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RESEARCH ARTICLE

Phytochemical analysis and antioxidant activity of leaf extracts of some selected plants of the family Acanthaceae

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ABSTRACT

The present era of scientific research has witnessed an enumerable amount of evidences to showcase the immense potential of medicinal plants. In the present investigation, the phytochemical analysis of *Phlogacanthus pubinervius* T. Anderson., *Adhatoda vasica* (L.) Nees,*Phlogacanthus thyrsiflorus* Nees, *Phlogacanthus curviflorus* (Wall.) Nees, and *Ruellia tuberosa* L. was carried out for the different plants extracted with methanol. Analysis was carried out to estimate the quantity of phenols, carbohydrates, tannins, flavonoids and proteins. The antioxidant property of these plants were analysed using DPPH method. The concentration of the plant samples required to decrease the DPPH concentration by 50% was calculated by interpolation from linear regression analysis and denoted IC_{50} value (µg/ml). The qualitative analysis showed the presence of alkaloids, tannins, saponins, proteins, carbohydrate and phenols in all the sample extracts. The highest amount of tannins and phenols was observed in *P. thyrsiflorus*. *P. pubinervius* (77.83%), *A. vasica* (74.81%), *P. curviflorus* (94.20%), and *R. tuberosa* (70.78%) which showed highest antioxidant activity of DPPH-scavenging at 150 µg/ml of methanol extract. The high percent of scavenging activities of those plants add value to their medicinal properties. The presence of the high amount of phytochemical compounds suggests that the plants have high amount of medicinal compounds and can be extensively used to extract the natural compounds.

Introduction

People started to use plant extracts as medicines in many of the traditional practices. Ethnopharmacology, one of the areas of Ethnobotany, is considered the scientific evaluation of traditional medicinal plants. It was suggested that ethno-mediated samples were succeeded to identify the drugs in the treatment of different ailments like gastrointestinal, inflammatory and dermatological complaints. Plant and animal products produced by different organisms have been isolated and characterized as biologically active compounds (1).

Phlogacanthus thyrsiflorus is considered to be a sacred plant in Manipur and in most of the north-eastern states of India. *P. thyrsiflorus* will usually grow up to 2.4 m, quadrangular stem, leaves of 13 to 35 cm long and oblanceolate, elliptic or oblong, acute or acuminate, and entire. Flowers are found in terminal elongated thyrsus. *Adhatoda vasica* is a common plant found in different gardens. However, the other two species, i.e., *Phlogacanthus pubinervius* and *Phlogacanthus curviflorus* are mainly forest plants and also found in wastelands. *Ruellia tuberosa* is found in the garden; but it is grown commonly as ornamental for its purple flower. *P. pubinervius,* an evergreen perennial shrub, is found to be very coloured in spring with its densely packed red-tubular flowers in its upright spike inflorescence. This shrub may grow up to 3 m tall, with quadrangular stem sand drooping leaves. *P. curviflorus* is a shrub, stems branched and grows up to 3–4 m tall. Leaves are arranged in opposite phyllotaxy and 8–9 inches long; flowers are borne on spike inflorescence at the end of the branches. Flowers are showy, tubular and reddish in colour. *R. tuberosa* is an erect, sub-erect or diffuse perennial herb which grows up to 60–70 cm tall, with four-angled stems, swollen and purplish at the nodes,

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and hairy. Roots are very slender, elongated, tuberous and white in colour. Leaves are used as liquid drink to cure gonorrhoea and ear diseases (2).

Boiled leaves of *P. pubinervius* are used to cure cough, cold, and asthma. *A. vasica* is used as a remedy for the treatment of cold, cough, chronic bronchitis and asthma (3). The principal active compounds found in *A. vasica* include alkaloids like vasicine and vasicinone. The vasicine is used as a herbal drug in India, as they are the derivatives of alkaloids, bromhexine and ambroxol (4). Another property of this herb is that it stops bleeding. All parts of this plant are used for the extraction of volatile oils and alkaloids, which show high medicinal properties (5, 6). The people use flowers of *P. thyrsiflorus* as vegetables. It shows antimicrobial properties. Fruits and leaves are used in the treatment of fever (7). In Manipur, boiled leaves of *P. curviflorus* are used as a remedy for cough and fever. Flowers are taken raw as a tonic. In Arunachal Pradesh, the people use the flowers as a condimental food. The young leaves and flowers of *P. curviflorus* var. *menchanens* are consumed as a vegetable by the people of Manipur (8). *R. tuberosa* is considered to be containing medicinal compounds that helps in stomach cancer. The dried root powder of the plant is reported to have the effect of causing abortion, and the decoction of leaves is used in treatment of bronchitis (9).

The present study also focussed on the properties of the antioxidant activities of the leaf extracts of the selected plants. As we know that in every living cell, oxygen is essential for the survival of all on this earth. Survival of life is not possible without oxygen. During the process of oxygen utilization in a normal physiological and metabolic process, approximately 5% of oxygen as per study gets univalently reduced to oxygen derived free radicals like superoxide, hydrogen peroxide, hydroxyl and nitric oxide radicals. All these radicals are known as reactive oxygen species (ROS) that exert oxidative stress towards the cells of human body rendering each cell to face about 10000 oxidative hits per second. In general, the effect of antioxidants is to break up the chains formed during the propagation process by providing a hydrogen atom or an electron to quench the free radical and receiving the excess energy possessed by the activated molecule (10).

The objective of the present study was to compare the phytochemical compounds present in the selected members of the family Acanthaceae. We also focussed on the quantification of bioactive compounds found in the plants. The study was also aimed at evaluating the antioxidant properties of the selected plants.

Materials and Methods

Collection of plant samples

Fresh and healthy plant parts of *P. pubinervius* and *P. thyrsiflorus* were collected in sterile polythene bags from Imphal east district called Tabungkhok in Manipur. *P. curviflorus* leaves were collected from Luwangsangbam near Matai Garden. *R. tuberosa* and *A. vasica* flower, stem and leaves were collected from Tabungkhok and Yaiskul of Imphal West. Collected plant parts were examined, identified and kept for experimental use in CHRIST (Deemed to be University) Botany laboratory.

Preparation of solvent extract

The cleaned, healthy plant materials were cut into small sections and dried under shade for a few days and then dried in a hot air oven. The dried materials were ground into a fine powder. The powder obtained was stored in a desiccator and kept in room temperature for extraction. The methanolic extracts of the samples were prepared by 2 g of dried powder in 20 ml of solvent. The extracts were filtered using filter paper. The extracts were tested for detecting various compounds such as flavonoids, tannins, saponins, protein, phenol, carbohydrates, etc. For quantification analysis, 5 g of the sample was extracted with methanol using soxhlet apparatus. The extracts were evaporated using a rotary evaporator (10).

Phytochemical analysis of plants extract

The preliminary phytochemical compounds were analysed using the filtered leaf extracts of the plants following the standard procedure (10).

Table 1. Qualitative phytochemical results of leaf extracts of different plant samples

Chemical compounds	Sample 1[a]	Sample 2[b]	Sample 3[c]	Sample 4[d]	Sample 5[e]
Alkaloids	++	++	++	++	++
Flavonoids	+	++	++	+	+
Tannins	++	+	++	++	+
Phenols	++	++	++	++	++
Proteins	++	+	++	++	--
Terpenoids	++	+	++	++	+
Saponins	++	++	++	++	+
Carbohydrates	++	++	+	++	++

Estimation of tannin content (TC)

Tannin content was estimated using Folin-Ciocalteu's assay (11). To 0.2 ml of the extract, 0.5 ml of the Folin-Ciocalteu's reagent was mixed and then 2 ml of sodium bicarbonate solution [20% (w/v)] was added slowly by stirring to the mixture and incubated for 2 h in dark at room temperature and the absorbance was measured at 725 nm. For quantification of the TC content in the sample, a standard calibration curve was plotted using Tannic acid (11).

Estimation of phenol content

The amount of phenol was determined by the slightly modified Folin and Ciocalteu method (11). To 200 µl of the sample extract, the volume was made up to 3 ml with distilled water, 0.5 ml of Folin Ciocalteu reagent mixture and 2 ml of 20% sodium carbonate were added. The solution was mixed thoroughly and for one min kept on boiling water bath, cooled and measured absorbance at 638 nm. Catechol dilutions were used as standard solutions. The results of phenols were expressed in terms of mg/ml of extract.

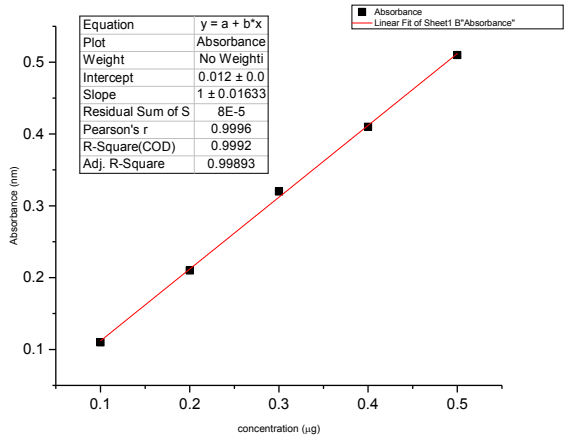

Fig. 1. Standard graph of Tannins at 725 nm wavelength using tannic acid in µg/ml.

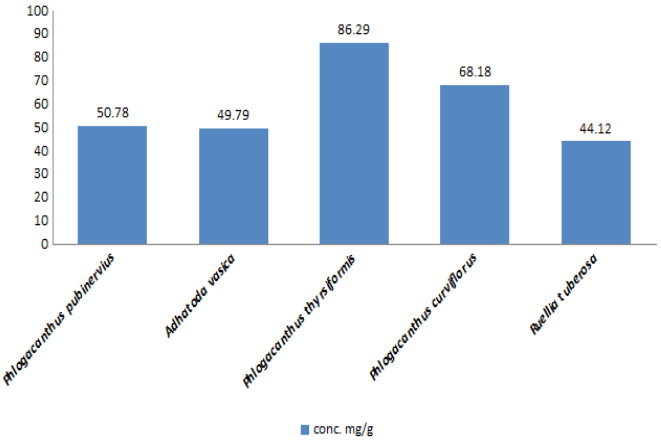

Fig. 2. The concentrations of tannins present in different plant samples.

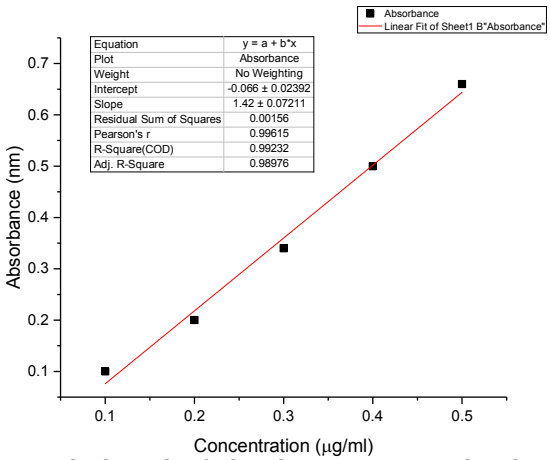

Fig. 3. Standard graph of Phenol at 638 nm wavelength using catechol in µg/ml.

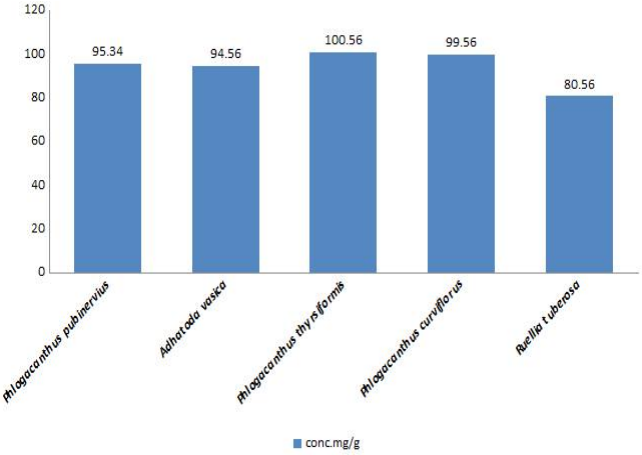

Fig. 4. Graphs showing the amount of phenol content (mg/g) in different plant samples.

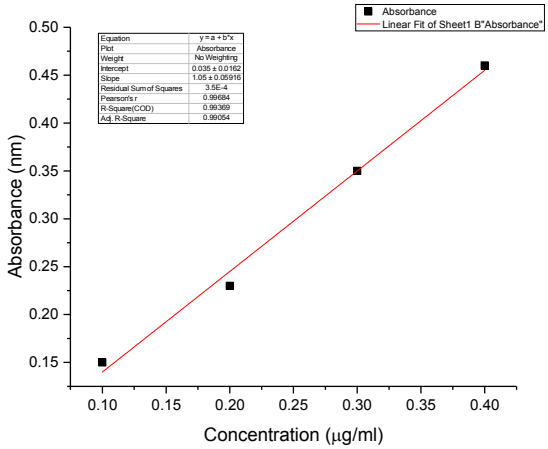

Fig. 5. Standard graph of protein showing absorbance at 595 nm using BSA µg/ml.

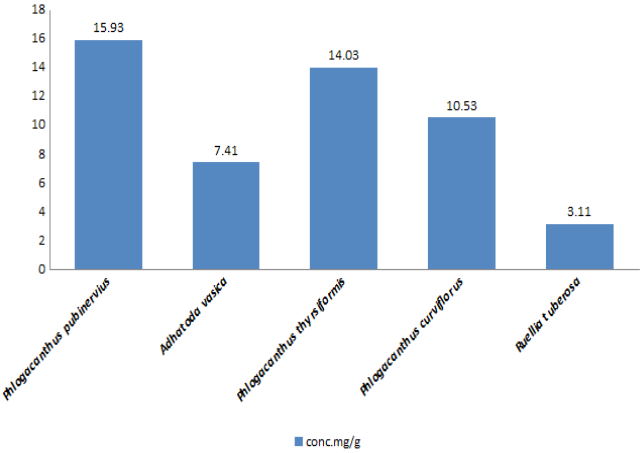

Fig. 6. Graph showing the amounts of protein content in different plant samples

Estimation of protein content

The total protein content was determined by using Bradford's method (12). To 100 µl of the sample extract, 3 ml of Bradford's reagent was added and incubated in the dark for 5 min. The absorbance was measured at 595 nm. Bovine serum albumin dilutions (0.1 to 0.5 mg/ml) were used as standard solutions.

Estimation of carbohydrate content

Phenol sulphuric acid method was used in which, 0.1 g sample was vortexed with 5 ml of 2.5 N HCl, placed the test tube in boiling water bath for 3 h, and cooled to room temperature. It was then neutralised with Na_2CO_3 until effervescence ceased. Volume was made up to 100 ml, centrifuged in 10000 rpm for 10 min and 0.2 ml of sample solution, made up to 1 ml, was kept in two separate tubes. Then, 1 ml phenol was added followed by 5 ml of 96% sulphuric acid and shaken well. After 10 min it was placed in a water bath at 25–30 °C for 20 min and absorbance was read at 490 nm (12).

Estimation of flavonoid content

The flavonoid content of each sample was carried out using the method described by Chang *et al.* (13). In 100 µl of plant sample extract, 1.5 ml of ethanol was added and was mixed thoroughly, 100 µl of 1M

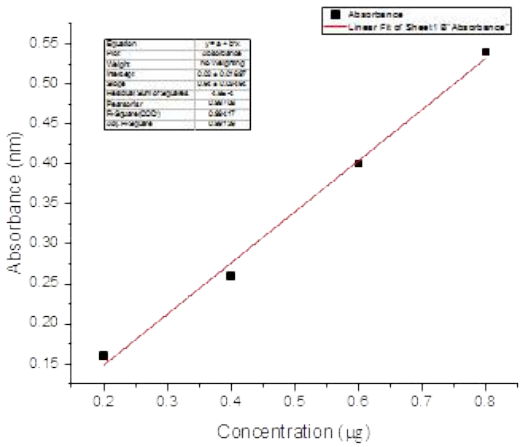

Fig. 7. Standard graph showing carbohydrates concentration using glucose in mg/ml.

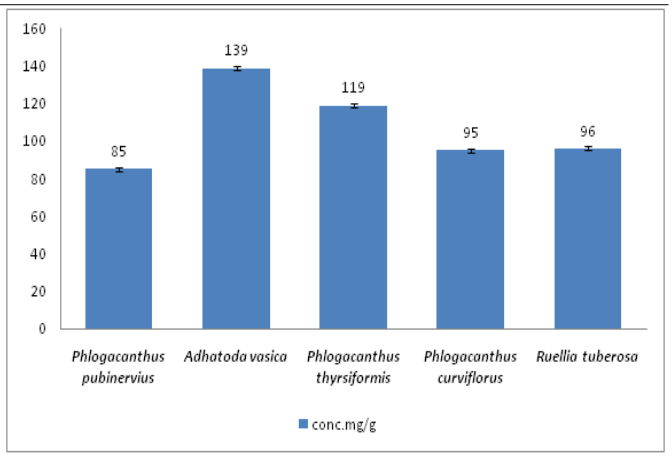

Fig. 8. Graphs showing different amounts of carbohydrates in different plant samples.

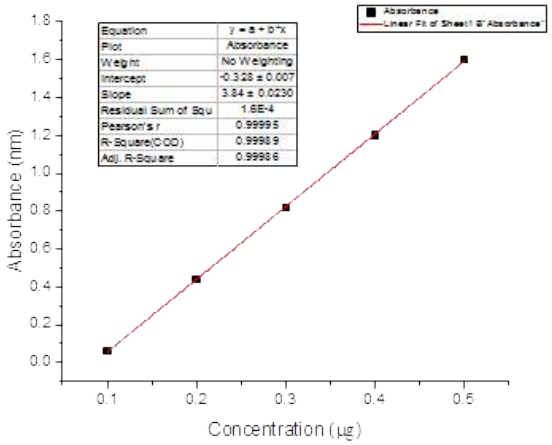

Fig. 9. Standard graph showing flavonoid content using Quercetin µg/ml.

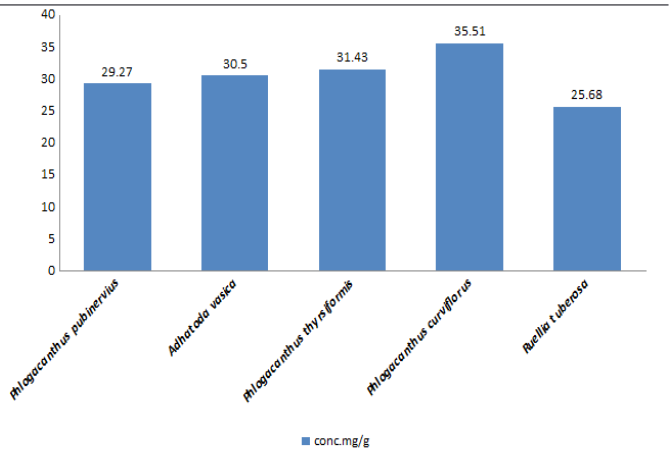

Fig. 10. Graphs showing the flavonoid content in different plant samples of crude extract.

potassium acetate was added, and 2 ml distilled water, the tubes were kept at room temperature for 40 min and read absorbance at 415 nm. Quercetin was used as the standard in a range of 2–10 µg.

GC-MS (Gas Chromatography - Mass Spectrometry) analysis

GC-MS analysis was carried out on a Shimadzu single quadrupole GCMS-QP2010SE gas chromatograph-mass spectrometer (USA) which includes an Auto sampler and a Quadrupole mass analyzer: The column used was SH-Rxi™-5Sil MS- Low-polarity phase: Crossbond™ silarylene phase 1,4-bis (dimethylsiloxy) phenylene dimethyl polysiloxane ID: 0.25 mm df: 0.25 µm Lenght: 30 m (Similar phases: DB-5ms UI, DB-5ms, VF-5ms, SLB-5ms) The carrier gas used was Helium at a flow rate of 1 ml/min.

Antioxidant activity using DPPH assay

Antioxidant activity was measured using DPPH radical-scavenging activity assay (14). Stock solution (1 mg/ml) diluted to dilutions series of 100–150–250 µg/ml with respect to solvent methanol. To an aliquot of each dilution, 3 ml of a methanolic solution of DPPH was mixed thoroughly. Mixtures were shaken well and then incubated at 37 °C in the dark for 30 min. The control contained 3 ml (v/v) methanol (1 ml) and methanolic solution of DPPH. The absorbance was measured at 517 nm against methanol blank.

Percentage of DPPH scavenging was calculated as:

DPPH radical scavenging activity (%) = [(Abs control-Abs sample)/Abs control] x 100

Results

Preliminary phytochemical analysis

Estimation of tannin

Tannins were present in all the samples extracted by methanol. More amount of tannins was present in *P. thyrsiflorus*, i.e., 86.298 ± 0.707 mg/g of crude extract. The less amount of tannins was observed in *R. tuberosa*, i.e., 44.121 ± 0.389 mg/g of crude extract of plant sample (Fig. 1, 2).

Estimation of phenol

Concentration of phenol was estimated using catechol as the standard (Fig. 3). The highest amount of phenol was present in *P. thyrsiflorus* (100.56 ± 0.94 mg/g) while the lowest amount was observed in *R. tuberosa* (80.56 ± 0.46 mg/g) (Fig. 4).

Estimation of protein

The protein present in the plants selected was measured at 595 nm absorbance. The highest amount of protein was present in *P. pubinervius* of 15.934 ± 0.213 mg in 1 g of plant sample while the lowest amount of protein in *R. tuberosa* with 3.113 ± 0.08 mg in 1 g of plant sample taken (Fig. 5, 6).

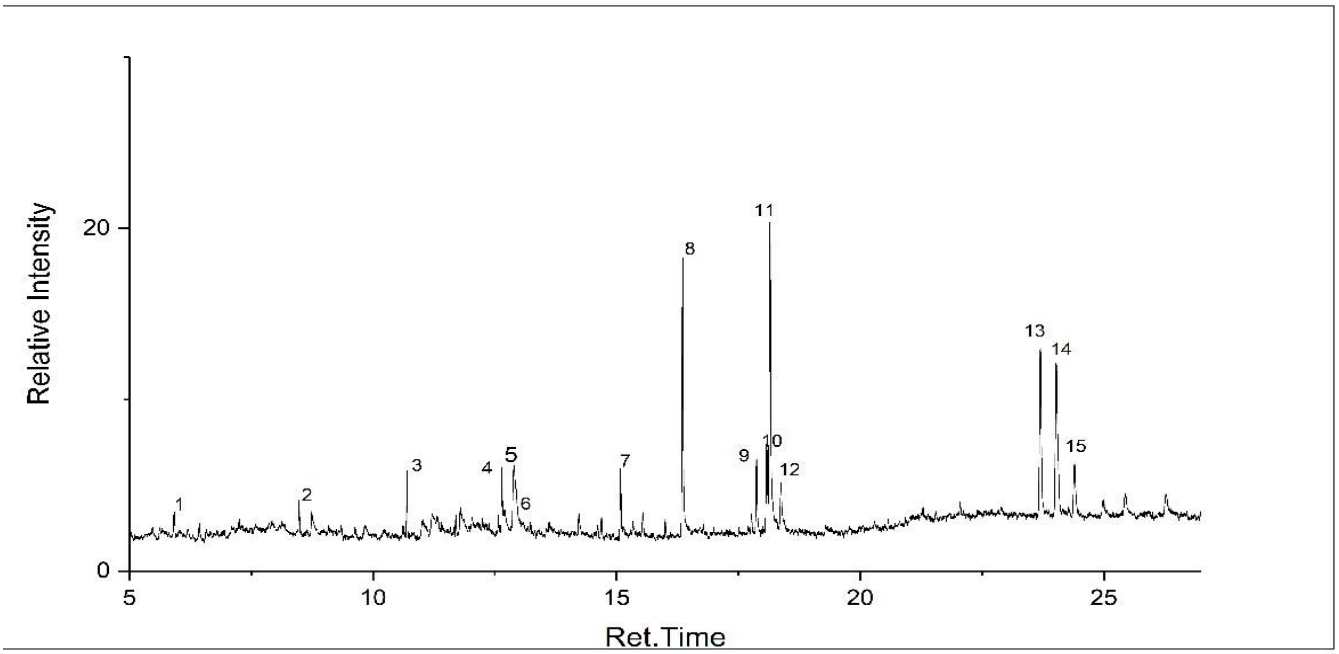

Fig. 11. GC-MS chromatogram of *Phlogacanthus thyrsiflorus.* The phytochemical compounds identified in the methanolic extract in GC-MS is given in Table 2 with respect to the peaks.

Table 2. Compounds identified in the methanolic leaf extract of *Phlogacanthus thyrsiflorus* in GC-MS

Peak#	R.Time	Area	Name
1	3.788	54.82	2-Ethylhexane
2	5.919	0.65	Decade
3	8.484	0.53	Dodecane
4	10.698	1.15	Tetradecane
5	12.646	1.06	Nonadecane
6	12.894	3.00	Hexanoic acid, 2-ethythexyl ester
7	12.945	0.55	2,4-Dimethyl-3-hexanone
8	15.083	1.48	Neophytadiene
9	16.354	6.45	n-Hexadecanoic acid
10	17.873	1.97	Phytol
11	18.081	2.48	Linoelaidic acid
12	18.147	9.48	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-
13	18.373	1.08	Octadecanoic acid
14	23.697	6.26	Chalepin
15	24.023	6.91	1,3,5(10)-Estratrien-3,17.beta,-diol, 17-acetate(ester)
16	24.400	2.14	5-Hydroxymethyl-1,1,4a-trimethyl-6-methylenedecahydronaphtl

Estimation of carbohydrate

Estimation of carbohydrates was done on different plant samples and its standard graph was prepared using glucose at 490 nm absorbance (Fig. 7). The highest amount of carbohydrates were present in *A. vasica* with 139 ± 0.20 mg in 1 g of plant sample. *P. pubinervius* exhibited less amount of carbohydrate (85 ± 0.15 mg/g of plant sample taken) (Fig. 8).

Estimation of flavonoid

Flavonoid was estimated from the selected plant samples using quercetin as standard in µg/ml with absorbance at 415 nm (Fig. 9). The highest amount of flavonoids were present in *P. curviflorus* with 105.32 ± 0.75 mg/g of plant sample and the least amount of flavonoids were found in *R. tuberosa* with 25.68 ± 0.03 mg/g of crude extract of plant sample taken (Fig. 10).

GC-MS analysis

The results of GC-MS analysis of the methanolic leaf extracts of *P. thyrsiflorus* showed the presence of various compounds such as 2-Ethylhexane, Decade, Dodecane, Tetradecane, Nonadecane, Hexanoic acid, 2-ethythexyl ester, 2,4-Dimethyl-3-hexanone, Neophytadiene, n-Hexadecanoic acid, Phytol, Linoelaidic acid, 9,12,15-Octadecatrienoic acid, (Z,Z,Z)-, Octadecanoic acid, Chalepin, 1,3,5(10)-Estratrien-3,17.beta,-diol, 17-acetate(ester) and 5-Hydroxymethyl-1,1,4a-trimethyl-6-methylenedecahydronaphtl. There were sixteen peaks with sixteen different phytochemical compounds identified and the composition determined for this plant extract corresponded to 100% of the entire GC-MS chromatogram (Fig. 11; Table 2).

The GC-MS analysis of methanolic leaf extracts of *P. pubinervius* lead to the identification of Tetradecane, Neophytadiene, 2-Pentadecanone, 6,10,14-trimethyl-, Hexadecanoic acid, methyl ester, n-Hexadecanoic acid, Phytol, 9,12-Octadecadienoic acid (Z,Z)-, 7-Tetradecenal, (Z)-, Squalene, 1,5-Cyclodecadiene and 1,5-dimethy1-8-(1-methylethylidene)-, (E,E). In the

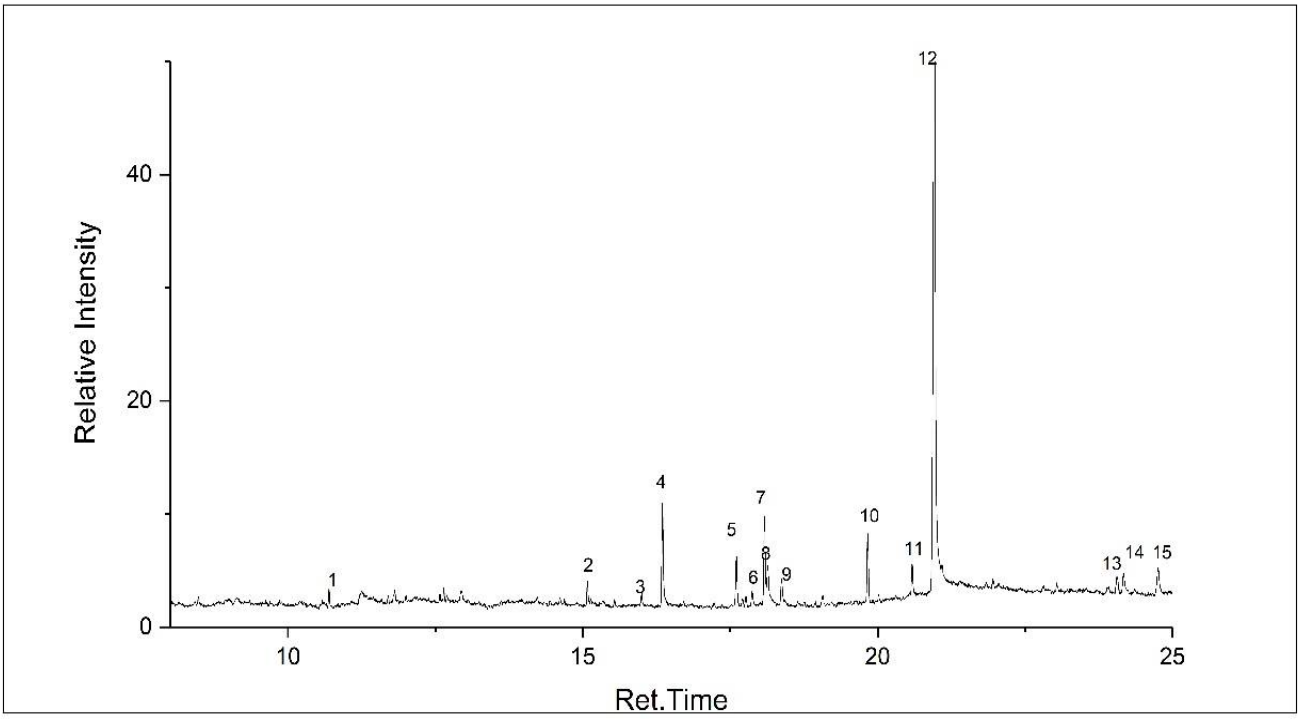

Fig. 12. GC-MS chromatogram of *Phlogacanthus pubinervius*. The phytochemical compounds identified in the methanolic extract in GC-MS is given in Table 3 with respect to the peaks.

Table 3. Compounds identified in the methanolic leaf extract of *Phlogacanthus pubinervius* in GC-MS

Peak#	R. Time	Area%	Name
1	10.696	1.69	Tetradecane
2	15.083	3.52	Neophytadiene
3	15.137	1.55	6,10,14-Trimethylpentadecan-2-one
4	16.000	2.49	Hexadecanoic acid, methyl ester
5	16.356	14.57	n-Hexadecanoic acid
6	17.872	10.76	Phytol
7	18.076	2.33	9,12-Octadecadienoic acid (Z,Z)
8	18.139	6.67	7-Tetradecenal, (Z)
9	24.069	49.51	Squalene
10	25.441	6.91	1,5-Cyclodecadiene, 1,5-dimethy1-8-(1-methylethylidene)-, (E,E)

present analysis ten phytocompounds were identified (Fig. 12; Table 3).

Phytochemical compounds such as Tetradecane, Neophytadiene, 2-Pentadecanone, 6,10,14-trimethyl-, Hexadecanoic acid, methyl ester, n-Hexadecanoic acid, Phytol, 9,12-Octadecadienoic acid (Z,Z)-, 7-Tetradecenal, (Z)-, Squalene, 1,5-Cyclodecadiene, and 1,5-dimethy1-8-(1-methylethylidene) were identified in the methanolic leaf extracts of *P. curviflorus* in GC-MS. This leaf extracts were also showed the presence of ten phytochemical compounds in the GC-MS analysis (Fig. 13; Table 4).

2-ethyhexane, Undecane, Dodecane, Tetradecane, Nonadecane, 2,4-Di--tert-butylphenol, Phthalic acid, ethyl pentadecyl ester, Nonadecane, 1-Nonadecene, Neophytadien.e, 3,7,11.15, Tetrameihy1-2-hexadecen-1-ol, 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester, Hexadecanoic acid methyl ester, n-Hexadecanoic acid, 9,12,15-Octadecattienoic acid methyl ester, (Z,Z,Z)-, 3,7,11,15-Tetramethy1-2-hexadecen-1-ol, Linoelaidic acid, 9,12,15, Octadecatrienoic acid, (Z,Z,Z)-, and Octadecanoic acid were identified in the GC-MS analysis of the leaf extracts of *Ruellia tuberosa.* This plant extract showed the presence of 19 compounds (Fig. 14; Table 5).

The results of GC-MS analysis of the methanolic leaf extracts of *A. vasica* showed the presence of bioactive compounds like 2-ethyhexane, Decane, Dodecane, Tetradecane, 2,4-Di-tert-butylphenol, 1-Tridecene, Nonadecane, 1-Tricosene, 2-Methyltetracosane, Neophytadiene, l Hexadecanoic acid, methyl ester, n-Hexadecanoic acid, 1H-Pyrrolo[2,1-b]quinazolin-9-one, 3-hydroxy-2,3-dihydro-, 8,11,14-Eicosatrienoic acid, methyl ester, Phytol, Linoelaidic acid, 8,11,14-Eicosatrienoic acid, (Z,Z,Z)-, Octadecanoic acid, Squalene, and gamma.-Sitosterol. This plant extract showed the presence of 20 compounds. More number of phytochemical compounds were found in this plant when compared to other plants studied in the present investigation (Fig. 15; Table 6).

Antioxidant activity using DPPH

The percentages of DPPH scavenging against the concentration of samples were plotted. The concentration of the samples required to decrease the

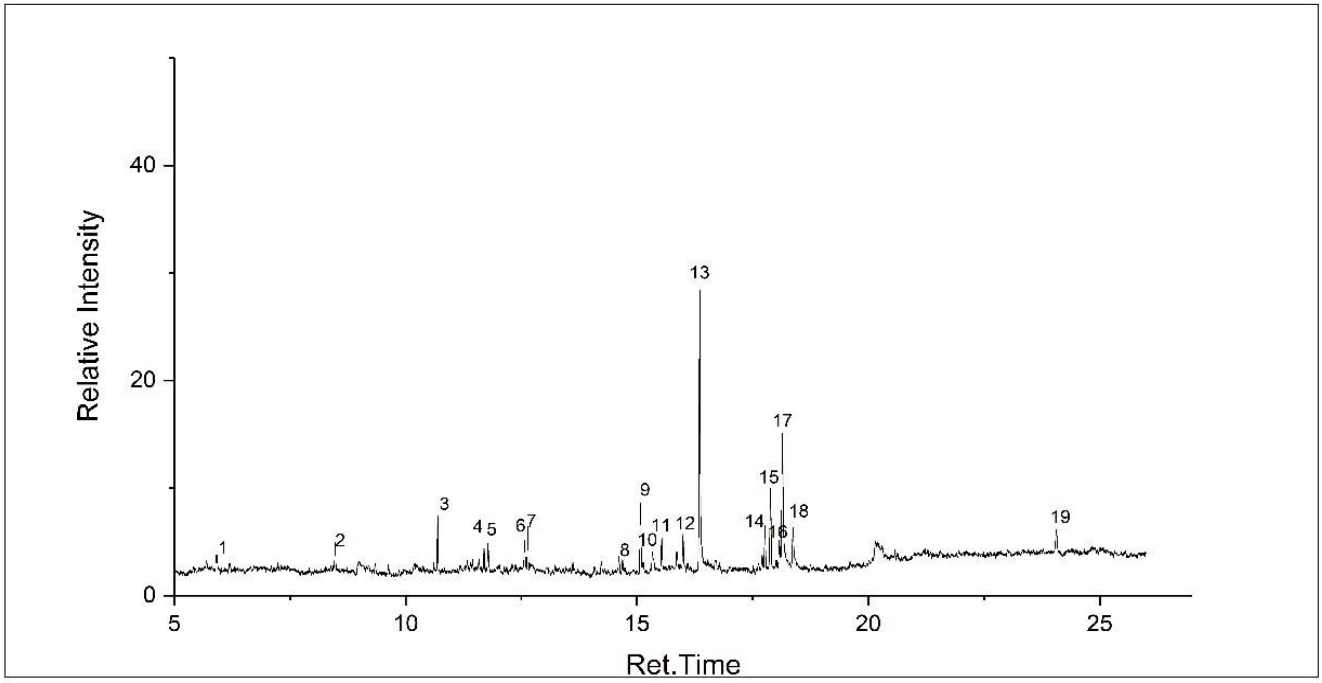

Fig. 13 . GC-MS chromatogram of *Phlogacanthus curviflorus.* The phytochemical compounds identified in the methanolic extract in GC-MS is given inTable 4 with respect to the peaks.

Table 4. Compounds identified in the methanolic leaf extract of *P. curviflorus* in GC-MS

Peak#	R.Time	Area%	Name
1	10.696	1.69	Tetradecane
2	15.083	3.52	Neophytadiene
3	15.137	1.55	6,10,14-Trimethylpentadecan-2-one
4	16.000	2.49	Hexadecanoic acid, methyl ester
5	16.356	14.57	n-Hexadecanoic acid
6	17.872	10.76	Phytol
7	18.076	2.33	9,12-Octadecadienoic acid (Z,Z)
8	18.139	6.67	7-Tetradecenal
9	24.069	49.51	Squalene
10	25.441	6.91	1,5-Cyclodecadiene, 1,5-dimethyl-8-(1-methylethylidene)-, (E,E)

DPPH concentration by 50% was estimated by the interpolation from linear regression analysis and denoted IC_{50} value (µg/ml). *P. pubinervius* (77.83%), *A. vasica* (74.81%), *P. curviflorus* (94.20%), and *R. tuberosa* (70.78%) have shown highest antioxidant activity of DPPH-scavenging at 150 µg/ml of methanol extract comparing with the ascorbic value percentage of (98.81%) (Table 3). *P. thyrsiflorus* (93.95%), *Oenanthe javanica* (94.45%) and *Enydra fluctuans* (93.45%) have shown highest percentage when 100 µg/ml of methanol crude sample extract was taken comparing with ascorbic value percentage of (97.53%) (Table 7). DPPH scavenging activity at 150 µg/ml was more suitable than 100 or 250 µg/ml (Table 7) and showed more antioxidant property when compared with ascorbic acid.

Statistical analysis

The statistical analysis was carried out by evaluating the lack of fit, coefficient of regression (R^2) and the Fisher test value (F-value) obtained from the analysis of variance (ANOVA) considering $P<0.05$ as significant using the software SPSS (Table 8). Three-dimensional response plots were generated by keeping one response as dependant variable and plotting it against two factors (independent variables). Statistical analysis clearly showed that there were no significant differences in the DPPH activity and the concentrations of phenol and flavonoids. The analysis indicated that in plant samples the percent of increase in DPPH activity were positively correlated with the increase in the concentration of phenols and flavonoids.

Discussion

The people in developing countries are very much depended on plants for their livelihood. The traditional herbal medicines play an important role in their health and in the treatment of many infectious diseases. The population of the rural areas are more exposed to traditional ways of treatment because of its easy availability and cheaper cost. Phytochemical screening is of very important in identifying the new sources of plant products which are therapeutically and industrially significant for its medicinal properties to make the best and judicious use of available natural wealth (15). The phytochemical screening of the extracts showed the presence of tannins, flavonoids, proteins, carbohydrates, phenol in methanolic extract of the plant materials.

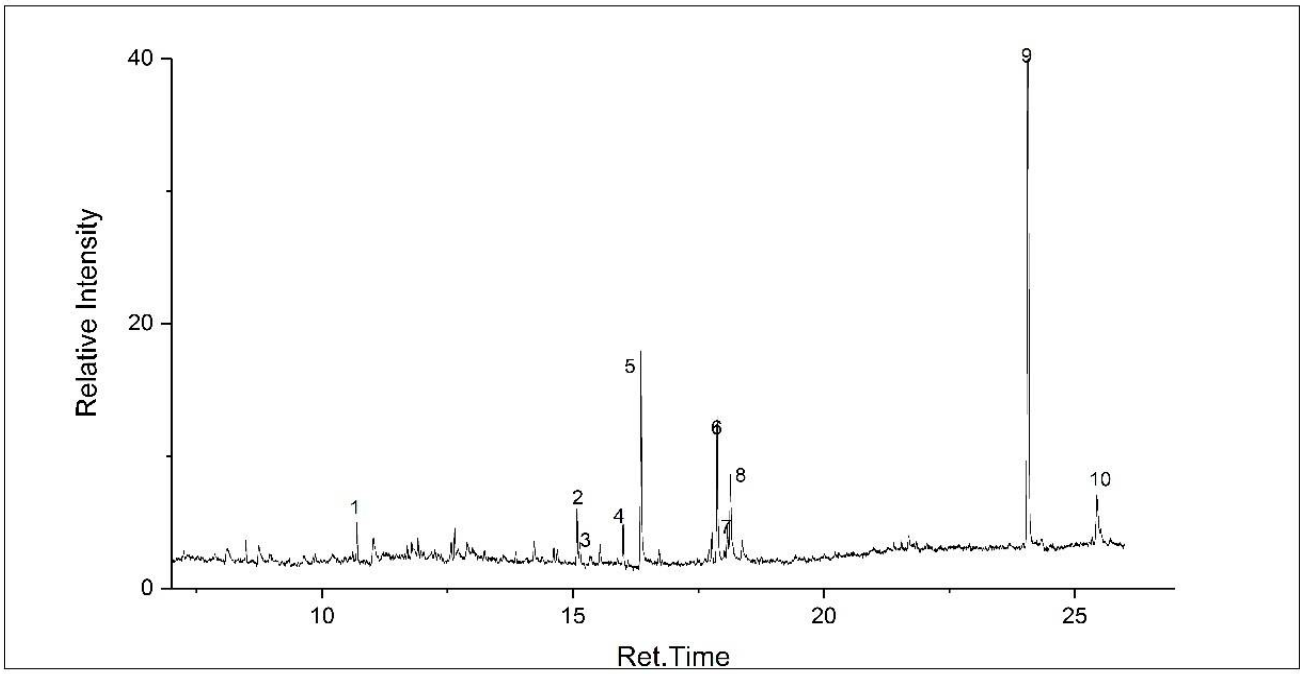

Fig. 14 . GC-MS chromatogram of *Ruellia tuberosa.* The phytochemical compounds identified in the methanolic extract in GC-MS is given in Table 5 with respect to the peaks.

Table 5. Compounds identified in the methanolic leaf extract of *R. tuberosa*in GC-MS

Peak	R. Tirne	Area %	Name
1	3.792	61.27	2-ethyhexane
2	5.916	0.65	Undecane
3	8.477	0.87	Dodecane
4	10.692	1.59	Tetradecane
5	11.695	0.74	Nonadecane
6	11.784	1.18	2.,4-Di--tert-butylphenol
7	12.575	0.97	Phthalic acid, ethyl pentadecyl ester
8	12.641	1.2	Nonadecane
9	14.613	0.55	1-Nonadecene
10	15.077	2.55	Neophytadien.e
11	15.534	1.1	3,7,11.15-Tetrameihy1-2-hexadecen-1-ol
12	15.861	0.52	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester
13	15.996	1.05	Hexadecanoic acid. methyl ester
14	16.355	10.98	n-Hexadecanoic acid
15	17.764	1.6	(Z,Z,Z)-9,12,15-Octadecatrienoic acid, methyl ester
16	17.886	3.33	3,7,11,15-Tetramethy1-2-hexadecen-1-ol
17	18.08	1.02	Linoelaidic acid
18	18.144	6.36	Octadeca-9,12,15-trienoic acid
19	18.37	1.63	Octadecanoic acid

The quantitative phytochemical analysis has revealed that *P. thyrsiflorus* contains a high amount of tannins. Tannins are phenol compounds with the benzene ring to which a hydroxyl group is attached. They are soluble in water and will precipitate alkaloids, nitrogenous bases and some glycosides when combined with other plant products in their powdered form. Tannins are often used to antidote alkaloid poisoning. This helps in pest prevention and attack of diseases on these plants. Total phenolic content in methanol extract as per our study showed high content in most of the samples but the highest amount was observed in *P. thyrsiflorus*. Phenols are reported as an essential plant compound because of their free radical scavenging ability due to their hydroxyl groups. Phenolic compounds may contribute directly to antioxidant action (11, 16).

Protein and carbohydrate estimation carried out in the plant samples can be related to maintaining stability and shelf life of the respective plant products. *P. pubinervius* had the highest protein content indicating that stability and shelf life is much well developed in *Phlogacanthus* while *A. vasica* had the high carbohydrate content.

From this study, it was concluded that the presence of various phytochemicals, mainly the flavonoids and phenols might be responsible for the

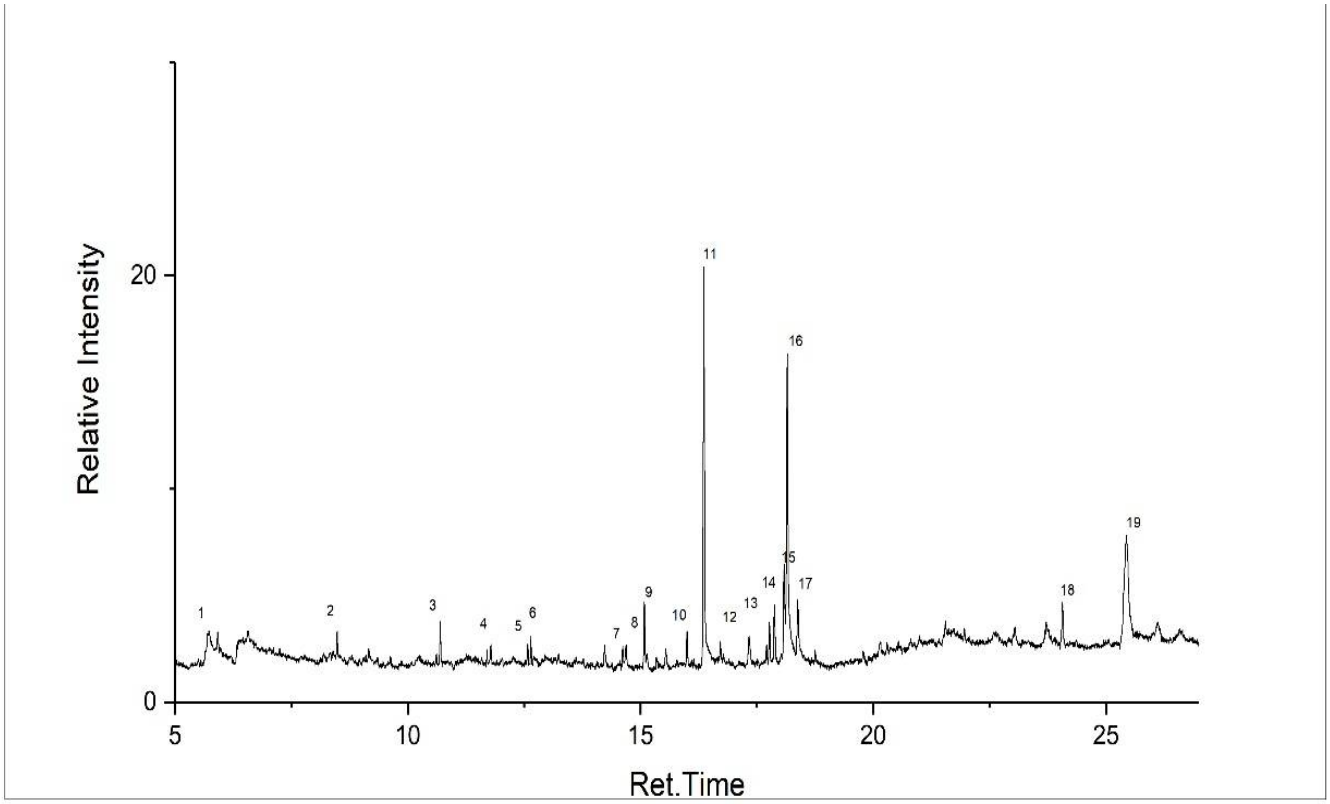

Fig. 15. GC-MS chromatogram of *A. vasica.* The phytochemical compounds identified in the methanolic extract in GC-MS is given in Table 6 with respect to the peaks.

Table 6. Compounds identified in the methanolic leaf extract of *A. vasica* in GC-MS

Peak#	R.Time	Area%	Chemical compounds
1	3.782	62.32	2-ethyhexane
2	5.918	0.44	Decane
3	8.484	0.40	Dodecane
4	10.697	0.66	Tetradecane
5	11.788	0.76	2,4-Di-tert-butylphenol
6	12.577	0.30	1-Tridecene
7	12.645	0.39	Nonadecane
8	14.617	0.43	1-Tricosene
9	14.690	0.55	2-Methyltetracosane
10	15.081	1.26	Neophytadiene
11	15.999	0.71	Hexadecanoic acid, methyl ester
12	16.360	8.99	n-Hexadecanoic acid
13	17.331	0.97	2,3-Dihydro-3-hydroxypyrrolo(2,1-b)quinazolin-9(1H)-one hydrochloride
14	17.767	0.82	8,11,14-Eicosatrienoic acid, methyl ester
15	17.875	1.34	Phytol
16	18.087	2.36	Linoelaidic acid
17	18.150	8.68	8,11,14-Eicosatrienoic acid
18	18.375	1.42	Octadecanoic acid
19	24.061	1.01	Squalene
20	25.436	6.18	Clionasterol (Gamma-Sitosterol)

free radical scavenging activity of the selected plants pointing to their use as a potential source of natural antioxidant for the treatment of free-radical and age-related diseases. In the present scenario, the study of antioxidants has got very significant role because of its health benefits. The antioxidants belong to a diverse group of chemical compounds which act as a protection to the body from oxidative damage induced by free radicals and reactive oxygen species by suppressing their formation acting as scavengers and acting as their substrate (14). The best known natural antioxidants include hydrophilic compounds like vitamin C, thiols and flavonoids; and lipophilic compounds, i.e., vitamin E, vitamin A, and carotenoids. Because of the complex nature of phytochemical extracts of plants, which contain different functional groups, polarity and chemical behaviour, it could lead to scattered results, depending on the test employed (16). The DPPH free radical scavenging assay is one of the most widely used methods to evaluate antioxidant activity of plant extracts. In the presence of an antioxidant, DPPH radical form a stable molecule by gaining one more electron or hydrogen atom from the

Table 7. Percentage of DPPH scavenging activity shown in different plant sample extracts of methanol in different concentrations

Sample	DPPH scavenging activity (%) at different concentrations		
	100 µg/ml	150 µg/ml	250 µg/ml
Phlogacanthus pubinervius	77.83 ± 0.535	57.93 ± 1.135	77.83 ± 1.207
Adhatoda vasica	74.81 ± 1.213	42.56 ± 1.300	74.81 ± 1.096
Phlogacanthus thyrsiflorus	92.94 ± 0.970	93.95 ± 0.592	92.94 ± 2.484
Phlogacanthus curviflorus	94.2 ± 0.529	75.81 ± 0.531	94.2 ± 3.078
Ruellia tuberosa	70.78 ± 4.410	35.76 ± 1.559	70.78 ± 1.556

(Mean ± standard deviation, n = 3)

Table 8. ANOVA Table showing the significant difference in DPPH scavenging activity between the different plant samples

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	4623.615[a]	6	770.603	4.607	.026
Intercept	85152.282	1	85152.282	509.076	.000
concentration	1373.904	2	686.952	4.107	.059[b]
plant samples	3249.711	4	812.428	4.857	.028[a]
Error	1338.147	8	167.268		
Total	91114.044	15			
Corrected Total	5961.762	14			

[a] Statistically significant difference is observed if P<0.05; [b] not statistically significant difference is observed if P>0.05

antioxidant and the UV absorbance decreases which signifies the scavenging activity of natural products as well as synthetic compounds. Pourmorad et *al.* (17, 18) reported that extracts of *Syzygium operculatus*, *Astilbe rivularis* and *Mallotus philippnensis* showed potent radical scavenging activity with EC_{50} close to that of ascorbic acid. Lower EC_{50} value indicates higher antioxidant activity. A linear relationship between total phenol and antioxidant activity of plant species have been reported in many of the plant species and it is attributed to the scavenging ability of their phenolic hydroxyl groups (15, 16, 19).

In the present study, antioxidant assays indicate that plant extracts showing higher antioxidant activity and higher phenol and flavonoid contents could be significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses and its related disorders. The medicinal plants used in this study have showed potent antioxidant activity but there is limited scientific evidence on those plants. So, further study is to be conducted to prove their potency. Further investigation is to be carried out to isolate and identify the components responsible for the antoxidative activity that are currently unclear.

Conclusion

In the present study, *P. thyrsiflorus* had more phytochemical contents than any other selected plants from Acanthaceae. It has most content in phytochemicals, and the same has been used for cooking, medicinal, oral ingestion, etc on various purposes. The present study has revealed the presence of phytochemical compounds present in the plant extracts. All of these medicinal plants are useful in new drug development and in pharmacological industries and for the benefit of today's world. However, there are many species of genus *Phlogacanthus* that are important and not fully recorded. With the ongoing research on these plants, it may be successful in finding out suitable compatible medicines from all these medicinal plants and thus helpful in the future.

Authors' contributions

Both authors have made a significant scientific contribution to the research described in the manuscript. Both authors performed the experiment, analysed the data and wrote the manuscript.

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Conflicts of Interest

The authors declares that they have no competing interests.

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