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SCREENING AND CHARACTERIZATION OF BIOACTIVE PHYTOCOMPOUNDS FROM ANDROGRAPHIS PANICULATA

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Abstract

To identify and characterize the phytoconstituents present in the different solvent systems of *Andrographis paniculata* extracts. *Andrographis paniculata* extracts were extracted from different solvents like acetone, methanol, and chloroform and petroleum ether by soxhlet extraction. The qualitative and quantitative phytochemical analyses were carried out. GC-MS analysis was performed to identify the bioactive compounds present in each extraction methods. In qualitative phytochemical analysis showed the presence of resins, flavonoids, tannins, alkaloids, carboxylic acids, proteins, and carbohydrates. Total flavonoids (2.13 mg/g), lipids (1.97 mg/g), and proteins (5.53 mg/g) are high in acetone, whereas total tannins (0.51mg/g), alkaloids (85.48 mg/g) are found to be high in chloroform extract. In GCMS analysis Andrographis *paniculata extracts*

revealed the presence of the important phytoconstituent Andrographolide in the methanol extraction, 1-Heptatriacotanol was obtained as the lead compound in acetone extract. Octadec-9-enoic acid was obtained from chloroform extract. 1,1,4,7-tetramethyldecahydro-1h-cyclopropa[e]azulen-4-ol was obtained in methanol and squalene was found in petroleum ether extractions. In our study, qualitative, quantitative and GC-MS analysis showed the presence of important therapeutic phytocompounds. These phytocompounds play an vital role in the treatment and prevention of various disease conditions.

Keywords: *Andrographis paniculata,* Andrographolide, GC-MS

Introduction

Andrographis paniculata belongs to the family Acanthceae, Kingdom Plantae and Genus Andrographis. *Andrographis paniculata* commonly called as "King of Bitters" and locally called as "Siriyanangai". It contains more than 40 species, out of these only few species are known to the common people. From the Ancient periods people are using this plant as a drug resource to treat various diseases. Origin of this plant is India and SriLanka, but it is widely present in Southeast Asia, United States of America, China and also in Christmas Island. The entire parts of the plant include stem, leaf, flower, fruits, seeds including roots has been used as a therapeutic agent in medicine (Okhuarobo et al., 2014). Various research works has concluded that *Andrographis paniculata* posses various activity like anti-microbial, anti-diabetic (Abas et al., 2016), anti-inflammatory, anti-oxidant and anti-angiogenic activity (Okhuarobo et al., 2014). The entire plant has been using to treat snake-bite and other poisonous bite. The aerial parts of the *Andrographis paniculata* have been used to treat malaria, hypertension and urinary tract infection. Research found that *Andrographis paniculata* also used to treat respiratory infections (Dirar et al., 2019). Phytoconstituents of *Andrographis paniculata* has anti-viral activity against dengue type 2 and 4, hepatitis virus, influenza virus (Kaushik et al., 2021) and chikungunya (Jain et al., 2020). The secondary compound named Andrographolide obtained from the *Andrographis paniculata* has a important role in inhibiting the DNA replication in Dengue type 2 virus (Kaushik et al., 2021). Ethanolic extract of *Andrographis paniculata* polyherbal formulation holds anti-viral property against chikungunya (Jain et al., 2020). Methanolic extract of *Andrographis paniculata* has anti-viral property against DENV-1(Tang et al., 2012). *Andrographis paniculata* maintains the cell morphology without causing cytopathic effects (CPE) (Tang et al., 2012). The phytochemical component Flavonoids in *Andrographis paniculata* has great anti-viral property (Özçelik et al., 2011). The important

phytoconstituents named Andrographolide has good binding and inhibiting activity against various viral diseases by inhibiting the DNA replication (Paemanee et al., 2019). Apart from these studies *Andrographis paniculata* will have some new compounds and other secondary compounds that uniquely extracted from other solvents. To identify those compounds in this study, we have performed extraction using acetone, methanol, chloroform and petroleum ether, qualitative and quantitative phytochemical studies and GC-MS analysis.

2. Materials and Methods

2.1 Collection of plant material

The plant material *Andrographis paniculata* was collected from Trichy Research Institute of Biotechnology Pvt Ltd, Thillai nagar (Latitude and Longitudes 10.8338° N, 78.6568° E) Tiruchirappalli, Tamil Nadu. Plant material was collected, shade dried and used for extraction.

2.2 Soxhlet extraction of Plant Material

Andrographis paniculata was shade dried and crushed using mortar and pestle, coarse powder was taken for the extraction process. Soxhlet apparatus was used for the extraction of *Andrographis paniculata* (Geow et al., 2021). Petroleum ether, Chloroform, Acetone, and methanol solvents were used for extraction process. *Andrographis paniculata* extracts were stored until use.

2.3 Qualitative phytochemical analysis

Qualitative phytochemical analysis was carried out for the Andrographis *paniculata* acetone, methanol, chloroform and petroleum ether extracts. The Phytochemical analysis was performed to identify the presence of phytoconstituents, such as carboxylic acids, steroids (Agidew, 2022), tannins (Nazir & Chauhan, 2019), flavonoids (Nagajothi et al., 2018), proteins by bradford's method, phenol by ferric chloride test, glycosides by born- trageru's test, saponins (Agidew, 2022), alkaloids by mayer's test, saponification test, gum test, flavanoglycoside, resins, carbohydrates and biuret test (Ayuba et al., 2021).

2.4 Quantitative phytochemical analysis

Quantitative analysis was performed to quantify the phytoconstituents present in the *Andrographis paniculata* acetone, methanol, chloroform and petroleum ether extracts. Quantitative analysis includes estimation of total flavonoids (Dirar et al., 2019), estimation of lipids by phospho vanillin method (Shankar et al., 2019), estimation of tannin by folin-ciocalteu method (Ricci et al., 2020), quantification of alkaloid (Rai et al., 2021) and estimation of protein by bradford's method (Nwachukwu & Aluko, 2019).

2.4.1 Estimation of total flavonoids

Total flavonoid content was measured by aluminium chloride colorimetric assay. For this experiment quercetin was used as a standard. Quercetin (50mg/ml) was used as a stock solution. About 100µl of test sample was mixed with 0.3 ml of 10% aluminium chloride after 5 miniutes. 2ml of 1M sodium hydroxide was added after 6 minutes. Finally the volume was made up to 10ml with distilled water. Orange yellowish color was developed. The absorbance was measured at 510nm using UV Spectrophotometer. The calibration curve was plotted against the concentration of quercetin (mg/ml) Vs. absorbance at 510nm (Dirar et al., 2019).

2.4.2 Estimation of protein

Protein was estimated by bradford's method using Bovine Serum Albumin (BSA) as a standard. To the 10µl of BSA and the test sample, 100µl of bradford's reagent was added and incubated for 10 minutes at dark. After incubation, the absorbance was measured at 595nm using UV Spectrophotometer. The calibration curve was plotted against the Concentration of BSA (mg/ml) Vs. Absorbance at 595nm (Nwachukwu & Aluko, 2019).

2.4.3 Estimation of tannin

Total tannin content was estimated by folin-ciocalteu method, using Tannic acid as a standard. To the 0.1ml of the extract 0.5ml of Folin-Ciocalteu phenol reagent, 1ml of 35% sodium carbonate solution was added and makes up to 10ml using distilled water. The mixture was incubated at room temperature for 30 minutes. After the incubation absorbance was measured at 700nm using UV Spectrophotometer. The

calibration curve was plotted against the concentration of tannic acid (mg/ml) Vs. absorbance at 700nm (Ricci et al., 2020).

2.4.4 Estimation of lipids

Lipids were estimated by phospho-vanillin method using cholesterol as standard. To the 100µl of test sample, 100µl of conc. sulphuric acid was added and incubated at dry heating bath at 90ºC for 10 minutes. After incubation 50µl of sulfo-phosphoric vanillin acid was added. After color development the absorbance was measured at 540nm using UV Spectrophotometer. The calibration curve was plotted against the concentration of cholesterol (mg/ml) Vs. absorbance at 540nm (Shankar et al., 2019).

2.4.5 Estimation of alkaloids

\ For estimating the alkaloids, to the 5g of sample, 200ml of 10% acetic acid was added in ethanol and 0.62g of sample. The solution was incubated for 4 hours and heated to one quarter of original volume. After heating solution was a filtered and 15 drop of conc. ammonium hydroxide was added allowed to rest for 3 hours. Then the precipitate was collected and washed with 20ml of 0.1m ammonium hydroxide. The amount of alkaloid present in the extract was determined by the formula,

% Alkaloid = Weight of the plant extract/ Weight of the precipitate (Alkaloid) × 100.

2.5 GC-MS analysis

The GC-MS analysis was carried out for *Andrographis paniculata* extract using a Clarus 500 Perkin-Elmer Gas Chromatograph equipped and coupled to a mass detector Turbo mass version 5. 2.0 – Perkin Elmer Turbomass 5.2 spectrometer with an Elite- (5%Phenyl 95% dimethyl polysiloxane), 30 m, and 250 µm capillary columns. The oven temperature was raised to 250°C, Injection port temperature was maintained at 280°C and Helium flow rate as 1 ml/min. The ionization voltage was 70 eV. The samples were injected in split mode at 1:10. The mass spectral scans range was set at 40-450 (MHz). Transfer line and source temperature: 200ºC, 250ºC, Sample injected was 1 mL (Thangavel et al., 2015).

3. Results

3.1 Qualitative phytochemical analysis of *Andrographis paniculata*

The presence of different 15 phytochemicals analyses were analyzed for the acetone, methanol, chloroform, and petroleum ether extracts of *Andrographis paniculata.*

Table 1: Phytochemical analysis of *Andrographis paniculata* extracted by acetone, methanol, chloroform and petroleum ether

S.no	Phytochemical compound	*Andrographis paniculata* extracted by different solvent system			
		Acetone	Methanol	Chloroform	Petroleum Ether
1.	Resins	++	++	++	-
2.	Carboxylic acid	++	++	++	-
3.	Tannins	+	+	+	+
4.	Steroids	-	++	-	+
5.	Flavonoid	+	+	+	+
6.	Carbohydrates	++	-	+	++
7.	Glycosides	-	-	-	-
8.	Saponification	++	++	-	-
9.	Protein	++	++	++	++
10.	Phenol	-	-	-	-
11.	Biuret	-	-	-	-
12.	Saponins	-	-	-	-
13.	Gum	-	-	-	++
14.	Flavanoglycosides	-	-	-	-
15.	Alkaloids	+	+	+	+

The results of qualitative phytochemicals were tabled in Table 1. (++) refers the presence of phytoconstituents, (+) refers presence of the phytoconstituents in milder amount. In out of 15 phytochemical analyses, acetone,

methanol, chloroform extracts showed the presence of resins, carboxylic acid, tannins, flavonoids, protein and alkaloids. Absence of glycosides, phenol, biuret, saponins, and flavanoglycosides was observed in acetone, methanol, and chloroform extracts. Presence of carbohydrates was observed at acetone, chloroform, and petroleum ether extracts. Steroids were present in methanolic extract and petroleum ether extracts confirmed the presence of gum and absence of saponification.

3.2 Quantitative phytochemical analysis of *Andrographis paniculata*

Table 2: Quantitative phytochemical analysis of *Andrographis paniculata*

S.no	Name of the Sample	Flavonoid content (mg/g)	Tannin content (mg/g)	protein content (mg/g)	Lipid content (mg/g)	Alkaloid content (%)
1	*Andrographis paniculata*- Petroleum Ether	0.39	0.1	1.31	0.08	70.80
2	*Andrographis paniculata*- Acetone	2.13	0.02	5.53	1.97	20.96
3	*Andrographis paniculata*- Chloroform	0.48	0.51	0.95	0.8	85.48
4	*Andrographis paniculata*- Methanol	0.16	0.41	0.1	0.11	11.29

3.2.1 Estimation of flavonoids

Flavonoids estimation was performed for *Andrographis paniculata* extracts, extracted by acetone, methanol, chloroform and petroleum ether using aluminium chloride method.

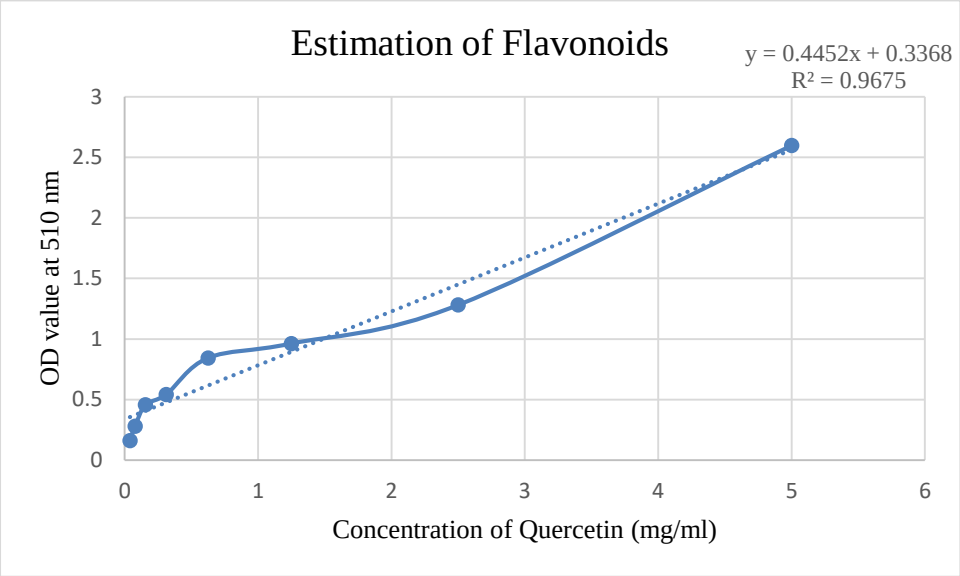

Figure 1: Concentration of quercetin (mg/ml) Vs. absorbance at 510 nm

Estimation of flavonoids were measured by plotting a graph between concentration of quercetin (mg/ml) Vs. absorbance at 510nm and the mean value of total flavonoid content was measured by taking average of triplicates (Figure 1). Total flavonoid content in *Andrographis paniculata* extracted by petroleum ether was found to be 0.39mg/g, for acetone 2.13 mg/g**,** for chloroform 0.48 mg/g and methanol was observed as 0.16 mg/g. The total flavonoid content was high in acetone extract with 2.13 mg/g (Table 2).

3.2.2 Estimation of proteins

Protein estimation was performed for *Andrographis paniculata* extracts, extracted by acetone, methanol, chloroform and petroleum ether by Bradford's method.

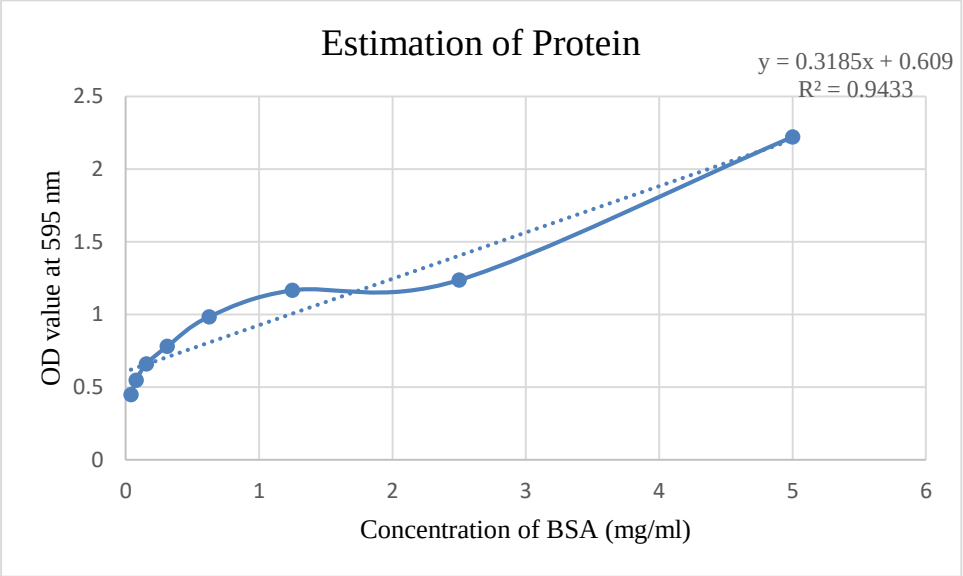

Figure 2: Concentration of BSA (mg/ml) Vs. absorbance at 595 nm

Total protein content in *Andrographis paniculata* extracted by petroleum ether was found to be 1.31 mg/g, acetone was 5.53 mg/g**,** chloroform as 0.95 mg/g, and methanol was 0.1 mg/g. The total protein content was high in acetone extract compared to other solvent extracts of *Andrographis paniculata* as shown in Table 2.

3.2.3 Estimation of tannin

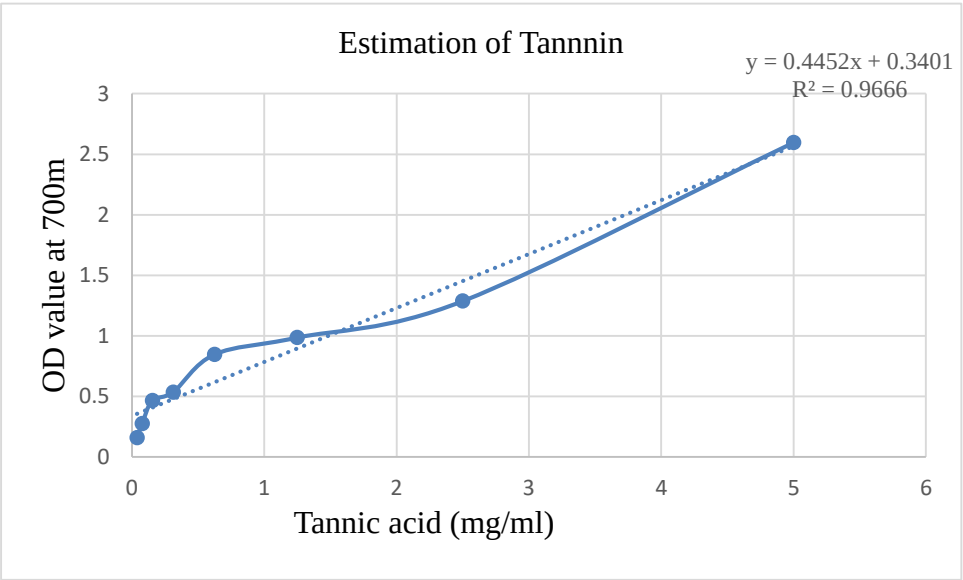

Figure 3: Concentration of Tannic acid (mg/ml) Vs. Absorbance at 700nm

Tannins was estimated by plotting a graph between concentration of tannic acid (mg/ml) Vs. absorbance at 700nm and the mean value of total tannin content was measured by taking average of triplicates (Figure 3).

Total tannin content present in *Andrographis paniculata* petroleum ether extract was found to be 0.1 mg/g, acetone extract was 0.02 mg/g**,** chloroform was 0.51 mg/g, and methanol extract was observed as 0.41 mg/g.

Among those extracts, the total tannins content was found to be high in chloroform extract as 5.53 mg/g were shown in Table 2.

3.2.4 Estimation of lipids

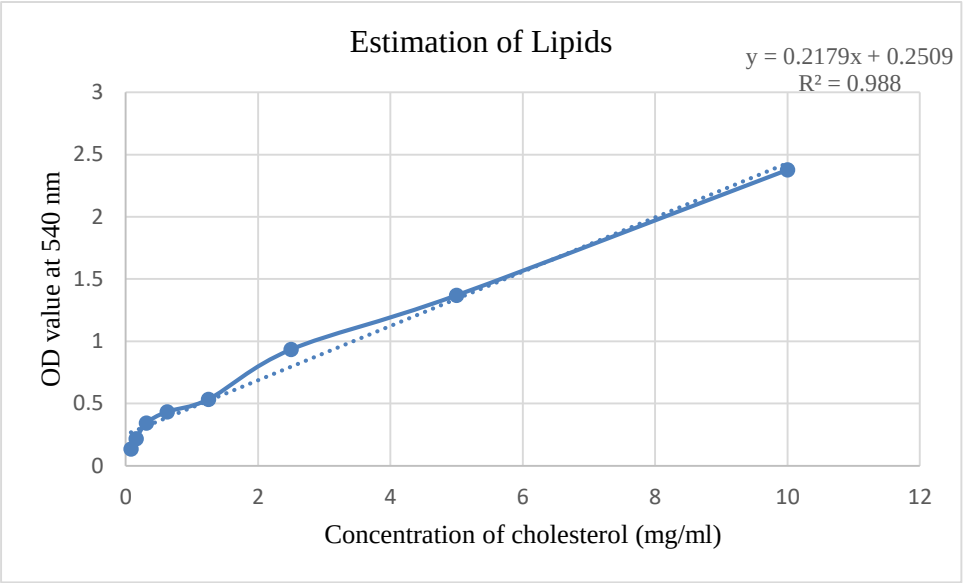

Figure 4: Concentration of cholesterol (mg/ml) Vs. absorbance at 540 nm

Lipids were estimated by phospho-vanillin method with cholesterol as standard. Total lipids content in *Andrographis paniculata* extracted by petroleum ether was found to be 0.08 mg/g, acetone (1.97 mg/g), chloroform (0.8 mg/g), methanol (0.11 mg/g). The lipid content was high in acetone extract with 1.97 mg/g.

3.2.5 Estimation of alkaloids

Total alkaloids content in *Andrographis paniculata* extracted by petroleum ether was found to be 70.80%, acetone was 20.96%, chloroform was 85.48%, and methanol was observed as 11.29%). Alkaloid content was higher in Chloroform extract with 85.48%.

3.3 GC-MS analysis

The GC-MS analysis was carried out for all the extracts of *Andrographis paniculata*.

3.3.1 GC-MS analysis *Andrographis paniculata* extracted by acetone

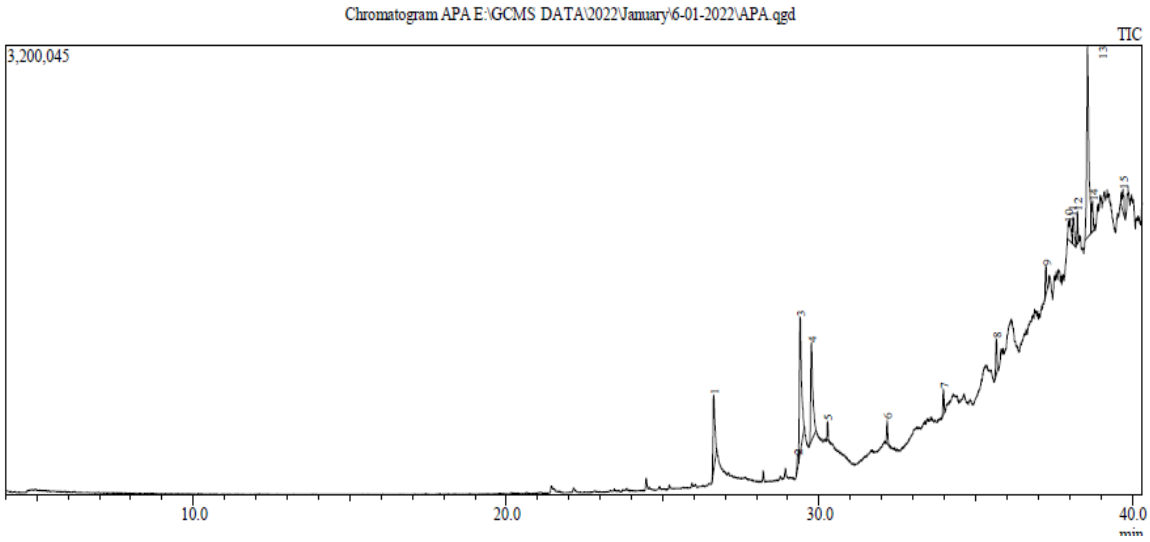

Figure 5: GCMS Chromatogram of *Andrographis paniculata* acetone extract

Table 3: GC-MS table with peak and name of the compound present in *Andrographis paniculata* acetone extract.

Peak	Retention time	Start time	End Time	Area %	Height %	Name
1	26.628	26.575	26.725	9.96	9.82	n-Hexadecanoic acid
2	29.305	29.27	29.34	1.39	1.73	9,12-Octadecadienoic acid (Z,Z)-
3	29.394	29.34	29.525	19.78	17.37	Octadec-9-enoic acid
4	29.752	29.695	29.9	14.23	12.68	Octadecanoic acid
5	30.268	30.235	30.305	1.37	2.42	Cyclononasiloxane, octadecamethyl-
6	32.176	32.135	32.23	1.79	3.05	Cyclononasiloxane, octadecamethyl-

7	33.971	33.935	34.015	1.91	3.26	Cyclononasiloxane, octadecamethyl-
8	35.664	35.615	35.715	3.08	4.6	Cyclononasiloxane, octadecamethyl-
9	37.246	37.2	37.29	2.46	3.78	Cyclononasiloxane, octadecamethyl-
10	37.96	37.935	38.085	3.79	2.14	1,54-dibromotetrapentacontane
11	38.121	38.085	38.205	3.39	3.52	Tetrapentacontane, 1,54-dibromo-
12	38.254	38.215	38.29	2.06	4.2	4,8,13,17,21-pentamethyl-4,8,12,16,20-docosapentaenal
13	38.578	38.49	38.695	28.41	24.69	1-Heptatriacotanol
14	38.74	38.695	38.79	3.19	3.83	Cyclononasiloxane, octadecamethyl-
15	39.713	39.64	39.78	3.18	2.91	14-.beta.-h-pregna

GC-MS analysis result for *Andrographis paniculata* acetone extract was listed in Table 3, In Figure 5, The GCMS chromatogram shows 15 different peaks, among 15 peaks, highest peak was obtained in 13th peak with the area of 28.41%, height of 24.69% and the obtained phytocompound was 1-Heptatriacotanol (Figure 6).

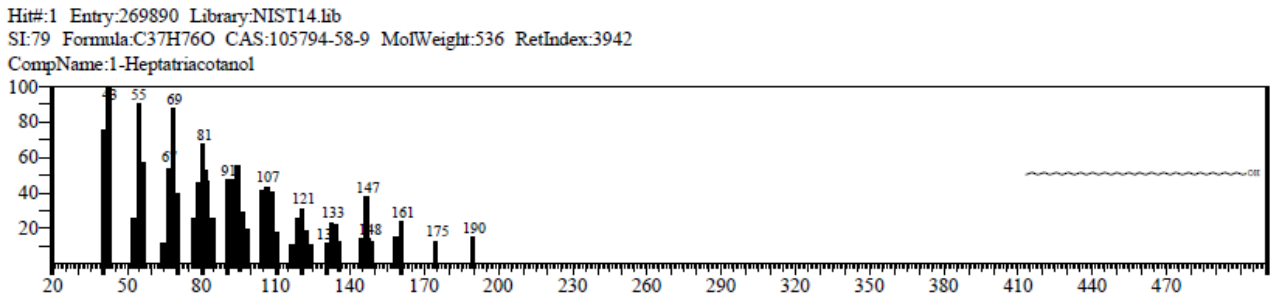

Figure 6: Peak and structure of 1-Heptatriacotanol.

3.3.2 GC-MS analysis of *Andrographis paniculata* extracted by chloroform

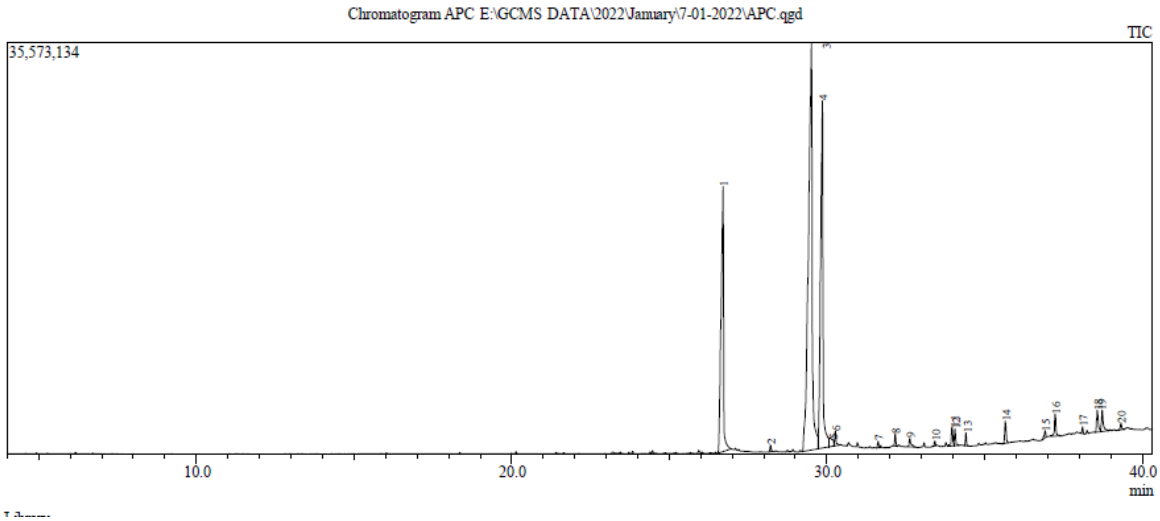

Figure 7: GCMS Chromatogram of *Andrographis paniculata* chloroform extract.

Table 4: GC-MS table with peak and name of the phyto compound present in *Andrographis paniculata* chloroform extract.

Peak	Retention time	Start time	End Time	Area %	Height %	Name
1	26.706	26.535	26.96	19.34	21.79	n-Hexadecanoic acid
2	28.209	28.155	28.285	0.26	0.56	Cyclododecasiloxane, tetracosamethyl-
3	29.511	29.215	29.715	45.27	33.28	Octadec-9-enoic acid
4	29.86	29.715	30.065	25.34	27.46	Octadecanoic acid
5	30.185	30.065	30.225	0.93	0.55	Oleyl alcohol, trifluoroacetate
6	30.271	30.225	30.415	0.62	1.12	Cyclononasiloxane, octadecamethyl
7	31.627	31.565	31.675	0.25	0.53	Glycidyl palmitate
8	32.173	32.115	32.235	0.41	1	Cyclononasiloxane, octadecamethyl

9	32.632	32.545	32.815	0.49	0.67	Eicosanoic acid
10	33.427	33.375	33.475	0.17	0.39	Oleoyl chloride
11	33.971	33.825	34.025	0.89	1.54	Cyclononasiloxane, octadecamethyl
12	34.065	34.025	34.195	0.64	1.38	Glycidyl oleate
13	34.411	34.355	34.505	0.42	1.03	Dotriacontane
14	35.662	35.575	35.765	0.93	1.78	Cyclononasiloxane, octadecamethyl
15	36.922	36.815	36.965	0.31	0.57	Nonacosane
16	37.243	37.115	37.425	0.99	1.8	Cyclononasiloxane, octadecamethyl
17	38.111	38.045	38.195	0.22	0.54	Tetracontane
18	38.58	38.505	38.675	1.09	1.74	13,15-Octacosadiyne
19	38.736	38.675	38.935	1.15	1.74	Cyclononasiloxane, octadecamethyl-
20	39.329	39.235	39.425	0.3	0.53	Celidoniol, deoxy

GC-MS analysis of *Andrographis paniculata* chloroform extract was shown in Table 4, Figure 7 shown 20 different peaks and their compounds. Among 20 peaks, highest peak was obtained in 3rd peak with the area of 45.27%, height of 33.28% and the obtained phytocompound was Octadec-9-enoic acid (Figure 8).

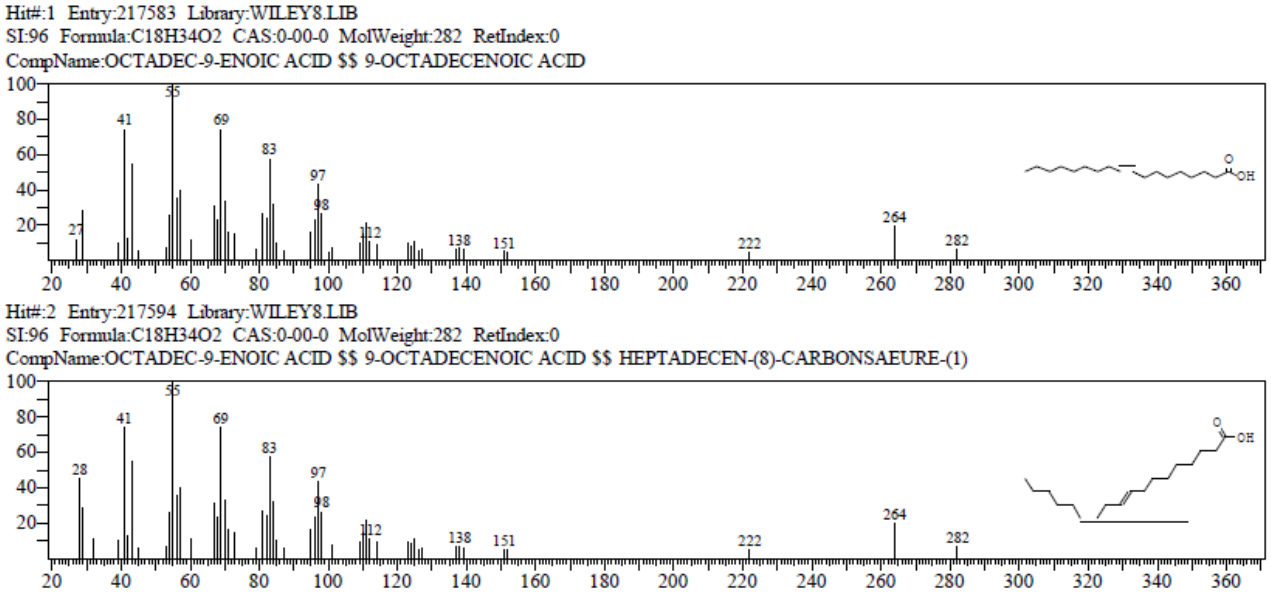

Figure 8: Peak and structure of Octadec-9-enoic acid.

3.3.3 GC-MS analysis *Andrographis paniculata* methanol extract

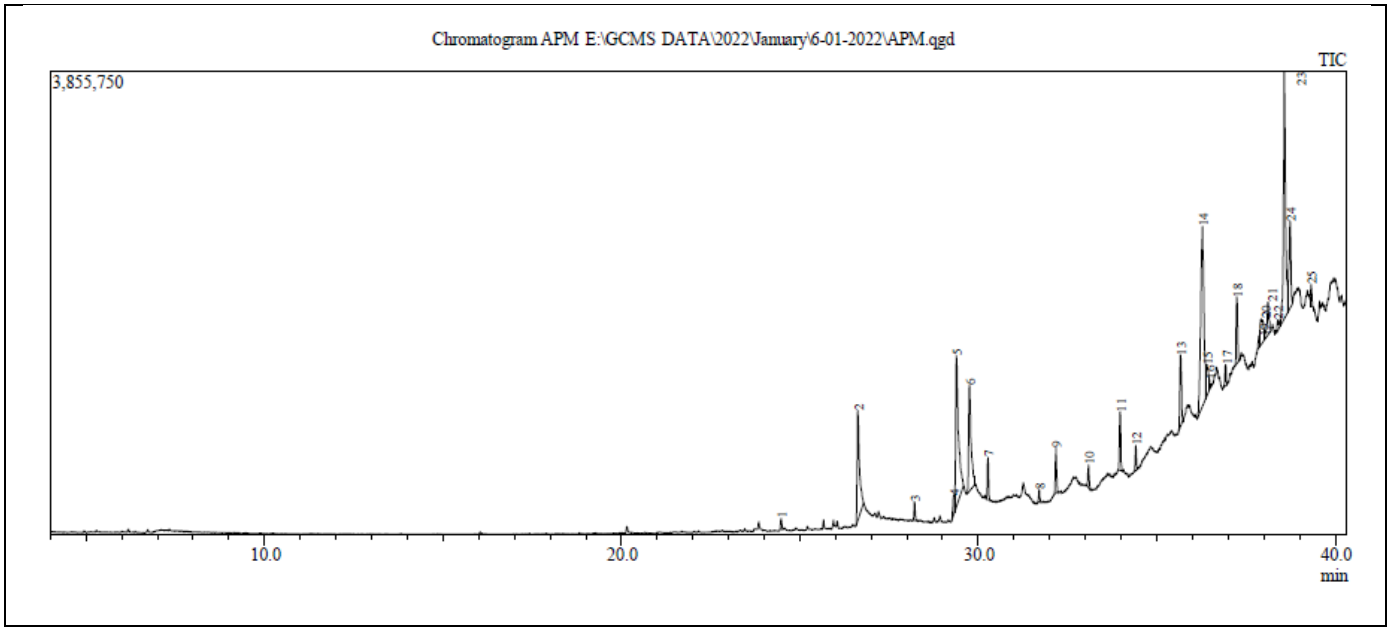

Figure 9: GCMS Chromatogram of *Andrographis paniculata* methanol extract

Table 5: GC-MS table with peak and name of the compound present in *Andrographis paniculata* extracted by methanol.

Peak	Retention time	Start time	End Time	Area %	Height %	Name
1	24.475	24.44	24.525	0.41	0.75	Neophytadiene
2	26.625	26.57	26.785	8.42	7.63	n-Hexadecanoic acid
3	28.211	28.175	28.26	0.69	1.28	Cyclododecasiloxane, tetracosamethyl
4	29.305	29.255	29.335	1.11	1.34	9,12-Octadecadienoic acid (Z,Z)-
5	29.387	29.335	29.585	13.94	10.51	Octadec-9-enoic acid
6	29.751	29.685	29.88	8.2	7.35	Octadecanoic acid
7	30.27	30.225	30.32	1.67	2.91	Cyclononasiloxane, octadecamethyl
8	31.704	31.675	31.75	0.45	0.9	Heneicosane
9	32.176	32.13	32.225	1.83	3.11	Cyclononasiloxane, octadecamethyl
10	33.089	33.045	33.135	0.89	1.6	Docosane
11	33.973	33.92	34.025	2.48	4.08	Cyclononasiloxane, octadecamethyl
12	34.414	34.375	34.455	0.94	1.75	Hexacosane
13	35.666	35.61	35.725	3.79	4.97	Cyclononasiloxane, octadecamethyl
14	36.279	36.135	36.375	19.18	12.54	Andrographolide
15	36.415	36.375	36.47	2.4	2.12	1,5-naphthalenediol, decahydro
16	36.48	36.47	36.505	0.23	0.72	Hexanedioic acid, di-2-propenyl ester
17	36.928	36.88	36.975	0.86	1.49	Docosane

18	37.244	37.19	37.295	2.99	4.65	Cyclononasiloxane, octadecamethyl
19	37.855	37.825	37.88	0.46	0.76	14-.beta.-h-pregna
20	37.93	37.88	38.025	2.76	1.78	Octatriacontyl pentafluoropropionate
21	38.119	38.025	38.2	2.28	2.31	Pentatriacontane
22	38.395	38.355	38.465	0.63	0.73	erythro-9,10-Dibromopentacosane
23	38.576	38.465	38.67	16.79	17.17	1,1,4,7-tetramethyldecahydro-1h-cyclopropa[e]azulen-4-ol
24	38.736	38.67	38.805	5.68	6.06	Silikonfett se30 (grevels)
25	39.324	39.3	39.37	0.91	1.5	Heptacosane

GC-MS analysis *Andrographis paniculata* extracted by methanol, was shown in Table 5, Figure 9 shows the presence of 25 different peaks and their compounds, among 25 peaks, highest peak was obtained in 23rd peak with the area of 16.79%, height of 17.17% and the phytocompound was 1,1,4,7-tetramethyldecahydro-1h-cyclopropa[e]azulen-4-ol (Figure 10). .

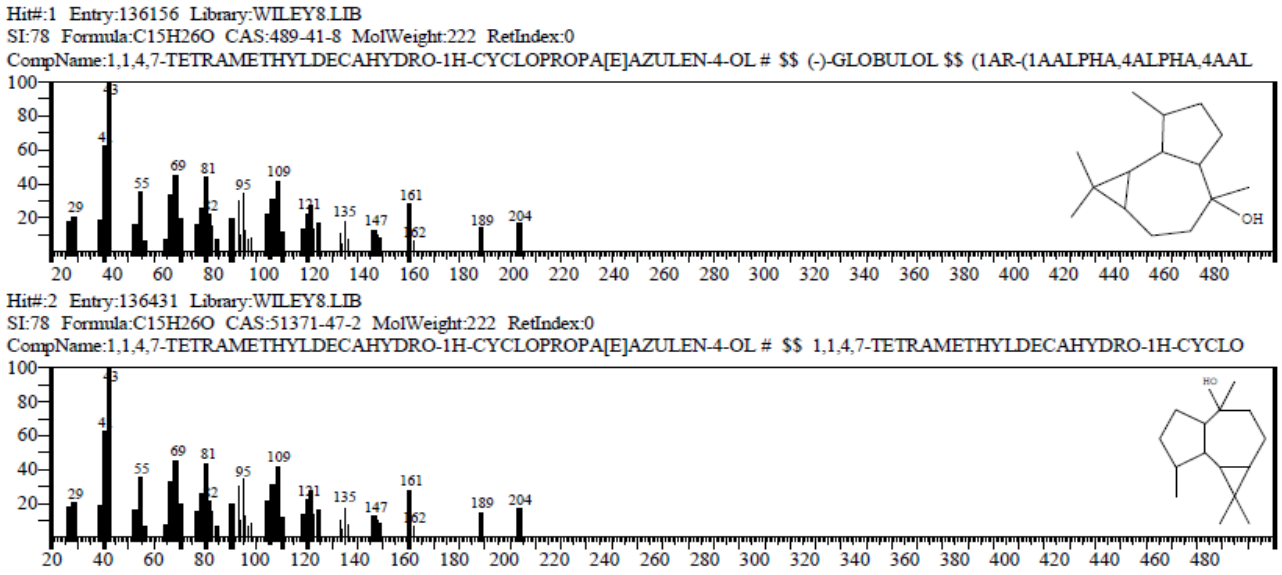

Figure 10: Peak and structure of 1,1,4,7-tetramethyldecahydro-1h-cyclopropa[e]azulen-4-ol.

3.3.4 GC-MS analysis *Andrographis paniculata* petroleum ether extract

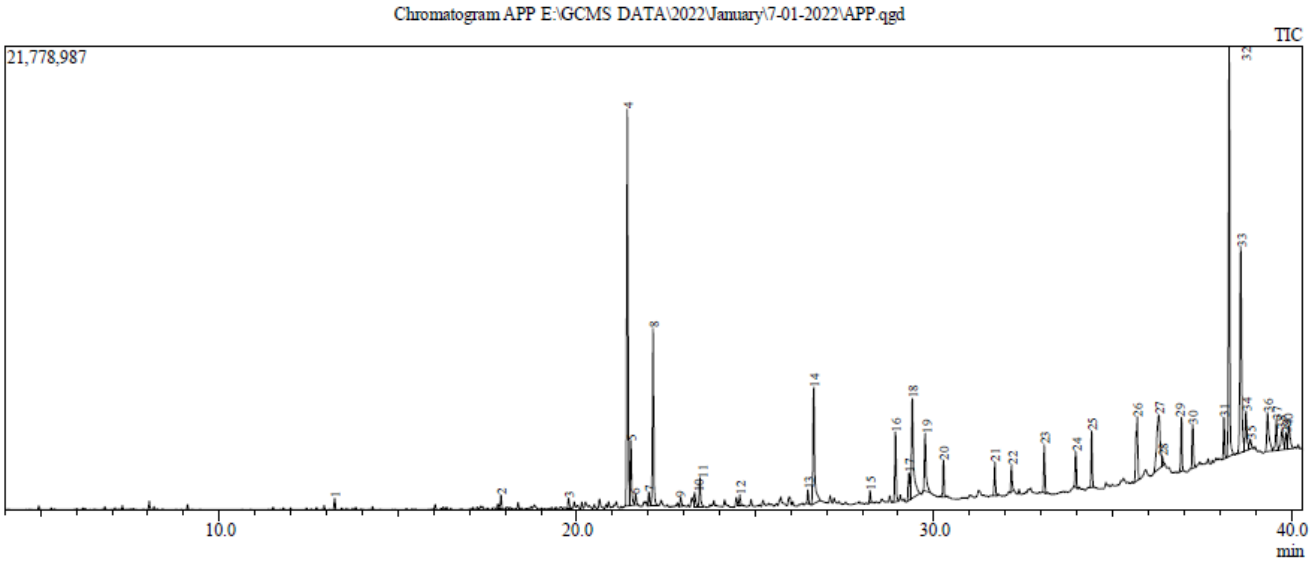

Figure 11: GCMS Chromatogram of *Andrographis paniculata* extracted by petroleum ether

Table 6: GC-MS table with peak and name of the compound present in *Andrographis paniculata* extracted by petroleum ether.

Peak	Retention time	Start time	End Time	Area %	Height %	Name
1	13.226	13.185	13.27	0.33	0.46	Hexadecane
2	17.882	17.825	17.93	0.48	0.55	Eicosane
3	19.775	19.735	19.845	0.37	0.41	2-Methyl-6-(p-tolyl)hept-2-en-4-ol
4	21.421	21.355	21.48	13.55	16.14	Ar-tumerone
5	21.518	21.48	21.61	2.49	2.65	Tumerone
6	21.653	21.61	21.715	0.4	0.43	1-tetradecanol
7	22.02	21.98	22.07	0.48	0.56	Eicosane
8	22.141	22.07	22.26	6.07	7.2	Curlone
9	22.914	22.87	22.975	0.37	0.37	(6r,7r)-bisabolone
10	23.302	23.27	23.37	0.62	0.57	Pentadecane, 2-methyl-2-phenyl-
11	23.45	23.37	23.545	1.09	1.17	(E)-atlantone
12	24.568	24.52	24.615	0.38	0.45	2-Pentadecanone, 6,10,14-trimethyl

13	26.474	26.435	26.535	0.45	0.56	Dibutyl phthalate
14	26.639	26.57	26.825	5.5	4.75	N-Hexadecanoic acid
15	28.209	28.165	28.26	0.41	0.51	Cyclooctasiloxane, hexadecamethyl
16	28.923	28.82	29.005	2.53	2.84	Phytol
17	29.298	29.25	29.34	1.25	1.13	9,12-Octadecadienoic acid (Z,Z)
18	29.396	29.34	29.61	5.71	4.06	Oleic acid
19	29.754	29.7	29.9	2.55	2.35	Octadecanoic acid
20	30.269	30.215	30.33	1.22	1.49	Silikonfett se30 (grevels)
21	31.706	31.65	31.765	1.12	1.37	Tetracosane
22	32.174	32.13	32.245	0.91	1.11	Cyclononasiloxane, octadecamethyl-
23	33.09	33.035	33.14	1.48	1.96	Tetracosane
24	33.971	33.92	34.025	1.19	1.46	Cyclononasiloxane, octadecamethyl-
25	34.417	34.37	34.465	1.78	2.29	Tetracosane
26	35.693	35.6	35.74	3.35	2.58	Tetracosane
27	36.295	36.155	36.39	5.18	2.19	2(3h)-furanone, 3-(2-(decahydro-6-hydroxy-5-(hydroxymethyl)-5,8a-dimethyl-2-methylene-1-naphthalenyl)ethylidene)dihydro-4-hydro
28	36.395	36.39	36.45	0.19	0.36	4-(2-chloroethoxy)-6-(methoxyamino)-n,n-dimethyl-1,3,5-triazin-2-amine #
29	36.929	36.87	36.98	1.78	2.18	Tetracosane
30	37.244	37.19	37.325	1.66	1.8	Cyclononasiloxane, octadecamethyl

31	38.118	38.07	38.18	1.38	1.69	Tetracontane
32	38.265	38.18	38.385	14.5	16.68	Squalene
33	38.589	38.5	38.685	9.59	8.38	13,15-octacosadiyne
34	38.736	38.685	38.8	1.85	1.61	Cyclononasiloxane, octadecamethyl
35	38.854	38.8	38.93	0.57	0.39	.Alpha.-tocospiro b
36	39.339	39.275	39.465	2.34	1.58	Nonacosane
37	39.586	39.465	39.635	1.38	1.25	Solanesol
38	39.747	39.635	39.805	1.3	0.85	2,2-dimethyl-3-[(3e,7e,11e,15e)-3,7,12,16,20-pentamethyl-3,7,11,15,19-henicosapentaenyl]oxirane
39	39.853	39.805	39.89	0.79	0.68	Geranyl linalool isomer b
40	39.938	39.89	40.045	1.4	0.93	(2z,6e,10e)-3,7,11,15-tetramethyl-2,6,10,14-hexadecatetraen-1-ol

GC-MS analysis of *Andrographis paniculata* petroleum ether extract was shown in Table 6, Figure 11 showed the presence of 40 different peaks and their compounds, Among 40 peaks, highest peak was obtained at 32nd peak with the area of 14.5%, height of 16.68% and the phytocompound was squalene (Figure 12).

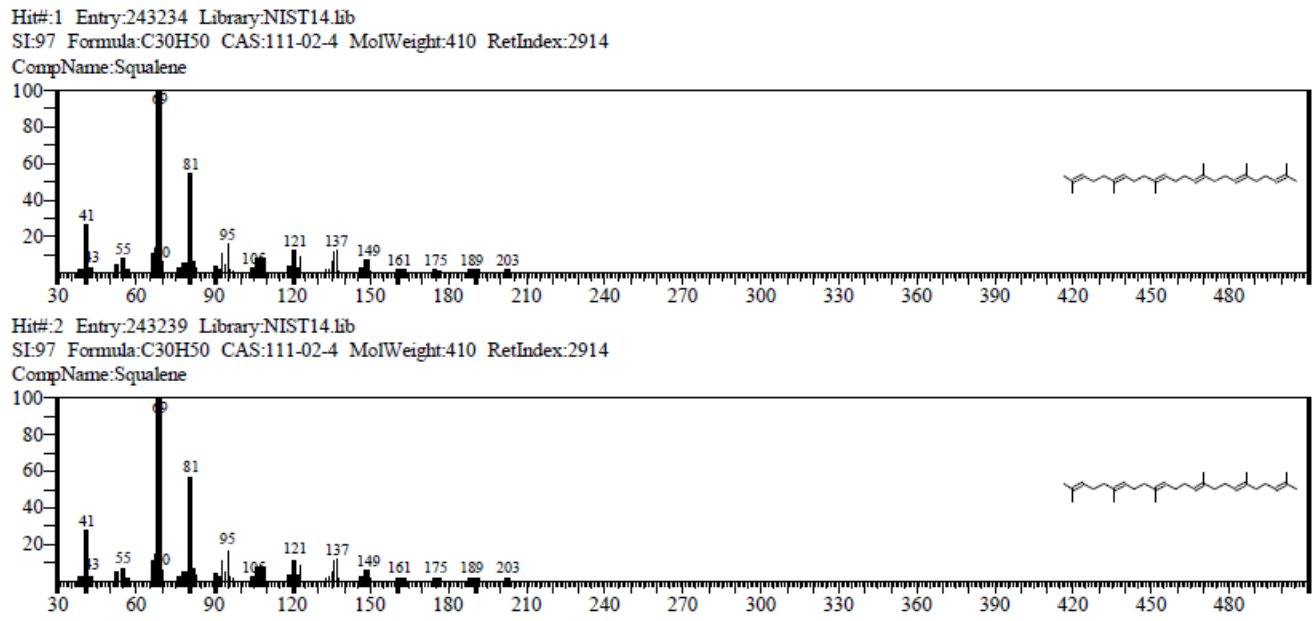

Figure 12: Peak and structure of squalene.

4. Discussion

Selection of solvents for extracting the phytoconstituents is more important for qualitative and quantitative analysis. Alcoholic solvents like acetone, methanol, chloroform and petroleum ether is known for its high efficiency to extract the phytoconstituents from plant sample. When compared to solvents like acetone, methanol, chloroform and petroleum ether, acetone is more polar, high polar phytoconstituents are precipitated in high dipole moment. In cold acetone extraction, Andrographolide was precipitated. Polyphenols have low efficiency in acetone(Bassey et al., 2019). Methodology used for *Andrographis paniculata* extraction acetone, methanol, chloroform and petroleum ether was similar to the study conducted by Chin Hong Geow et al (Geow et al., 2021).

In *Andrographis paniculata* acetone extract, showed the presence of resins, carboxylic acid, carbohydrates, saponification, and protein were confirmed by qualitative phytochemical methods and in quantitative phytochemical analysis, total flavonoids was found to be 2.13 mg/g, total tannins was 0.02 mg/g, total protein was 5.53 mg/g, total lipids was 1.97 mg/g and total alkaloids was obtained as 20.96%. 15 different peak compounds were obtained in GCMS analysis with 1-Heptatriacotanol as peak compound with the area of 28.41%, and height of 24.69%. 1-Heptatriacotanol has various therapeutic activities against hypocholesteroloic, acts as anti inflammatory and anti oxidant agent. Anti oxidant property protects the cells from oxidative damage(Kalaimagal, 2019). Mishra et al., reported the antibacterial property of *Andrographis paniculata* ethanolic extract against various gram positive and gram negative bacteria (Mishra et al., 2009).

Hung et al., reported the inhibition of TNF α using *Andrographis paniculata* ethanolic extract (Lin et al., 2019). Andri et al., elucidated the andrographolide from ethanolic extract of *Andrographis paniculata* dried leaves (Kumoro et al., 2019).

Similarly, methanolic extract of *Andrographis paniculata* showed positive results for resins, carboxylic acid, tannins, steroids, flavonoid, saponification, protein, and alkaloids. In addition quantitative phytochemical analysis, showed 0.16.g/g of total flavonoids, 0.41mg/g of total tannins, 0.1 mg/g of total proteins, 0.11 mg/g of total lipids, and 11.29 mg/g of total alkaloids. GCMS analysis of methanolic extract revealed the presence of 25 different phytocompounds and the highest peak was observed at 23rd peak, 1,1,4,7-tetramethyldecahydro-1h-cyclopropa[e]azulen-4-ol with the area of 16.79%, and height of 17.17%. Andrographolide the important therapeutic phytocompound was obtained at 14th peak with the area of 19.18% and height of 12.54%. Koteswara et al., also confirmed the presence of andrographolide in *Andrographis paniculata* methanolic extract (Koteswara Rao et al., 2004). Sriram et al., reported the cell differentiating property in mouse myeloid leukemia cells, antipoliferative response against human cancers using *Andrographis paniculata* methanolic extract (Rajagopal et al., 2003). Pubali and Kakoti reported the presence of Terpenoids, alkaloids, tannins, and flavonoids and anti-oxidant properties in methanolic extract of *Andrographis paniculata* (Borgohain & Kakoti, 2019).

Chloroform is less polar in nature, these solvents are used to extract the derivatives of terpenoids(Chua et al., 2019), In the present study chloroform extract of *Andrographis paniculata* showed the positive results in resins, carboxylic acid, tannins, flavonoids, carbohydrates, proteins, and alkaloids. *Andrographis paniculata* chloroform extract exhibited the 0.48mg/g of flavonoids, 0.51 mg/g of tannins, 0.95 mg/g of proteins, 0.8 mg/g of total lipids and 85.48 mg/g of total alkaloids by quantitative phytochemical analysis. Screening of phytocompounds present in the chloroform extract was studied using GCMS analysis method. There were 20 various types' phytocompounds were observed as shown in table 4. The maximum area of 45.27% and height 33.28% containing peak was found at 3rd peak, Octadec-9-enoic acid (Figure 8). Presence of phenols, aromatic carboxylic acid, esters were elucidated in GCMS analysis method and elucidation of Octadec-9-enoic acid in *Andrographis paniculata* chloroform extract was reported by Soma Roy et al (Roy et al., 2010). Presence of steroids, terpenoids, saponins, glycosides, and carbohydrates in Chloroform leaf extracts of *Andrographis paniculata* was reported by Pande et al (Pande et al., 2011). Parvataneni and Koduru

studied and reported the antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *E.coli*, *Proteus vulgaris*, *Aspergillus niger* and *Penicillium chrysogenum* using chloroform extracts of root and stem of *Andrographis paniculata* (Radhika & Lakshmi, 2010).

In our study, petroleum ether extract of *Andrographis paniculata* showed positive results for tannins, steroids, flavonoid, carbohydrates, protein, gum and alkaloids in out of 15 qualitative phytochemical analyses. Quantification of pharmacologically active phytochemicals such as flavonoids (0.39 mg/g), tannins (0.1 mg/g), proteins (1.31 mg/g), lipids (0.08 mg/g), and alkaloids of 70.80% were studied for the *Andrographis paniculata* pet extract. Furthermore, characterizations of phytocompounds were elucidated by GCMS method. As shown in Table 6, *Andrographis paniculata* petroleum ether extract showed 40 types of phytocompounds, among that squalene was found to be highest peak value with the area of 14.5%, and height of 16.68%. Squalene acts as a good reducing substance in reducing the free radical oxidative damage, it acts as oxygen scavenging agent, it inhibits the skin tumorigenesis(Kim & Karadeniz, 2012). Studies reported that squalene posses anticancer, antioxidant, and drug carrier in animal studies and *in-vitro* studies(Huang et al., 2009). Presence of flavonoids, phenols in *Andrographis paniculata petroleum ether* extract was reported by Pubali and Kakoti (Borgohain & Kakoti, 2019). Presence of steroids in Petroleum ether extract was confirmed by Pande et al (Pande et al., 2011).

5. Conclusion

In our study qualitative, quantitative phytochemical analysis and GC-MS analysis of *Andrographis paniculata* extracts was studied and observed that the yield of *Andrographis paniculata* extracts was high in acetone and methanol extraction methods. Flavonoids, lipids and proteins are high in acetone extraction. Tannins and alkaloids were found to be high in chloroform extraction methods. GC-MS analysis revealed the presence of therapeutic important phytocompounds such as Andrographolide, 1-Heptatriacotanol, 1,1,4,7-tetramethyldecahydro-1h-cyclopropa[e]azulen-4-ol, Octadec-9-enoic acid and squalene in different solvent systems, which makes *Andrographis paniculata* as excellent biosource for the control of various life threatening diseases.

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Conflicts of interest: The authors declare that they have no conflict of interest.

6. References

Abas, F., Ismail, A., Lajis, N. H., & Shaari, K. (2016). Anti-Diabetic Activity and Metabolic Changes Obese Diabetic Rats. *Molecules*, *21*(8), 1026. https://doi.org/10.3390/molecules21081026

Agidew, M. G. (2022). Phytochemical analysis of some selected traditional medicinal plants in Ethiopia. *Bulletin of the National Research Centre*, *46*(1), 87. https://doi.org/10.1186/s42269-022-00770-8

Ayuba, S. A., Shehu, A., Isah, M., Ibrahim, T., & Muhammad Labbo, A. (2021). Extraction and Estimation of Protein from Ginger (Zingiber officinale) and its Interaction with Glucose Molecule. *Asian Food Science Journal*, *20(10)*(September), 95–99. https://doi.org/10.9734/afsj/2021/v20i1030365

Bassey, E. E., Ifeayinwa, O., Onuorah, C., & Chioma, L. (2019). Photochemical screening and in vitro antimicrobial analysis of chloroform, methanol and acetone extracts of leaves of Andrographis paniculata. *International Journal of Molecular Biology: Open Access*, *4*(2), 38–44. https://doi.org/10.15406/ijmboa.2019.04.00096

Borgohain, P., & Kakoti, B. B. (2019). Phytochemical screening and study of in vitro antioxidant activity of two different extracts of Andrographis paniculata Nees (Family : Acanthaceae). *The Pharma Innovation Journal*, *8*(5), 303–306.

Chua, L. S., Abdullah, F. I., & Azlah, M. A. F. (2019). Phytochemical Profile of Andrographis paniculata Extract from Solvent Partition and Precipitation. *Journal of Biologically Active Products from Nature*, *9*(3), 238–249. https://doi.org/10.1080/22311866.2019.1646671

Dirar, A. I., Alsaadi, D. H. M., Wada, M., Mohamed, M. A., Watanabe, T., & Devkota, H. P. (2019). Effects of extraction solvents on total phenolic and flavonoid contents and biological activities of extracts from Sudanese medicinal plants. *South African Journal of Botany*, *120*, 261–267. https://doi.org/10.1016/j.sajb.2018.07.003

Geow, C. H., Tan, M. C., Yeap, S. P., & Chin, N. L. (2021). A Review on Extraction Techniques and Its Future Applications in Industry. *European Journal of Lipid Science and Technology*, *123*(4), 2000302. https://doi.org/10.1002/ejlt.202000302

Huang, Z. R., Lin, Y. K., & Fang, J. Y. (2009). Biological and pharmacological activities of squalene and related compounds: Potential uses in cosmetic dermatology. *Molecules*, *14*(1), 540–554. https://doi.org/10.3390/molecules14010540

Jain, J., Kumar, A., Narayanan, V., Ramaswamy, R. S., Sathiyarajeswaran, P., Shree Devi, M. S., Kannan, M., & Sunil, S. (2020). Antiviral activity of ethanolic extract of Nilavembu Kudineer against dengue and chikungunya virus through in vitro evaluation. *Journal of Ayurveda and Integrative Medicine*, *11*(3), 329–335. https://doi.org/10.1016/j.jaim.2018.05.006

Kalaimagal, C. (2019). Identification of bioactive compounds in flower of Tabernaemontana divaricata (l.) Using gas chromatography–mass spectrometry analysis. *Asian Journal of Pharmaceutical and Clinical Research*, *12*(9), 129–132. https://doi.org/10.22159/ajpcr.2019.v12i9.34559

Kaushik, S., Dar, L., Kaushik, S., & Yadav, J. P. (2021). Identification and characterization of new potent inhibitors of dengue virus NS5 proteinase from Andrographis paniculata supercritical extracts on in animal cell culture and in silico approaches. *Journal of Ethnopharmacology*, *267*, 113541. https://doi.org/10.1016/j.jep.2020.113541

Kim, S.-K., & Karadeniz, F. (2012). Biological Importance and Applications of Squalene and Squalane. In *Advances in Food and Nutrition Research* (Vol. 65, pp. 223–233). Elsevier Inc. https://doi.org/10.1016/B978-0-12-416003-3.00014-7

Koteswara Rao, Y., Vimalamma, G., Venkata Rao, C., & Tzeng, Y.-M. (2004). Flavonoids and andrographolides from Andrographis paniculata. *Phytochemistry*, *65*(16), 2317–2321. https://doi.org/10.1016/j.phytochem.2004.05.008

Kumoro, A. C., Hasan, M., & Singh, H. (2019). Extraction of Andrographolide from Andrographis paniculata Dried Leaves Using Supercritical CO2 and Ethanol Mixture. *Industrial and Engineering Chemistry Research*, *58*(2), 742–751. https://doi.org/10.1021/acs.iecr.8b02243

Lin, H.-C., Li, C.-C., Yang, Y.-C., Chiu, T.-H., Liu, K.-L., Lii, C.-K., & Chen, H.-W. (2019). Andrographis paniculata diterpenoids and ethanolic extract inhibit TNFα-induced ICAM-1 expression in EA.hy926 cells. *Phytomedicine*, *52*, 157–167. https://doi.org/10.1016/j.phymed.2018.09.205

Mishra, A., Kumari, R., Murthy, P., & Naik, B. (2009). Antibacterial Activity of Ethanol Extract of Andrographis paniculata. *Indian Journal of Phamaceutical Sciences*, *71*(4), 436–438. www.ijpsonline.com

Nagajothi, S., Mekala, P., & Senthilkumar, P. (2018). Andrographis paniculata: qualitative and quantitative phytochemical analysis. *Journal of Pharmacognosy and Phytochemistry*, *7*(4), 1251–1253.

Nazir, I., & Chauhan, R. S. (2019). Qualitative phytochemical analysis of Allium sativum (Garlic) and Curcuma longa (Turmeric). *Journal of Entomology and Zoology Studies*, *7*(1), 545–547.

Nwachukwu, I. D., & Aluko, R. E. (2019). A systematic evaluation of various methods for quantifying food protein hydrolysate peptides. *Food Chemistry*, *270*(July), 25–31. https://doi.org/10.1016/j.foodchem.2018.07.054

Okhuarobo, A., Falodun, J. E., Erharuyi, O., Imieje, V., Falodun, A., & Langer, P. (2014). H arnessing the medicinal properties of A ndrographis paniculata for diseases and beyond : a review of its phytochemistry and pharmacology. *Asian Pacific Journal of Tropical Disease*, *4*(3), 213–222. https://doi.org/10.1016/S2222-1808(14)60509-0

Özçelik, B., Kartal, M., & Orhan, I. (2011). Cytotoxicity, antiviral and antimicrobial activities of alkaloids, flavonoids, and phenolic acids. *Pharmaceutical Biology*, *49*(4), 396–402. https://doi.org/10.3109/13880209.2010.519390

Paemanee, A., Hitakarun, A., Wintachai, P., Roytrakul, S., & Smith, D. R. (2019). Biomedicine & Pharmacotherapy A proteomic analysis of the anti-dengue virus activity of andrographolide. *Biomedicine & Pharmacotherapy*, *109*(October 2018), 322–332. https://doi.org/10.1016/j.biopha.2018.10.054

Pande, J. N., Moulisha, B., & Gupta, B. (2011). Thin layer chromatographic studies and evaluation of analgesic activity of andrographis paniculata leaf extracts in Pharmacologyonline 2 : 391-396 (2011). *Pharmacologyonline*, *2*(May 2014), 391–396.

Radhika, P., & Lakshmi, K. R. (2010). Antimicrobial Activity of the Chloroform Extracts of the Root and the Stem of Andrographis paniculata Nees. *Research Journal of Microbiology*, *1*(2), 37–39.

Rai, R., Singh, R. K., & Suthar, S. (2021). Production of compost with biopesticide property from toxic weed Lantana: Quantification of alkaloids in compost and bacterial pathogen suppression. *Journal of Hazardous Materials*, *401*(May), 123332. https://doi.org/10.1016/j.jhazmat.2020.123332

Rajagopal, S., Kumar, R. A., Deevi, D. S., Satyanarayana, C., & Rajagopalan, R. (2003). Andrographolide, a potential cancer therapeutic agent isolated from Andrographis paniculata. *Journal of Experimental*

Therapeutics and Oncology, *3*(3), 147–158. https://doi.org/10.1046/j.1359-4117.2003.01090.x

Ricci, A., Iaccheri, E., Benelli, A., Parpinello, G. P., Versari, A., & Ragni, L. (2020). Rapid optical method for procyanidins estimation in red wines. *Food Control*, *118*(April), 107439. https://doi.org/10.1016/j.foodcont.2020.107439

Roy, S., Rao, K., Bhuvaneswari, C., Giri, A., & Mangamoori, L. N. (2010). Phytochemical analysis of Andrographis paniculata extract and its antimicrobial activity. *World Journal of Microbiology and Biotechnology*, *26*(1), 85–91. https://doi.org/10.1007/s11274-009-0146-8

Shankar, M., Chhotaray, P. K., Gardas, R. L., Tamilarasan, K., & Rajesh, M. (2019). Application of carboxylate protic ionic liquids in simultaneous microalgal pretreatment and lipid recovery from marine Nannochloropsis sp. and Chlorella sp. *Biomass and Bioenergy*, *123*(February), 14–24. https://doi.org/10.1016/j.biombioe.2019.02.005

Tang, L. I. C., Ling, A. P. K., Koh, R. Y., Chye, S. M., & Voon, K. G. L. (2012). Screening of anti-dengue activity in methanolic extracts of medicinal plants. *BMC Complementary and Alternative Medicine*, *21*(1), 3. https://doi.org/10.1186/1472-6882-12-3

Thangavel, M., Umavathi, S., Thangam, Y., Thamaraiselvi, A., & Ramamurthy, M. (2015). Original Research Article GC-MS Analysis and Larvicidal Activity of Andrographis paniculata (Burm . F) Wall . Ex Nees . against the Dengue Vector Aedes aegypti (L) (Diptera : Culicidae). *International Journal of Current Microbiology and Applied Sciences*, *4*(7), 392–403.