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15. Phytochemical Analysis, Antioxidant Activity and Trace Element Analysis of *Phlogacanthus thyrsiformis* Nees

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Abstract:

Herbal drugs have gained a great popularity in recent years. Phlogacanthus thyrsiformis is an important traditional medicinal plant that has been used against various diseases. Qualitative phytochemical content was done using standard protocols. Quantitative analysis of Protein, carbohydrate, total phenol and total flavonoid were done using standard protocols. The antioxidant properties were measured by ferric reducing antioxidant power assay, 1,1-diphenyl-2-picryl-hydrazyl, and 2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulfonate) assays. The analysis of elements was performed through atomic absorption spectrophotometry. The flower extract showed rich source of protein (323.4±8.62µg protein/mg crude extract) carbohydrate (be 224.67±2.162µg carbohydrate/mg crude extract) phenolics (123.68±2.95 µgGAE/mg plant extract) and flavonoids (45.85±1.26 µg quercetin/mg plant extract). The extract also shows a potent antioxidant activity. Trace element analysis shows that the tested elements were below the permissible limits. This suggests P. thyrsiformis as a great source of medicine, though further research needed.

Keywords:

P. thyrsiformis, edible flower, phytochemicals, trace element, GC-MS.

15.1 Introduction:

Development of drugs from medicinal plants or natural products has gained popularity, notably in the pharmaceutical industry. The use of herbal products may be attributed to unmet therapeutic requirements, lesser side-effects, effective therapy, diverse biological activities and use of novel bioactive natural compounds as biochemical probes, the development of novel active compounds (Hosseinzadeh *et al*., 2015). *Approximately* 80% of the world's population relies on traditional practitioners for remedy and cure of various diseases (Sandhya *et al*., 2009). The healing property of the plants might be due to their phytochemical content and presence of secondary metabolites (Savithramma *et al*., 2011). Phytochemicals are not directly related to human health but are necessary to remove harmful chemicals from our bodies. Free radicals generated, as a result of stress and metabolic activity in our body results in various diseases and the endogenous antioxidant system is not sufficient to give protection to excess harmful free radicals (Poljsak *et al*., 2013). Supplementation of exogenous antioxidants might be a promising way to combat the

undesirable effects of free radicals (Ozougwu, 2016). Plants have an abundant source of antioxidants and phytonutrients that help plants to fight against several diseases (Sen *et al*., 2010). Since ancient times, ethnic tribal groups include a variety of plants in their food. In recent years, there is a rise in interest in edible flowers as important sources of dietary antioxidants (Kaisoon *et al*., 2011). *Phlogacanthus thyrsiformis* Nees commonly known as 'titaphul' (Assamese) or 'basikhar' (Bodo) belonging to the family Acanthaceae, is a 4-7 ft. shrub, native to India with great medical importance. The bitter taste of the flower not only suits the taste buds of different people but also has a tremendous medicinal value which has been traditionally used to cure many diseases (Daimari *et al*., 2019; Swargiary *et al*., 2019). The wild edible plant is widely consumed by the dwellers of Assam. Traditionally, the flower part of *P. thyrsiformis* is boiled with powdered rice grains and 5-6 tablespoon alkali solution. Different parts of *P. thyrsiformis* are known for the treatment of intestinal worm, rheumatism, cough, cold diabetes and many more (Dutta *et al*., 2016; Daimari *et al*., 2019). Heavy metals are used in many industrial applications and are widely spread. Excess amounts of accumulation in agricultural land irrigated by wastewater may affect the quality of the food. (Chandel *et al*., 2020). It is very much necessary to check the heavy metals of edible food plants on a time-to-time basis. Therefore, the present study was designed to explore its phytochemicals, antioxidant properties, heavy metal analysis, and its probable active compounds.

15.2 Materials and Methods:

15.2.1 Collection and Identification of Plant:

The flower part of *P. thyrsiformis* (BUBH2018028) was collected from Debargaon area of Kokrajhar district, Assam the help of taxonomic expert. The collected plant part was cleaned with distilled water and allowed to dry in a hot-air oven at ≥50° C.

The dried flower was ground to smaller fine particles with the help of mechanical grinder immersed fully in 80% methanol and stand for 72 hours with constant stiring. The solvent was filtered and the filtrate so obtained was concentrated using Rotavapour. The semi-solid, *P. thyrsiformis* methanolic extract (PTME) so yield is stored at -20℃ for further use.

Qualitative Phytochemical Tests:

PTME was tested for the presence of phenol, flavonoids, saponins, reducing sugar, tannins, protein, alkaloids, and glycosides, using the methods of Trease and Evans (2002) and Sofowara (1993).

15.2.2 Quantitative Phytochemical Analysis:

Protein test:

Protein content of PTME was done using the Folin-phenol method (Lowry *et al*., 1951). The values were expressed in µg/mg crude extract.

Carbohydrate test:

The carbohydrate content of PTME was done following the method described by Sadasivam and Manickam (2008). The values were expressed in µg/mg crude extract.

Test for Phenol:

The estimation of total phenolic content (TPC) was done following Iloki-Assanga *et al.* (2013). The amount of phenolic content was calculated from the standard curve of gallic acid, and the result was expressed as µg gallic acid equivalent (µgGAE) per mg PTME.

Test for Flavonoid:

Flavonoid content of PTME was done using the method described by Ordonez *et al*. (2006). The TFC was calculated from the standard curve of quercetin. The values are represented as µg quercetin equivalent (µgQE) per mg PTME.

15.2.3 Antioxidant study:

Total antioxidant Capacity (TAC):

TAC was estimated following the method of Huda-Faujan *et al*. (2009). TAC was expressed as µg ascorbic acid equivalent (µgAAE) per mg PTME.

Ferric- reducing power assay (FRAP):

FRAP assay was conducted following the method of Benzie and Strain (1999). The standard curve of FeSO4 was prepared and the absorbance was read at 593nm. The values were expressed as µg Fe^{2+}equivalent (FE)/mg plant extract.

Diphenyl -1- picryl-hydrazyl-hydrate assay (DPPH):

DPPH free radical scavenging activity of PTME was estimated following Mamta *et al*. (2015). The absorbance was read at 517nm. The scavenging activity of PTME was calculated as follows:

$$\text{DPPH activity} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}}$$

Where, Abs control is the absorbance of DPPH and methanol
Abs sample is the absorbance of DPPH and ascorbic acid or plant extract.

Thiobarbituric acid reactive species assay (TBARS):

Lipid perodixation scavenging activity of the plant was studied following the method of Ohkawa *et al*., (1979). To measure the lipid peroxide formation, egg yolk homogenate was used as lipid-rich media. Lipid peroxidation scavenging activity was calculated following the same calculation of the DPPH assay.

2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulfonate) assay (ABTS):

ABTS activity of PTME was studied following Re *et al*., (1999) using gallic acid as a standard. The ABTS activity was calculated following the calculation of the DPPH assay.

15.2.4 Trace element analysis:

Four harmful elements, such as Chromium (Cr), Nickel (Ni), Lead (Pb), Cadmium (Cd) and three less toxic elements such as Copper (Cu), Zinc (Zn) and manganese (Mn), were analysed using Atomic Absorption Spectrophotometer (AAS) following Zheljazkov and Nielson (1996). Briefly, 1 g of powder of PTME was digested with conc. HNO_3, at 90˚c for 45 minutes.

The temperature is then increased up to 100˚c and boiled for 6-7 hours by adding 5 ml HNO_3 up to total digestion. The digestion was continued until the extract becomes colourless. The clear solution was then filtered by Whatman filter No.1 and make upto 100ml with distilled water. The values were expressed in parts per million.

15.2.5 GC-MS analysis:

The phytochemical components of PTME were analyzed by GC-MS system (TQ-8030 Shimadzu Corporation Kyoto, Japan) (Kalita *et al*., 2016). The Mass spectral scan range was set at (0-700) m/z. The identification of compound was done comparing the spectra with the databases (NIST-11) using probability-based algorithm.

15.2.6 Statistical Analysis:

Statistical analysis for all the data was carried out using in MS. Excel 2007, Origin Pro-8.5 software (OriginLab Corp., USA).

All the experiments were performed in triplicates and data are presented as mean standard deviation (SD). Significant study was performed using one way ANOVA, followed by Tukey test.

Table 15.1 Qualitative analysis of Phytochemicals

Test	Methodology	Observation	Presence/Absence
Protein	Ninhydrin test	Violet color	+
Carbohydrate	Fehling's test	Red brick ppt.	+
Phenol	Folin-phenol	Blue-green color	+
Flavonoid	Acids test	Orange color	+
Saponin	Vigorous shaking	Constant foam	-
Tannin	Ferric chloride test	Blue-black, green or blue green.	+

Test	Methodology	Observation	Presence/Absence
Alkaloids	Mayer's test	Yellow cream ppt.	+
Glycosides	Salkowski test	Upper layer blueish red to violet color lower layer yellow to green	+

'+' indicates presence and '–' indicates absence of tested phytochemicals

15.3 Results and Discussion:

Plants are rich in phytochemicals. The screening of Phytochemicals was done for the presence of protein, carbohydrates, phenol, flavonoid, saponins, tannins, glycosides, and alkaloids. The presence of tested phytochemicals was seen in PTME except saponin which was seen to be absent (Table 15.1). Plants are thought to have therapeutic properties because of the phytochemicals present in them. *P. thyrsiformis* was analyzed for its moisture content. The moisture content of the plant was found to be 43±13.29 % and the crude extract so obtained was 45±5.3 % after the three rounds of extraction. Fig. 15.1 shows the moisture content, crude extract, total protein content, and carbohydrate content. In a similar study conducted by Primitivo *et al.*, 2022, moisture content was reported to be 39.14 ± 1.34 % in the whole flower of *Helichrysum italicum*. On the contrary, range of moisture content was found to be 75.7-96.2% in a study conducted on thirteen edible flowers in Japan (Chensom *et al.*, 2019). The crude extract was seen to possess a high amount of protein (323.4±8.62µg protein/mg crude extract). In a similar study conducted in Mexico, in wild edible flowers, the protein content ranges from 113 to 275 ug/mg (Sotelo *et al.*, 2006). Our study revealed a carbohydrate content of 224.67±2.162µg carbohydrate/mg crude extract. A significant difference was observed between carbohydrate and protein content of *P. thyrsiformis* at P≥0.05

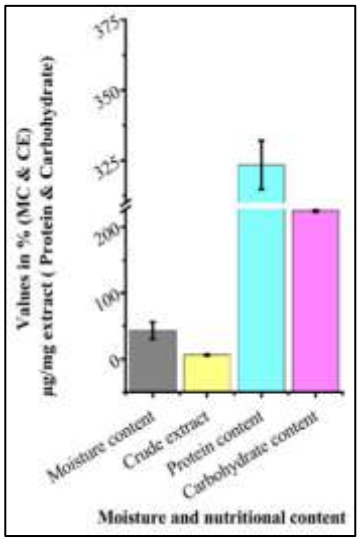

Figure 15.1. Phytochemical content of the plant. Values are represented as mean of triplicates with ± standard deviation.

PTME also shows a substantial amount of Phenol and flavonoid content. Our study revealed the phenolic and flavonoid content to be 123.68±2.95 µgGAE/mg plant extract and 45.85±1.26 µg quercetin/mg plant extract respectively. The phenolic content was seen to be higher than the Flavonoid content. In a similar study conducted by Zheng *et al*., 2018 in the flower of *Helichrysum bracteatum*, the phenolic content (72.38 ± 3.32 mg/GAE) was almost 2.5 times the flavonoid content (28.59 ± 0.4mg CAE/g). Many researchers confirmed the phenolic compounds to be comparatively higher than the flavonoid content. (Pang *et al*., 2018; Siatka and Kašparová 2010;).

Antioxidant activity of any substance is primarily due to its redox characteristics, which is crucial for the adsorption and neutralisation of free radicals, quenching of singlet or triplet oxygen or the breakdown of peroxides (Saritha and Saraswati, 2014). Additionally, PTME is reported to have good antioxidant properties. The IC_{50} value for PTME and standard reference chemical gallic acid for DPPH assay were found to be 23.34±0.34 µg/ml and 3.44±0.20µg/ml respectively. In a similar study conducted by Badakhshan Mahdi-Pour *et al*., 2012) the flower extract of *Lantana camara* had a good DPPH activity with an IC_{50} of 28.92±0.19 µg/ml. A concentration-dependent inhibition of lipid peroxidation was observed for TBARS assay The IC_{50} of the plant extract and standard chemical was found to be 153.46±1.24 µg/ml and 37.1±0.13 µg/ml respectively. For, ABTS, the IC_{50} was 27.13±0.151 µg/ml and 1.76±0.05 µg/ml for standard chemical gallic acid. Roy *et al*., 2015 studied the ABTS inhibitory property of *Pyrostegia venusta* flower extract and found an IC50 value of 18 µg/ml, which is almost in similar to our study (Roy *et al*., 2011). Similarly, TAC and FRAP property of PTME was reported to be 118.7±3.5µgAAE/mg extract and 349.08±5.52 µgFAE/mg PTME respectively. The plant's high antioxidant activity may be linked to the prevention of many diseases. Assays such as DPPH, ABTS, TBARS, TAC, and FRAP have demonstrated that the plant has potent antioxidant activity which may be beneficial in preventing free radical-mediated pathological damage.

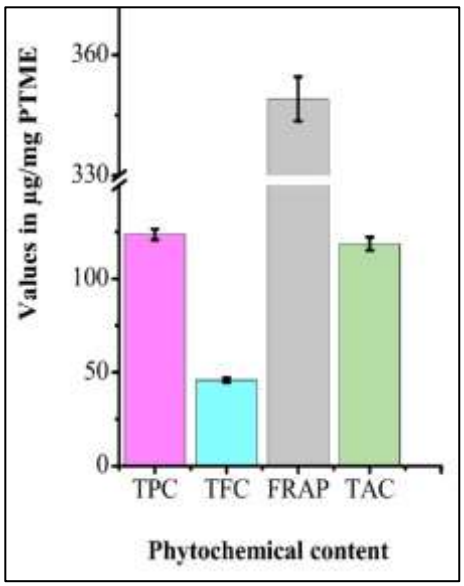

Figure 15.2: Phytochemical content and Antioxidant assays.

TPC- Total phenolic TFC- Total Flavonoid content. FRAP- Ferric oxide reducing antioxidant potential. The values of TPC are represented as gallic acid equivalent (GAE/mg) PTME and TFC is represented as quercetin equivalent (QE)/ mg PTME. The values of FRAP assay were expressed as µg Fe^{2+} equivalent (FE)/mg plant extract. For TAC, the values are expressed as µg ascorbic acid equivalent per milligram PTME (AAE/mg). Values are represented as mean of triplicates with ± standard deviation.

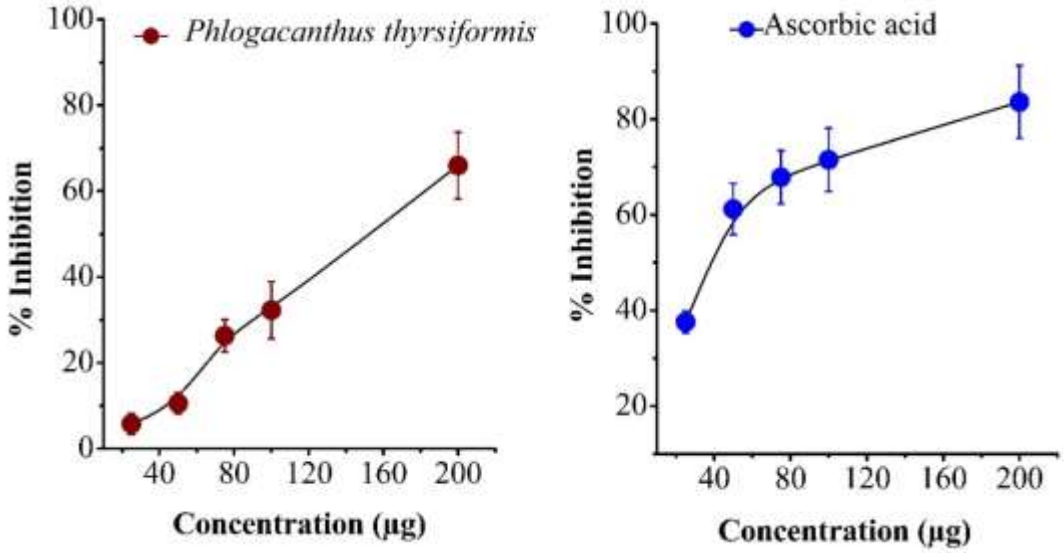

Figure 15.3: TBARS assay. Values are represented in percentage of mean of triplicates ± Standard deviation

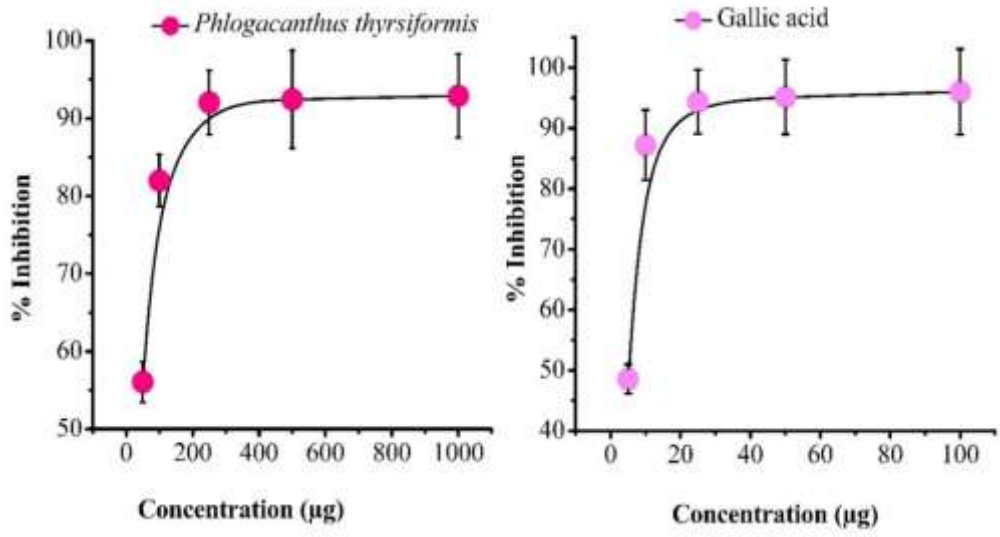

Figure 15.4: Dose-dependent percentage inhibition of DPPH assay of the plant. Values are represented as mean of triplicates with ± standard deviation.

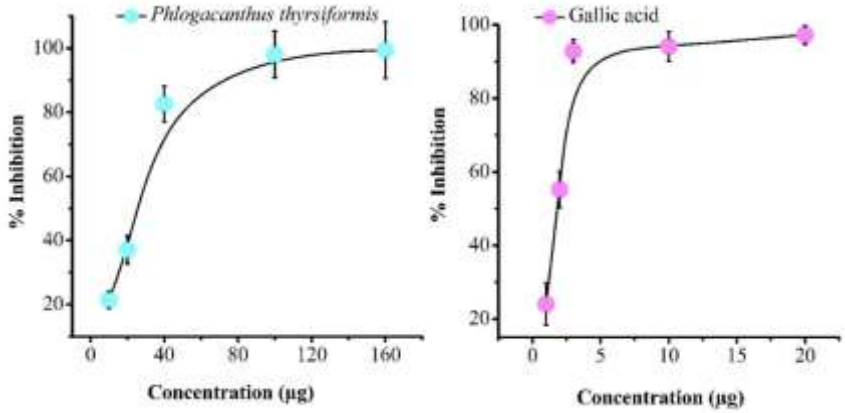

Figure 15.5: Dose-dependent percentage inhibition of ABTS assay. Values are represented as mean of triplicates± standard deviation.

Metals play a crucial role in human health and wellness. These elements when consumed in lesser quantity have a beneficial effect but can be harmful when exceeds the daily requirement of the individuals. Seven elements i.e., Cd, Mn, Cr, Zn, Pb, Ni, and Cu were analyzed. Zinc was found to be in highest concentration among the seven tested elements, but was below the range of daily intake recommendation by WHO. Zinc is a cofactor to more than 300 enzymes and has a major role in the stabilization of the structure of a huge number of proteins (Chasapis *et al*., 2021). Zn is followed by Ni, Cr, Cu, and Mn. All the elements were below the permissible limit. Heavy toxic element Pd and Cd was not detected (Table 15.2.) Pd when consumed or exposed to higher levels, can affect the gastrointestinal tract, hematologic system causing anaemia, basophilic stripling erythroblast and can also affect the Central Nervous System (Srianujata 1998). Even a low dose of Cadmium has a high toxicity in the liver kidney and other organs (Genchi *et al*., 2020). PTME did not show any traces of Cadmium and lead or was below the detectable limit. The use of GC-MS analysis to identify several potential bioactive plant components has grown. Six probable compounds were identified through GC-MS system (Table 15.3). Plants are known for various active components which may be beneficial for medicinal purposes. Many inorganic gases and aromatic solvents are detected through GC-MS system. The construction of chromatographic fingerprints aims at evaluating the quality of Herbal Medicines (Yi-Zeng *et al*., 2004).

Table 15.2. Trace element analysis of *Phlogacanthus thyrsiformis*

Sample name	**Cd**	**Mn**	**Cr**	**Zn**	**Pb**	**Ni**	**Cu**
Phlogacanthus thyrsiformis (µg)/g plant extract	ND	0.01	0.026	0.0323	ND	0.042	0.0161
WHO daily permissible limit (mg/kg plants)	0.02	1.8-2.3mg	1.30	0.60	2	10	10

ND – Not Detected; Cd- Cadmium; Mn- Manganese; Cr- Chromium; Zn- Zinc; Pb- Lead; Ni- Nickel; Cu- Copper. Values are represented in parts per million

Table 15.3. GC-MS profiles of the compounds identified from *Phlogacanthus thyrsiformis*

Name of the compound	Retention time	MW (g/mol)	Area (%)	Height (%)	m/z	MF
Tungsten, dicarbonyl-bis (eta. -4-R(+)-pulegone)	4.130	544.33	4.81	9.00	527.00	$C_{22}H_{32}O_4W$
Chromium (III) tris(undecane-5,7-dione)	4.169	604.8	15.20	39.27	603.00	$C_{33}H_{60}CrO_6$
6-Chloro-12H-tetrachlorodibenzo[d,g][1,3,2]phosphorin-6-sulfide	6.500	434.489	6.73	8.72	122.00	$C_{13}H_6Cl$
Ethyl 4,4,6,6-tetramethyl-9-oxo-3,5,7,10-tetraoxa-4,6-disiladodecan-1-oate	9.014	338.50	33.37	16.26	265.00	$C_{12}H_{26}$
Cyclopenta[d,E]anthracene, 5,7-dichloro-1,2(1H,2H)-dioxo-	17.076	301.1	30.79	14.411	278.00	$C_{16}H_6Cl_2O_2$
Ruthenium, tricarbonyl [(3, 4-.eta.)-4,5-diethyl-2,2-dimethyl-3-(1-methylethenyl)-1-selena-2-sila-5-boracyclopent-3-ene]-	20.573	501.2	9.09	12.35	400.00	$C_{16}H_6BOSSi$

MW – molecular formula; MF – molecular formula

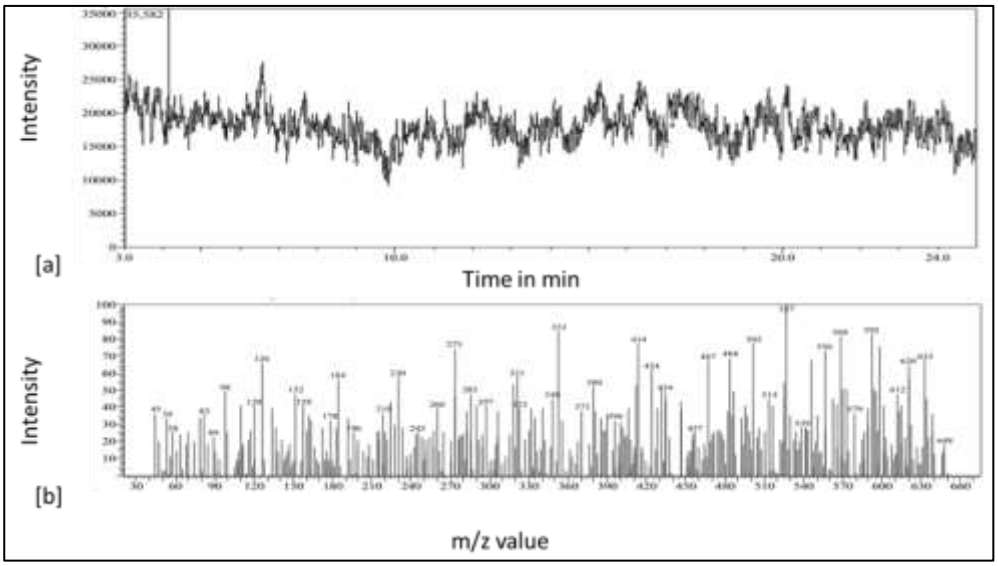

Figure 15.6: Chromatogram of *Phogacanthus thyrsiformis* methanolic extract

15.4 Conclusion:

Phlogacanthus thyrsiformis is a widely distributed plant in the Indian subcontinent, and several parts of the plant are known for medicinal properties and the flower part specifically consumed by the tribals of Northeast India. *P. thyrsiformis* showed rich phytochemical content and great antioxidant potential. Besides these, elemental analysis showed the safe use of the flower as a food source or medicine. *P. thyrsiformis* could be an alternative source of medicine for various diseases. However, further investigation is needed to understand the exact mode of action.

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Conflict of Interest:

Authors declares no conflict of Interest.

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