Chromatography) instrumentation :

The HPTLC system (CAMAG, Switzer land) is consisted of a Linomat 5 automatic sample applicator, a TLC Scanner III equipped with WinCATS software, a 100µl Hamilton syringe, and a nitrogen gas supply. Chromato graphy was carried out on pre-coated silica gel 60 F_{245} TLC plates (E. Merck, Germany). The TLC plates were pre-washed with methanol and thoroughly air dried before use. Samples were applied as bands 10.0 mm wide using the Linomat 5 applicator. The mobile phase used for development was a mixture of toluene:ethyl acetate:formic acid in the ratio 5:3.5:0.5 (v/v/v). Prior to development, the CAMAG twin-trough chamber was saturated with the mobile phase. After development, the plates were air-dried and analyzed using the CAMAG TLC Scanner III. The chromatographic bands were visualized using the CAMAG Visualizer, and images were captured under UV light at 254 nm and 366 nm.

GC–MS (Gas Chromatography–Mass Spectrometry) :

Gas Chromatography–Mass Spectrometry (GC–MS) was employed to separate and identify the bioactive constituents responsible for antibacterial activity. The analysis was performed at the Sophisticated Instrumentation Centre for Applied Research and Training (SICART), Vallabh Vidyanagar, Gujarat, using the electron impact ionization (EI) method. An AutoSystem XL Gas Chromatograph (Perkin Elmer Instruments, Germany) coupled with a TurboMass Mass Spectrophotometer (Perkin Elmer Instruments, Germany) was used for the analysis.The GC separation was carried out on an RTx-5MS capillary column (30 m × 0.25 mm i.d. × 0.5 µm film thickness). The oven temperature program was as follows: initial temperature of 80 °C (held for 3 min), increased to 250 °C at 10 °C/min, followed by a further ramp to 280 °C at 15 °C/min, and held at this final temperature for 23 minutes. The injection port was maintained at 250 °C, and a 2 µL sample was injected in split mode with a split ratio of 10:1. Helium was used as the carrier gas at a flow rate of 1.21 mL/min. Mass spectral detection was carried out in electron ionization (EI) mode. The identification of chemical constituents was achieved by comparing the obtained mass spectra with those available in the NIST 107 mass spectral library database.

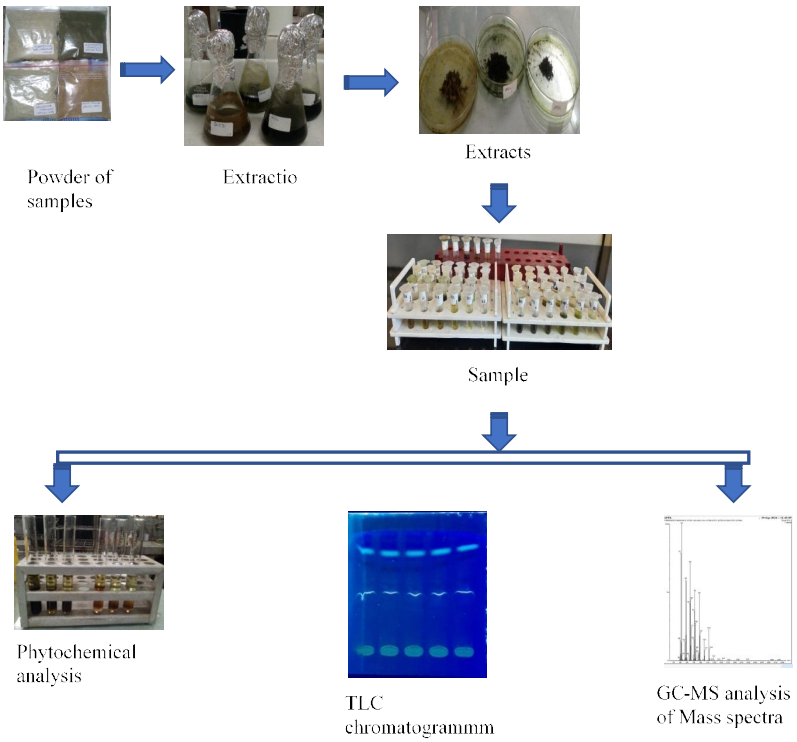

Fig. 1. Diagram of samples preparation to samples analysis of various biological assay

HPTLC :

The present study was undertaken to develop and validate a sensitive, rapid, and reproducible high-performance thin-layer chromatography. HPTLC method for the analysis of the roots, stems, and leaves of *Andrographis paniculata*. HPTLC (chromatogram) for 3 samples denoted (Figures 1 to 4) by tracks 1 to 3, there with table 1 to 3 for APEL, APES, APER.

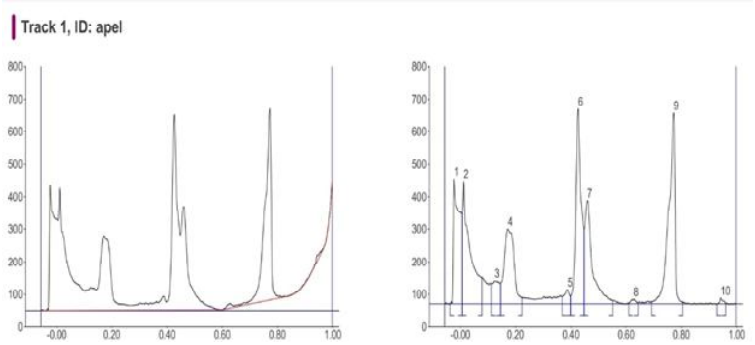

Fig. 2 HPTLC chromatogram for APEL

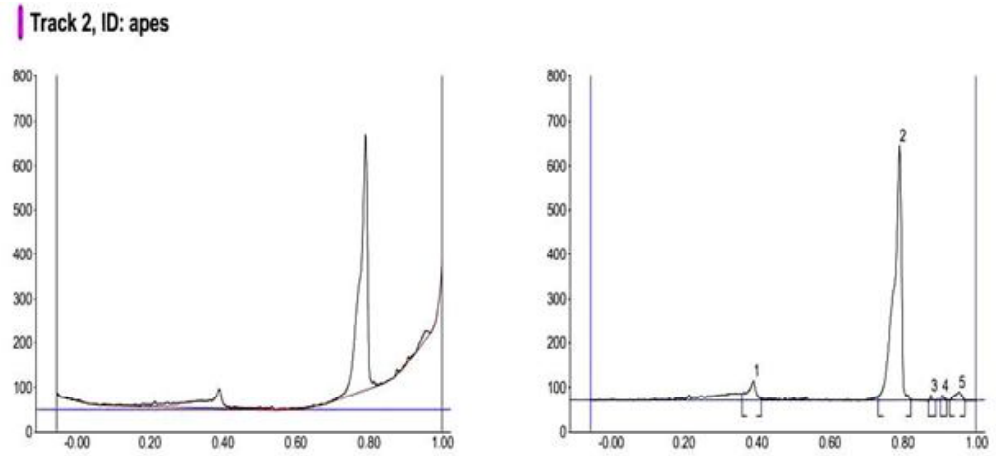

Fig. 3. HPTLC chromatogram for APES

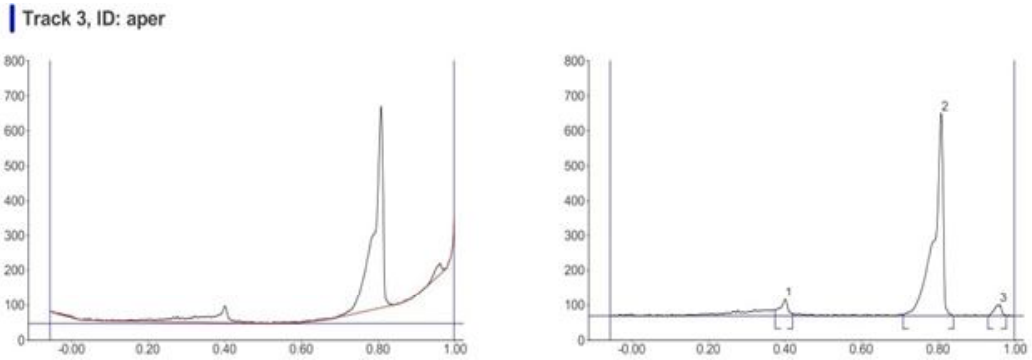

Fig. 4. HPTLC chromatogram for APER

Table-1. HPTLC chromatogram for APEL peak and Rf values

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.04	0.4	-0.02	384.2	14.51	0.01	276.8	8843.3	13.41	unknown *
2	0.01	283.7	0.01	377.7	14.26	0.08	80.6	10470.4	15.88	unknown *
3	0.11	62.1	0.12	71.0	2.68	0.14	64.1	1950.0	2.96	unknown *
4	0.15	64.8	0.17	229.6	8.67	0.22	19.6	8624.3	13.08	unknown *
5	0.37	23.6	0.39	43.6	1.65	0.40	25.7	893.1	1.35	unknown *
6	0.40	27.7	0.43	600.3	22.67	0.45	231.3	11867.1	18.00	unknown *
7	0.45	231.7	0.46	319.0	12.05	0.55	11.4	9054.2	13.73	unknown *
8	0.61	2.8	0.63	14.9	0.56	0.64	3.9	280.1	0.42	unknown *
9	0.69	6.2	0.77	588.0	22.21	0.81	5.5	13704.5	20.79	unknown *
10	0.93	0.1	0.94	19.4	0.73	0.96	2.8	234.9	0.36	unknown *

Table-2. HPTLC chromatogram for APES peak and Rf values

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.36	16.0	0.39	44.1	6.64	0.41	4.6	1007.8	7.37	unknown *
2	0.73	9.2	0.79	575.5	86.79	0.82	1.8	12110.2	88.57	unknown *
3	0.87	0.1	0.88	11.2	1.69	0.89	0.0	67.4	0.49	unknown *
4	0.90	1.4	0.91	12.3	1.85	0.92	1.8	89.6	0.66	unknown *
5	0.93	0.3	0.95	20.1	3.03	0.97	0.5	397.3	2.91	unknown *

Table-3 HPTLC chromatogram for APER peak and Rf values

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.38	16.6	0.40	46.3	7.07	0.42	7.2	951.9	6.03	unknown *
2	0.71	3.5	0.81	577.8	88.27	0.84	0.3	14229.3	90.07	unknown *
3	0.93	0.9	0.96	30.5	4.66	0.98	0.9	617.3	3.91	unknown *

The chromatogram of APEL (Figure b and table-1) shows the separation profile of the alcoholic extract, with distinct peaks observed across the Rf range. Ten prominent peaks were detected, indicating the presence of multiple phytoconstituents. Peaks at Rf values was around 0.38 (peak 6) particularly sharp and intense, suggesting a higher concentration of specific compound. The chromatogram of APES (Figure c and Table-2) illustrated the separation profile of the alcoholic extract, revealing distinct peaks across the Rf range. A total of five prominent peaks were detected, indicating the presence of multiple phytoconstituents. Notably, the peak at an Rf value around 0.73 (Peak 2) was particularly sharp and intense, with a maximum height of 575.5 and representing 88.57% of the total area, suggesting a significantly higher concentration of a specific compound compared to others. The chromatogram of APER (Figure d and table-3) demonstrated the separation profile of the alcoholic extract, displaying distinct peaks across the Rf range. Three major peaks were detected, indicating the presence of multiple phytoconstituents. Among them, the peak at an Rf value around 0.81 (Peak 2) was notably sharp and intense, with a maximum height of 577.8 and contributing 90.07% to the total area, suggesting a predominant compound in the extract.

According to Pawar *et al*.,[10] study of *Andrographis paniculata* in determination of andrographanoide with mobile phase (toluene: ethyl acetate: formic acid), found Rf value of 0.38. However, 3 samples (APEL, APES and APER) from *Andrographis paniculata* with same mobile phase gave Rf values of 0.37, 0.36 and 0.38, respectively which was with dark gray colour (0.38±0.02) respectively. Among the various mobile phases tested, the

combination of Toluene: Ethyl acetate: Formic acid (5:3.5:0.5, v/v) effectively separated the active compound Andrographolide, which appeared as a dark grey band at an Rf value of 0.38, from other constituents present in the ethanolic extracts extract of *Andrographis paniculata* (whole plant). These values closely aligned with the reference Rf, suggesting a high probability that the compound identified in the samples is andrographolide.

GC–MS :

GC–MS (Gas Chromatography–Mass Spectrometry) analysis is carried out because it is one of the most powerful techniques for identifying, separating, and quantifying chemical compounds in complex. The compounds present in the ethanolic extract of *Andrographis paniculata* were identified by GC-MS analysis showed in table 4 - 6.

Table-4. The GC-MS profile of compounds identified in ethanol leaf extract of *Andrographis paniculata*

Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	936	906	3,7,11,15-TETRAMETHYL-2-HEX ADECEN-1-OL	296	C20H40O	102608-53-7
2	908	88	PHYTOL	296	C20H40O	150-86-7
3	903	880	1-OCTADECYNE	250	C8H34	629-89-0
4	899	889	1-OCTADECYNE	250	C18H34	629-89-0
5	898	838	HEPTADECANAL	254	C17H34O	900376-70-0
6	895	835	Z-2-OCTADECEN-1-OL	268	C18H36O	900131-11-0
7	895	846	18-NONADECEN-1-OL	282	C19H38O	900142-89-2
8	893	833	HEXADECANAL	240	C16H32O	629-80-1
9	892	861	E-2-TETRADECEN-1-OL	212	C14H28O	900130-83-7
10	891	876	1-HEXADECYNE	222	C16H30	629-74-3
11	890	831	PENTADECANAL-	226	C15H30O	2765-11-9
12	886	813	OCTADECANAL	268	C18H36O	638-66-4
13	885	826	HEXADECANAL	240	C16H32O	629-80-1
14	883	872	1-HEPTADECYNE	236	C17H32	26186-00-5
15	882	825	CIS-9,10-EPOXYOCTADECAN-1-OL	284	C18H36O2	13980-12-6
16	881	870	1-HEXADECYNE	222	C16H30	629-74-3
17	881	822	TETRADECANAL	212	C14H28O	124-25-4 18
18	881	808	OCTADECANAL	268	C18H36O	638-66-4
19	880	756	16-HEPTADECENAL	252	C17H32O	900144-57-9
20	879	786	BICYCLO[4.1.0]HEPTANE, 7-UTYL-	152	C11H20	18645-10- 8
21	943	918	N-HEXADECANOIC ACID	256	C16H32O2	57-10-3
22	924	894	N-DECANOIC ACID	172	C10H20O2	334-48-5
23	924	866	UNDECANOIC ACID	186	C11H22O2	112-37-8
24	916	901	UNDECANOIC ACID	186	C11H22O2	112-37-8

25	906	870	PENTADECANOIC ACID	242	C15H30O2	1002-84-2
26	904	877	TRIDECANOIC ACID	214	C13H26O2	638-53-9
27	903	855	OCTADECANOIC ACID	284	C18H36O2	57-11-4
28	899	864	N-DECANOIC ACID	172	C10H20O2	334-48-5
29	895	85	NONADECANOIC ACID	298	C19H38O2	646-30-0
30	894	868	N-DECANOIC ACID	172	C10H20O2	334-48-5
31	894	813	EICOSANOIC ACID	312	C20H40O2	506-30-9
32	891	847	HEPTADECANOIC ACID	270	C17H34O2	506-12-7
33	888	854	N-DECANOIC ACID	172	C10H20O2	334-48-5
34	887	847	TRIDECANOIC ACID	214	C13H26O2	638- 53-9
35	887	859	DODECANOIC ACID	200	C12H24O2	143-07-7
36	885	834	PENTADECANOIC ACID	242	C15H30O2	1002-84-2
37	883	858	N-DECANOIC ACID	172	C10H20O2	334-48-5
38	882	829	NONADECANOIC ACID	298	C19H38O2	646-30-0
39	880	827	OCTADECANOIC ACID	284	C18H36O2	57-11-4
40	876	826	N-HEXADECANOIC ACID	256	C16H32O2	57-10-3

Table-5. The GC-MS profile of compounds identified in ethanol stem extract of *Andrographis paniculata*

Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	944	909	N-HEXADECANOIC ACID	256	C16H32O2	57-10-3
2	927	883	N-DECANOIC ACID	172	C10H20O2	334-48-5
3	914	880	TRIDECANOIC ACID	214	C13H26O2	638-53-9
4	913	871	PENTADECANOIC ACID	242	C15H30O2	1002-84-2
5	912	882	UNDECANOIC ACID	186	C11H22O2	12-37-8
6	909	850	OCTADECANOIC ACID	284	C18H36O2	57-11-4
7	906	833	UNDECANOIC ACID	186	C11H22O2	112-37-8
8	900	813	EICOSANOIC ACID	312	C20H40O2	506-30-9
9	898	844	N-DECANOIC ACID	172	C10H20O2	334-48-5
10	898	847	HEPTADECANOIC ACID	270	C17H34O2	506-12-7
11	898	852	NONADECANOIC ACID	298	C19H38O2	646-30-0
12	897	849	TRIDECANOIC ACID	214	C13H26O2	638-53-9
13	896	860	DODECANOIC ACID	200	C12H24O2	143-07-7
14	893	836	PENTADECANOIC ACID	242	C15H30O2	1002-84-2
15	892	849	N-DECANOIC ACID	172	C10H20O2	334-48-5
16	888	838	N-DECANOIC ACID	172	C10H20O2	334-48-5
17	887	829	OCTADECANOIC ACID	284	C18H36O2	57-11-4
18	887	849	N-DECANOIC ACID	172	C10H20O2	334-48-5

19	887	829	NONADECANOIC ACID	298	C19H38O2	646-30-0
20	885	829	N-HEXADECANO	256	C16H32O2	57-10-3
21	933	873	OLEIC ACID	282	C18H34O2	112-80-1
22	922	810	4-TETRADECENE, (E)-	196	C14H28	41446-78-0
23	920	809	7-TETRADECENE	196	C14H28	10374-74-0
24	919	805	ERUCIC ACID	338	C22H42O2	112-86-7
25	919	775	8-HEPTADECENE,1-CHLORO-	272	C17H33Cl	56554-80-4
26	919	832	2-TRIDECENAL, (E)-	196	C13H24O	7069-41-2
27	918	806	4-TETRADECENE, (Z)-	196	C14H28	41446-65-5
28	915	802	HEXADECENOIC ACID, Z-11-	254	C16H30O2	2416-20-8
29	915	781	7-HEPTADECENE, 1-CHLORO-	272	C17H33Cl	56554-78-0
30	913	817	4-TRIDECENE, (Z)-	182	C13H26	41446-54-2
31	911	770	7-HEPTADECENE, 17-CHLORO-	272	C17H33Cl	56554-79-1
32	903	787	E-2-HEXADECACEN-1-OL	240	C16H32O	900131-10-1
33	902	791	9-HEXADECENOIC ACID	254	C16H30O2	2091-29-4
34	902	786	CIS-2-METHYL-7-OCTADECENE	266	C19H38	35354-39-3
35	900	783	E-11-TETRADECENOIC ACID	226	C14H26O2	900130-96-2
36	898	802	Z-8-METHYL-9-TETRADEC-ENOIC ACID	240	C15H28O2	900130-84-5
37	897	768	3-DODECENE, (Z)-	168	C12H24	7239-23-818
38	896	789	9-EICOSENOIC ACID, (Z)-	310	C20H38O2	29204-02-2
39	892	801	2-DODECENAL, (E)-	182	C12H22O	20407-84-5
40	888	774	3-DODECENE, (Z)-	168	C12H24	7239-23-

Table-6. The GC-MS profile of compounds identified in ethanol root extract of *Andrographis paniculata*

Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	935	897	N-HEXADECANOIC ACID	256	C16H32O2	57-10-3
2	921	877	N-DECANOIC ACID	172	C10H20O2	334-48-5
3	903	877	UNDECANOIC ACID	186	C11H22O2	112-37-8
4	900	837	UNDECANOIC ACID	186	C11H22O2	112-37-8
5	897	852	PENTADECANOIC ACID	242	C15H30O2	1002-84-2
6	896	847	N-DECANOIC ACID	172	C10H20O2	334-48-5
7	891	854	TRIDECANOIC ACID	214	C13H26O2	638-53-9
8	889	850	N-DECANOIC ACID	172	C10H20O2	334-48-5
9	889	829	OCTADECANOIC ACID	284	C18H36O2	57-11-4
10	888	806	EICOSANOIC ACID	312	C20H40O2	506-30-9

11	885	838	N-DECANOIC ACID	172	C10H20O2	334-48-5
12	881	829	NONADECANOIC ACID	298	C19H38O2	646-30-0
13	881	841	N-DECANOIC ACID	172	C10H20O2	334-48-5
14	880	831	TRIDECANOIC ACID	214	C13H26O2	638-53-9
15	880	824	HEPTADECANOIC ACID	270	C17H34O2	506-12-7
16	877	841	DODECANOIC ACID	200	C12H24O2	143-07-7
17	874	811	NONADECANOIC ACID	298	C19H38O2	646-30-0
18	873	812	PENTADECANOIC ACID	242	C15H30O2	1002-84-2
19	870	809	N-HEXADECANOIC ACID	256	C16H32O2	57-10-3
20	869	806	OCTADECANOIC ACID	284	C18H36O2	57-11-4

In the present study, GC-MS analysis revealed the presence of various phytochemial constituents in two plant samples which accounted for the pharmacological activity of the plant. The identification of the phytoconstituents was conformed based on peak, peak area in percentag, retention time and molecular formula are shown in table 4 to 6. The GC-MS chromatographic analysis of the ethanolic extract of *Andrographis paniculata* leaves revealed a total of 20 prominent peaks Among these, peak 16.25 (min) and peak 17.56 (min) were subjected to detailed mass spectral analysis presented in table 4. From these two peaks alone, a total of 40 chemical constituents were identified. However, several novel compounds were found to be repeated**.** Many of the compounds showed antibacterial, antimicrobial, antifungal agents. In this observation, 27 bioactive compounds namely 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, phytol, 1-Octadecyne, Heptadecanal, Z-2-octadecen-1-ol, 18-Non-adecen-1-ol, Hexadecanal E-2-tetradecen-1, 1-Hexadecyne, Pentadecanal-, Octadecanal, 1-Heptadecyne, CIS-9,10-Epoxyoctadecan-1-ol, Tetradecanal, 16-Heptadecenal, Bicyclo [4.1.0] heptanes, 7-butyl, N- hexadecanoic acid, N-decanoic acid, Undecanoic acid, Pentadecanoic acid, Tridecanoic acid, Octadecanoic acid, Non- adecanoic acid O, Eicosanoic acid, Heptadecanoic acid, Dodecanoic acid etc were identified.

Gas Chromatography–Mass Spectrometry (GC-MS) analysis of the ethanolic extract of *Andrographis paniculata* stem revealed 12 chromatographic peaks. Among these, the peaks at retention times 17.54 minutes and 19.22 minutes were subjected to detailed mass spectral analysis. From these two peaks alone, a total of 40 chemical constituents were identified; however, several compounds appeared repeatedly across both spectra in table 5. Some compounds showed various types of biological activities. The list denoted 29 active constituents such as, N-hexadecanoic acid, N-decanoic acid, Pentadecanoic acid, Undecanoic acid, Octadecanoic acid, Eicosanoic acid, Heptadecanoic acid, Nonadecanoic acid, Onadecanoic acid, Dodecanoic acid, Oleic acid, 4-Tetradecene (E)-, 7-Tetradecene, Erucic acid, 8-Heptadecene, 1-Chloro-, 2-Tridecenal (E)-, 4-Tetradecene (Z)-, Hexadecenoic acid-11, 7-Heptadecene, 1-Chloro 4- Tridecene, (Z), 7-Heptadecene,

17-Chloro- E-2-Hexadecacen-1-OL, 9-Hexadecenoic acid, CIS- 2-Mithyl-7-Octadecene, E-11-Tetradecenoic acid, Z-8-Methyl-9-Tetradecenoic acid, 3- Dodecene(Z), 9-Eicosenoic acid, (Z)-, 2-Dodecenal, (E)-,3-DODECENE,(Z)-.

Gas Chromatography–Mass Spectrometry (GC-MS) analysis of the ethanolic extract of *Andrographis paniculata* root in the presence of 17 distinct chromatographic peaks, indicating the presence of multiple bioactive compounds. Among the 17 chromatographic peaks detected in the GC-MS analysis. Peaks were observed at retention time 17.54 minutes and 22.63 minutes were selected for detailed mass spectral analysis. From these two peaks alone, a total of 40 chemical constituents were identified, which was presented in table-6. However, several compounds were found to be common to both spectra, indicating overlapping constituents or fragment similarities. Identified compound name are N-hexadecanoic acid, N-decanoic acid, Undecanoic acid, Pentadecanoic acid, Tridecanoic acid, Octadecanoic acid, Eicosanoic acid, Nonadecanoic acid, Heptadecanoic acid, Dodecanoic acid, 2-Propylhept-3-enoic acid, Phenylthio ester, Cyclohexanecarboxylic acid, 4-Propyl-,2,3-dicyano-4-(Pentyloxy)phen, Dimethylmalonic acid, Nnonyl 2,4,6-trichlorophenyl, ester, Cyclohexanecarboxilic acid,4-propyl-, 4-Cyanophenyl ester, Trans-cyclohexanecarboxylic acid, ANS-, 4-Propyl-, 2,3-dicyano-4-ethoxy-phenyle, Tetrahydroedulan cyclohexanecarboxylic acid, -2,3-dicynophenyle isophthalic acid, Decyl methyl ester,2,5-tris-o-(trifluoracetyl)-3- deoxy-.alpha.-dl-threo-pentofuranose, 3-desoxy-1,2,5- tris-o-(trifluoracetyl)-. beta.dl-threo-pentofuranose, Cyclopentane, 1-Butyl-2-pentyl-, D-(-)-erythrose,tris(trifluoroacetate) (isomer 1), D-(-)-Erythrose,Tris(trifluoroacetate) (isomer2), L-(+)-threose, tris(trifluoroacetate) (isomer1), Dodecanoic acid, 1, 1'-biphenyl-4-ylcarbonyl-methyl ester, 6-tetradecanol acetate, 3 – Butyryl -6 – propyl - 2, 3 - dihydropyran-2, 4-dione, N- [2- cyclododecylaminoethyl] aziridine, Cyclohexane,1,2,3,4,5,6hexaethyl-, 5-Cyclohexadiene-1,4- Dione, 2,5-Dihydroxy-3-methyl-6-(1-methylethyl)-.

Some novel compounds reported from ethanolic extracts (root, stem and leves) of *Andrographis paniculata* include **3,7,11,15-tetramethyl-2-hexadecen-1-ol, cis-9,10-epoxyoctadecan-1-ol, bicyclo [4.1.0] heptane, 7-butyl-, Z-8-methyl-9-tetradecenoic acid, cyclohexanecarboxylic acid, 4-propyl-, 4-cyanophenyl ester, trans-, and 3-desoxy-1,2,5-tris-O-(trifluoroacetyl)-β-DL-threo-pentofuranose,** which belong to diterpenoids, alcoholic compounds, aliphatic acids, ester derivatives, and poly fluoroalkyl substances. These compounds have been associated with antimicrobial, antibacterial, antioxidant, anti-inflammatory, anticancer properties, and potential pharmaceutical applications.

Despite centuries of ethnomedicinal use, *Andrographis paniculata* still holds significant untapped potential in modern drug discovery and development. In this research study, the roots of *Andrographis paniculata* were found to contain the highest number of secondary metabolites (32). In this study, six novel compounds were identified in the ethanolic extracts of the root, stem, and leaves of Andro-graphis paniculata, highlighting the

plant's rich phytochemical diversity and its potential for further pharmacological exploration**.** With the growing global interest in phytopharmaceuticals and integrative medicine, systematic research integrating phytochemistry, molecular biology, and clinical validation could facilitate the development of novel therapeutic agents derived from this potent medicinal plant.

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The first author SP performed data creation, formal analysis, investigation, methodology, and writing—original draft; co-author RN executed supervision, conceptualization, and writing—review and editing; co-author KI contributed in supervision and review.

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