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GC-MS analysis and quantitative phytochemical analysis of *Phyllanthus reticulatus* Poir

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Abstract
Phyllanthus reticulatus Poir is a medically important plant in the family Euphorbiaceae. Traditionally they are utilized for their various medicinal properties. The present study deals with the GC-MS analysis and quantitative phytochemical analysis of ethanolic extract of *Phyllanthus reticulatus* poir. By GC-MS analysis, thirty compounds including Pyranone, dodecane, guanosine, loliolide, and vitamin E were identified. Quantitative phytochemical analysis for alkaloids, flavonoids, and phenol was performed, and the total alkaloid content was determined using colorimetry using atropine as standard and was found to be 96.48 µg/ml. The total flavonoid content of ethanolic extract was determined using an Aluminium chloride colorimetric assay by comparing it with the standard absorbance curve of quercetin was found to be 171.71 µg/ml. The total phenolic content of ethanolic extract was determined using the Folin-Ciocalteu method by using standard absorbance cure of Gallic acid and was found to be 131.39µg/ml.

Keywords: *Phyllanthus reticulatus* poir, Neeroli, GC-MS analysis, Phytochemical analysis

1. Introduction
Using medicinal plants to prevent and treat illnesses is known as herbal medicine. It can take many forms, from the usage of standardized and titrated herbal extracts to traditional and widely used medicines from around the world. Generally speaking, a traditional medical system's enduring and widespread use rooted in culture may indicate the safety of treatments, but not their efficacy. This is particularly true in the case of herbal medicine, where traditional practices almost exclusively rely on remedies that contain active ingredients at extremely low or ultralow concentrations or that rely on magical-energetic principles [1].
The genus Phyllanthus, which contains 600 species, is a member of the Euphorbiaceae family. The shrub tree *Phyllanthus reticulatus* Poir can reach a height of ten feet. The plant can be found growing in tropical and subtropical regions in Bangladesh, India, China, Malaysia, Indonesia, the Malay Islands, northern Australia, and Africa. The plant is used in traditional medicine to treat a wide range of conditions, such as inflammation, diabetes, smallpox, diarrhea, asthma, syphilis, and gum bleeding. Analgesic and anti-inflammatory, antioxidant, antidiabetic, antidiarrheal, hepatoprotective, anti-plasmodial, antiviral, and skin-healing properties were reported by the pharmacological study on P. reticulatus [2, 3, 4].

Fig 1: *Phyllanthus reticulatus* Poir

Materials and Methods

Collection of plant material

The leaves of *Phyllanthus reticulatus* poir were collected from available surroundings and identified with standard literature and authenticated with valid voucher specimens. The Botanist, Dr. VB Sreekumar, Senior scientist, Department of Forest Botany, KFRI, Peechi, taxonomically identified the plant material. The plant material was powdered after drying for seven days in shade and stored in an air-tight container.

Extract preparation

The plant material was coarsely pulverized after being shade-dried. After weighing, moistening, and packing about 100g of dried powder in the Soxhlet extractor, the material was extracted using 600 ethanol for eighteen hours. After that, the extract was concentrated after being filtered through Whatman No. 1 filter paper.

GC-MS Analysis

Gas chromatography Mass spectroscopy analysis of ethanolic extract was performed using Shimadzu GC-MS Model number: QP2010S equipped with Column - ELITE-5MS, 30-meter length, 0.25 mm ID,0.25 µm thickness. The oven temperature was programmed from 70.000 C which is given in Table 1. An electron ionization system was used; details of the GC program are given in Table 2. Helium gas was used as the carrier gas Details of the GC-MS program are given in Table 3. Program specifications regarding Mass Spectra are depicted in Table 4. GC-MS Software is GCMS Solutions, Libraries used are NIST 11& WILEY [2, 5, 6].

Quantitative phytochemical analysis

- **Estimation of total alkaloid**

Procedure

The test sample (1 mg) was dissolved in 1 ml dimethyl sulphoxide (DMSO), added 1 ml of 2N HCl, and filtered. This solution was transferred to a separating funnel, and 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3, and 4 ml chloroform by vigorous shaking and collected in a 10-ml volumetric flask, and diluted to the volume with chloroform. A set of reference standard solutions of atropine (20, 40, 60, 80, and 100 µg) were prepared in the same manner as described earlier. The absorbance for test and standard solutions was determined against the reagent blank at 470 nm with a UV/Visible spectrophotometer.

Table 1; Temperature programme of oven.

Rate	Temperature(°c)	Hold time(min)
-	70.0	2.00
10.00	200.0	5.00
5.00	280.0	15.00

Table 2: GC programme (GC 2010).

GC-Parameters	programme
Column oven temperature	70.00 °C
Injection temperature	260.00°
Injection mode	Split less
Sampling time	2.00 min
Flow control mode	Linear velocity
Pressure	61.5 kPa
Total flow	54.0 mL/min
Column flow	1.00 mL/min
Linear velocity	36.7 cm/sec
Purge flow	3.0 mL/min
Split ratio	50.0

Table 3: GC Programme (GCMS-QP 2010)

GC-MS Parameters	Programme
Ion source temperature	200.00 °C
Interface temperature	280.00 °C
Solvent cut time	6.50 min
Detector gain mode	Relative
Detector gain	1.01 k V+0.00kV
Threshold	1000

Table 4:MS Program

Mass spectroscopy parameters	Program
Start time	6-7 min
End time	51.00 min
ACQ time	Scan
Event time	0.50 sec
Scan speed	1000
Start m/z	50.00
End m/z	500.00
Sample inlet unit	GC

- **Estimation of total flavonoids**

Procedure

10 mg Quercetin was weighed and made upto 10 ml with methanol in a 10 ml standard flask. From the above solution (1mg/ml), 1 ml was pipetted out and made up to 10 ml with methanol to get 100 µg/ml Quercetin standard solution (stock solution). From the stock solution, 0.25, 0.50, 0.75, 1.25, 1.5, 1.75, and 2ml was pipetted out and made up to 2 ml with water to get 25, 50, 75, 100, 125, 150, 175, and 200 µg/ml solutions respectively. To each of these, 4ml water was added followed by 0.3 ml 5% sodium nitrate. After 5 minutes, 0.3ml of 10% aluminum chloride solution and at the 6th minute 2ml of 1 M sodium hydroxide was added. The total volume was made up to 10ml with distilled water. A blank was prepared without the addition of aluminium chloride solution. Sample solution is also prepared similarly by taking 1 ml of 1 mg/ml solution of the sample. The solutions were mixed well and the absorbance was measured against the blank at 510nm using a UV-VIS spectrophotometer. A standard graph was plotted using concentration and absorbance.

- **Estimation of total phenol**

Procedure

10mg Gallic acid was weighed and made upto 10ml with methanol in a 10ml standard flask. From the above solution (1mg/ml), 1ml was pipetted out and made up to 10ml with methanol to get 100µg/ml Gallic acid standard solution (stock solution). From the stock solution, 0.20, 0.40, 0.60 upto 1.6 ml was pipetted out and made up to 2 ml with water to get 20, 40, 60, 80, 100, 120, 140, and 160 µg/ml solutions respectively. To the above solution, 5 ml of Follin Ciocalteau reagent was added and 4ml of 7.5% sodium carbonate solution was added after 5 minutes. The sample solution is also prepared similarly by taking 1 ml of 1 mg/ml solution of the sample. It was stirred and incubated at room temperature for 2 hours. After 2 hours, the absorbance of the solution was measured at 750nm using a UV-VIS spectrophotometer. The absorbance values were plotted against concentration and the standard graph was obtained [7, 8, 9, 10].

ResultS and Discussion

GC-MS analysis

Thirty chemicals were found in the ethanolic extract of

Phyllanthus reticulatus Poir by GC-MS analysis, shown in Table 5. Among the various compounds found are loliolide (0.82%), which has anticancer, cellular protection, and antioxidant qualities; pyranone (0.84%), which has antitumor, antibiotic, antibacterial, and antiallergic activities; and glutamic acid (0.88%), which prevents nerve damage and treats low blood sugar and epilepsy. Guanosine (15.80%) can lower oxidative stress and neuroinflammation. Phytol (10.61%) possesses anti-inflammatory qualities. Antioxidant and anti-inflammatory, vitamin E prevents platelet aggregation. Squalene (0.76%) moisturizes and shields the skin from damaging external factors.

Table 5: GC-MS analysis of ethanolic extract of *Phyllanthus reticulatus* Poir

Peak#	R. Time	Area	Area%	Height	Height%	Name	Base m/z
1	7.711	142890	0.84	52891	1.26	Pyranone	144.00
2	8.552	30977	0.18	18692	0.44	DODECANE	57.10
3	8.916	150216	0.88	45325	1.08	5-(HYDROXYMETHYL)-2-FURALDEHYDE	97.00
4	9.145	150849	0.88	49747	1.18	GLUTAMIC ACID	84.05
5	11.285	42928	0.25	30367	0.72	ACETIC ACID, NONYL ESTER	70.10
6	11.394	123253	0.72	78634	1.87	Dodecane	57.05
7	11.841	2699743	15.80	380263	9.05	GUANOSINE	57.00
8	13.010	112317	0.66	74279	1.77	Benzoic acid, 4-ethoxy-, ethyl ester	121.05
9	13.378	100422	0.59	55054	1.31	DODECANOIC ACID	73.05
10	13.810	121347	0.71	62806	1.49	HEXADECANOIC ACID, ETHYL ESTER	88.05
11	13.912	108767	0.64	71391	1.70	Undecane, 4,7-dimethyl-	57.10
12	15.741	178366	1.04	85043	2.02	Tetradecanoic acid	60.00
13	16.030	140705	0.82	67594	1.61	(-)-LOLIOLIDE	105.05
14	16.886	1297204	7.59	472583	11.24	2,6,10-TRIMETHYL,14-ETHYLENE-14-PENTADECNE	68.05
15	17.261	1013338	5.93	416375	9.91	1,2-BENZENEDICARBOXYLIC ACID, BIS(2-METHYLPROPYL) ESTER	149.00
16	17.596	444648	2.60	139534	3.32	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	81.05
17	19.089	855243	5.01	186834	4.45	9-OCTADECENOIC ACID (Z)-	73.00
18	19.886	292859	1.71	60184	1.43	HEXADECANOIC ACID, ETHYL ESTER	88.05
19	22.676	1812894	10.61	477110	11.35	Phytol	71.05
20	23.241	473349	2.77	123699	2.94	cis, cis, cis-7,10,13-Hexadecatrienal	79.05
21	23.906	279112	1.63	93461	2.22	ETHYL (9Z,12Z)-9,12-OCTADECADIENOATE #	79.05
22	28.377	187229	1.10	63746	1.52	Diisooctyl adipate	129.05
23	33.633	90483	0.53	38019	0.90	2-methyloctacosane	57.10
24	35.284	129872	0.76	52939	1.26	Squalene	69.05
25	36.567	200575	1.17	80921	1.93	2-methyloctacosane	57.05
26	40.600	1374473	8.04	291639	6.94	Vitamin E	165.05
27	43.659	655225	3.83	133162	3.17	OCTADECANAL	57.05
28	44.567	496149	2.90	96675	2.30	PALMITALDEHYDE, DIISOPROPYL ACETAL	57.10
29	45.245	628198	3.68	102353	2.44	Gamma.-Sitosterol	55.05
30	45.558	2753924	16.12	301751	7.18	N-HENTRIACONTANOL-1	57.05
		17087555	100.00	4203071	100.00		

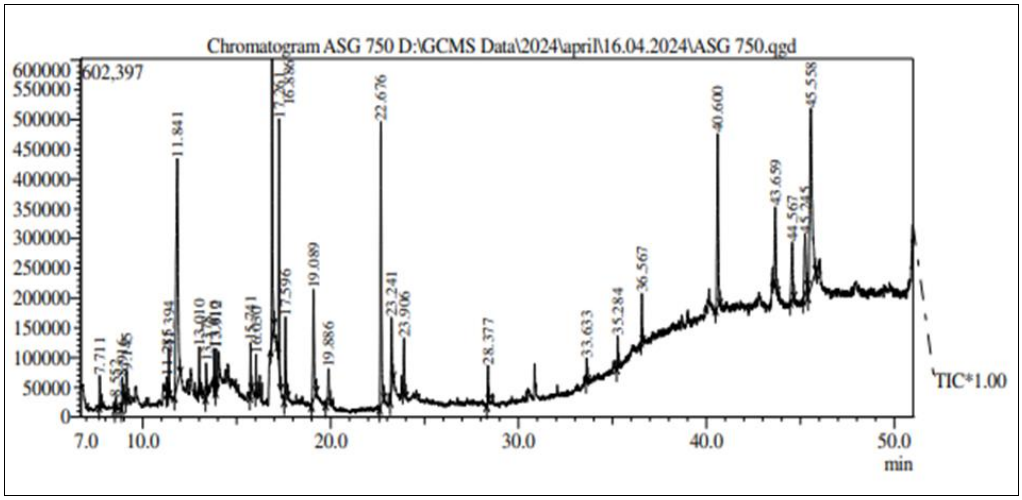

Fig 2: GC-MS chromatogram of ethanolic extract of *Phyllanthus reticulatus* Poir.

- **Estimation of total alkaloid content**

The total alkaloids in the ethanolic extract were determined using the standard Atropine (100 µg\ml).

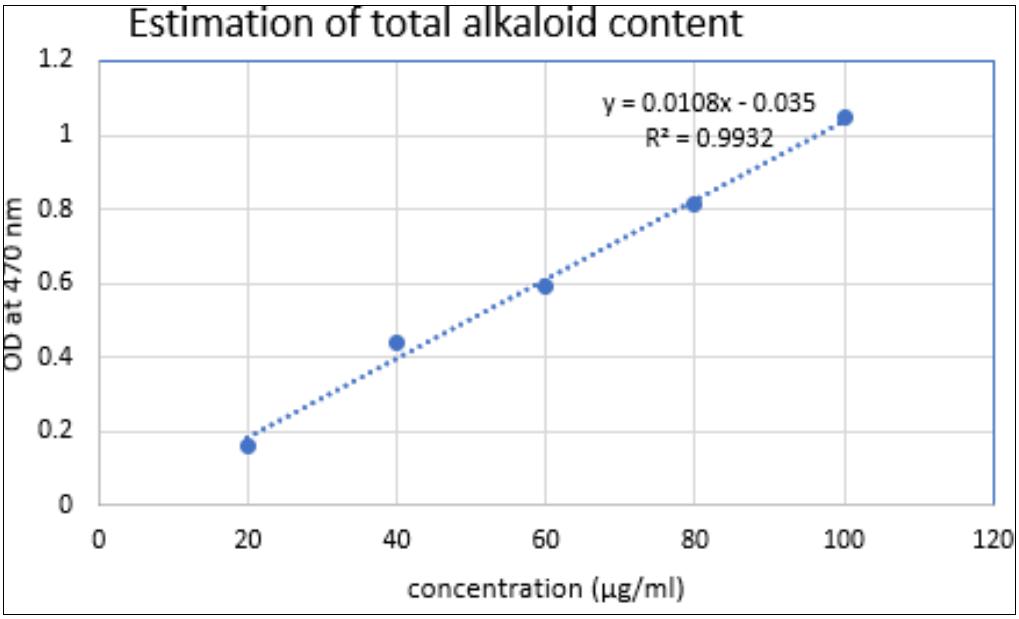

Fig 3: Standard graph of atropine

The concentration of alkaloids in atropine equivalent µg/ml of extract was found to be 66.38 µg/ml.

- **Estimation of total flavanoid content**

The total flavonoid content in the ethanolic extract was determined using the standard Quercetin (100 µg/ml).

Table 6: OD of atropine at different concentration.

Sl. No	Concentration(µg/ml)	OD
1	20	0.347
2	40	0.413
3	60	0.487
4	80	0.627
5	100	0.816
Sample		0.752

Table 7: OD of quercetin at different concentrations

Sl. NO	Concentration	OD
1	25	0.022
2	50	0.057
3	75	0.065
4	100	0.107
5	125	0.110
6	150	0.122
7	175	0.140
8	200	0.143
Sample		0.139

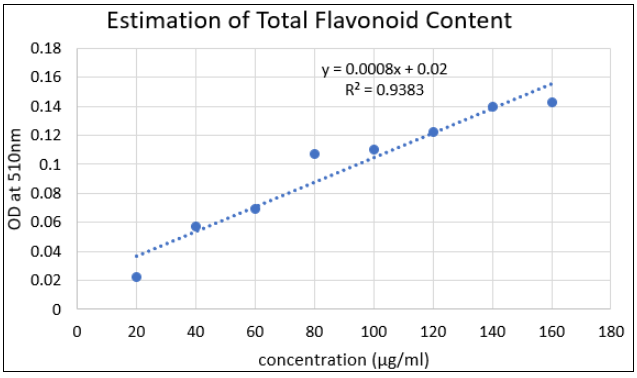

Fig 4: Standard graph of Quercetin

The concentration of flavonoid in Quercetin equivalent µg/ml of extract was found to be 148.75 µg/ml.

- **Estimation of total phenol content**

The total phenol content in the ethanolic extract was determined using the standard Gallic acid (100 µg/ml)

Table 8: The concentration of phenol in gallic acid equivalent to µg/ml of extract was found to be 131.39 µg/ml.

Sl. No.	Concentration	OD
1	20	0.16
2	40	0.44
3	60	0.59
4	80	0.81
5	100	1.05
6	120	1.22
7	140	1.37
8	160	1.48
Sample		1.28

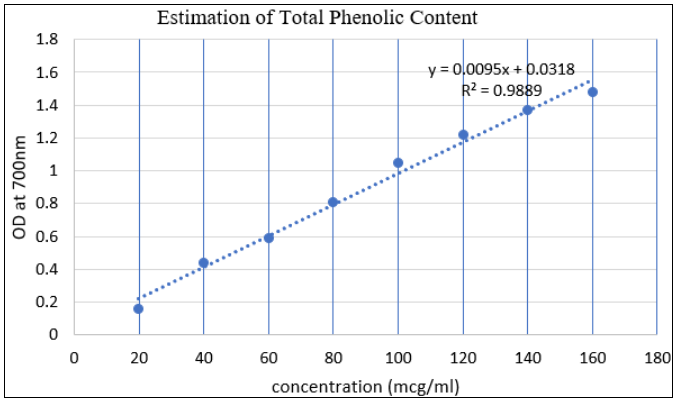

Fig 5: Standard graph of gallic acid

Conclusion

In the present study, by GC-MS analysis thirty chemical constituents were identified in ethanolic extract of *Phyllanthus reticulatus* Poir and they have various activities that can be utilized to treat various ailments. Quantitative phytochemical analysis for alkaloids, flavonoids, and phenol was performed, and the total alkaloid content was determined using colorimetry using atropine as standard and was found to be l5.349µg/ml. The total flavonoid content of ethanolic extract was determined using an Aluminium chloride

colorimetric assay by comparing it with the standard absorbance curve of quercetin was found to be 171.71µg/ml. The total phenolic content of ethanolic extract was determined using the Folin-Ciocalteu method by using a standard absorbance curve of Gallic acid and was found to be 7.20µg/ml.

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