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GC-MS ANALYSIS, PHYTOCHEMICALS AND *IN-VITRO* ANTIOXIDANT PROPERTIES OF ROOT EXTRACTS OF *PHLOGACANTHUS THYRSIFLORUS* NEES., WESTERN ASSAM, INDIA

Hankhray Boro, Kuntala Mashahary and Sandeep Das *

Department of Biotechnology, Bodoland University, Kokrajhar - 783370, Assam, India.

Keywords:

Phytochemicals, Antioxidants, GC-MS analysis, *Phlogacanthus thyrsiflorus* Nees

Correspondence to Author:
Sandeep Das

Associate Professor, Department of Biotechnology, Bodoland University, Kokrajhar - 783370, Assam, India.

E-mail: daskokrajhar@gmail.com

ABSTRACT: The *Phlogacanthus thyrsiflorus* roots have been used by the tribal population of western Assam to treat the hepatic disorder. The present study focuses on phytochemical screening, GC-MS analysis and *in-vitro* antioxidant activities using different extracts. Plant total phenolics, flavonoids, reducing power assay and antioxidant activity was evaluated. DPPH, FRAP, ABTS, Metal chelating activity, and H_2O_2 free radical scavenging activity were studied. The screening from extracts showed the presence of phenols, flavonoids, tannins, resins, terpenoids, alkaloids, glycosides, cardiac glycosides, steroids. The highest concentration of total phenolics, flavonoids, reducing power and total antioxidant activity was found in the hydro-alcoholic extract of RoPt. The results of the radical scavenging activity in 70% ethanolic extract showed better IC_{50} value in DPPH (265.87 ± 17.58), ABTS (17.89 ± 1.18) and H_2O_2 (17.89 ± 1.05) µg/ml respectively. EC_{50} value of 535.16 ± 34.82 was found in 70% ethanolic against 1471.32 ± 19.77 µg/ml of acetone extract whereas the FRAP showed high $FeSO_4.7H_2O$ concentration in acetone extract with 820 ± 9.1 µM/ml. The major chemical constituents that were identified in GC-MS analysis were 2R-Acetoxymethyl-1,3,3-Trimethyl-4T-(3-Methyl-2-Buten-1-YL)-1T-Cyclohexanol (71.243%) and 2,4,4-Trimethyl-3-Hydroxymethyl -5A-(3-Methyl-But-2-Enyl)- Cyclohexene (88.107%) in hydro-alcoholic and acetone extracts respectively. The study showed an enhanced phenolic and flavonoid concentration and radical scavenging activity in the 70% ethanol extracts of RoPt which are the good source of antioxidants showing potential in preventing much free radical-mediated disorder.

INTRODUCTION: Recently, there has been a noticeable change towards the mass acceptance of herbal remedy mainly because of the persistence of lesser-known side effects. However, due to urbanization as well as continuous unsustainable exploitation of the herbal reserves, the plant resources along with their related traditional knowledge are depleting day by day [1].

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Medicinal plants have a wide range of natural constituents *viz*., phenolics, flavonoids, tannins, terpenoids, glycosides, alkaloids, *etc*., some of which are very good antioxidants associated with lower incidence of several human diseases [2]. Polyphenols play a significant role in the prevention of free radicals. In addition to this, their natural origin itself is an advantage in contrast to synthetic antioxidants which is limited in use due to their potent carcinogenicity [3].

Phlogacanthus thyrsiflorus Nees. under the family Acanthaceae, is the fast-growing evergreen shrub which grows up to 8 ft in height, red or blackish red tubular flowers, leaves are oblong-ovate & deep green, 15-20 cm long and 6-8 cm broad. Their

distribution is common along tracts of moist deciduous forest and is found widely in India and South East Asia, commonly known as Barsikha / Bahika by local people of Kokrajhar, Assam, India.

The flower is edible and is used as a vegetable, and the traditional healers of the area use the root of this plant in curing hepatic disorders [4]. No scientific records are available at present based on the traditional utility of root of this plant which makes the present research a valuable one.The present study focuses on qualitative screening, *in-vitro* antioxidant property and their radical scavenging activity of root of *Phlogacanthus thyrsiflorus* (RoPt) by different solvent extracts.

MATERIALS AND METHODS:

Collection and Identification: Fresh whole roots (**Image 1**) of the plants were collected in the winter season (February-March, 2014) from Sukhanjhara area (Lat: 26˚23'26.18"N / Lon: 90˚12'18. 61"E) of the Kokrajhar, Assam. The plant was identified having authentication number- BSI/ERC/2014/Plant authentication/538 and specimen call number BU.002, at B.S.I. Shillong, Meghalaya, India before initiation of the said work.

IMAGE 1: PHOTO OF THE PLANT *PHLOGACANTHUS THYRSIFLORUS* AND ITS ROOTS

Chemicals: Chemicals used for the study were of analytical grade and were purchased from Sigma Aldrich.

Extract Preparation of Sample: The sample was washed properly with clean water and is oven dried at 40 ºC for 7-14 days. After that, they were crushed up and ground to get fine homogeneous powder by a grinder and stored at airtight container for further use [5]. 50 g of dried root powder is extracted twice in 200 ml of ethanol (70%) and acetone for 48 h. Filtered through Whatman filter paper no. 42. The filtrate was concentrated by evaporating in the rota-evaporator until fully dried and store at 4 °C for their further studies.

Phytochemical Screening: The qualitative phytochemical screening of root of *Phlogacanthus thyrsiflorus* by 70% ethanol (RoPt-EE) and acetate (RoPt-AE) solvents were accomplished. The plant phytochemicals were screened mainly phenols [6], flavonoids [6], tannins [7], resins [6], terpenoids [8], alkaloids [6], glycosides [7], cardiac glycosides [7], reducing sugar [9], steroids [9], anthraquinone [5] and saponins [10].

Total Phenolic Content: The folin-ciocalteau method [11] was used for the determination of total phenolic content which was adopted from Swain and Hillis (1959). Briefly, in a test tube 100 µl of the extract was taken, to which 1600 µl of doubled distilled water was added and then 100 µl of folin-ciocalteau reagent (0.25 N) was added and mixed properly. The above sample mixture was allowed to react for 3 min. After that, 150 µl of Na_2CO_3 (1N) solution was added. The mixture was incubated at room temperature in the dark for 2 h. The absorbance was taken at 725 nm using a PC based double beam spectrophotometer (Systronics) by taking Gallic acid as standard and were expressed in milligram of gallic acid equivalent (GAE)/gm of dried extract.

Total Flavonoid Content: Total flavonoid content was determined by aluminum chloride method [12]. From both the extracts, 0.1 mg/ml of extracts were prepared in double distilled water and were reacted with 1.5 ml ethanol (95%), 0.1 ml aluminum chloride hexahydrate (10%), 0.1 ml potassium acetate (1 M) and 2.8 ml of doubled distilled water for 40 min @ RT. Finally, the absorbance of the

above mixture was measured at 415 nm taking quercetin as standard.

Total Reducing Power Assay: The reducing power assay was determined by the method adopted by Hsieh *et al.* [12] An aliquot of 1ml of the sample was reacted with 0.5 mL of phosphate buffer (0.2 M) and 0.5 mL of potassium ferricyanide (1%). The above reaction mixture was then incubated at 50 ºC for 20 min. After the cooling, 0.5 mL of trichloroacetic acid (10%) was added. Now, 2 mL of distilled water was mixed with 2 mL of the above reaction liquid and 0.2 mL of iron (III) chloride (0.1%). Finally, the absorbance was measured at 700 nm. Butylated hydroxyanisole (BHA) was used as a positive control.

Total Antioxidant Capacity: Phosphomolybdate method was adopted for the determination of total antioxidant capacity [13]. In brief, 0.3 ml of each extract was added to 3 ml of phosphomolybdate reagent (0.6 M H_2SO_4, 0.028 M sodium phosphate, 0.004 M ammonium molybdate). This reaction mixture was incubated at 95 °C in a water bath for 90 min. After cooling to room temperature, the absorbance was measured at 765 nm. Ascorbic acid served as standard. Results were expressed in milligram of ascorbic acid equivalent ((AAE)/gm of dried extract.

DPPH Radical Scavenging Assay: The antioxidant ability of root extracts of *Phlogacanthus thyrsiflorus* was determined by their capacities to neutralize radicals of DPPH [di (phenyl)- (2,4,6-trinitrophenyl) iminoazanium] [14]. The antioxidants in the sample scavenge the free radical and turn it into yellow. A working solution of DPPH (0.004%) was prepared freshly in methanol. 1 ml of sample and standard dilution of various concentrations (10, 20, 40, 80 and 160 µg/ml) was added to 3 ml of DPPH working solution. After 30 min of incubation in the dark at room temperature, 25 °C ± 2, change in color from violet to yellow was recorded at 517 nm with UV-VIS Spectrophotometer (Systronic). Ascorbic acid was used as a positive control. 1 ml of methanol with 3 ml of working DPPH solution serves as a control. The capability to scavenge DPPH radical activity was calculated by-

$$\% \text{ inhibition} = Ac - As / Ac \times 100$$

Where, Ac is the absorbance of the control and As is the absorbance of samples or ascorbic acid. Lower values represent higher antioxidant ability.

ABTS Radical Cation Scavenging Activity: ABTS (2, 2 azobis, 3-ethyl benzothiazoline-6-sulphonic acid) radical cation scavenging activity was determined by Shah *et al*., methodology [13]. Briefly, ABTS (7 mM) solution was allowed to react with potassium persulfate (2.45 mM) overnight in the dark for generation of dark-colored ABTS radicals. For the analysis, the ABTS solution was diluted with 50% ethanol to obtain an initial absorbance of 0.7 ± 0.05 at 745 nm. For the determination 100 µl sample of different dilution was added to 1 ml of ABTS solution. The decrease in absorbance was measured at 745 nm after one min and 6 min of mixing. The difference was calculated and compared with control. The butylated hydroxytoluene (BHT) taken as positive control. The formula calculated ABTS radical % inhibition:

$$\% \text{ inhibition} = Ac - As / Ac \times 100$$

Iron Chelating Capacity: For the evaluation of ferrous ion chelating potential of the extracts of RoPt was done by the method adopted by Sasikumar *et al.* [15] In a reaction mixture, 1 ml of various concentration of RoPt extracts (200-1000 µg/ml) and 2 mM $FeCl_2$ (0.05 ml) was taken. The control contains all the reagents except for the sample. The reaction was initiated after the addition of 5 mM Ferrozine (0.2 ml). Shaken vigorously and left in the room temperature for 10 min. The absorbance of both reaction mixture and control was taken at 562 nm. Lower the absorbance higher will be the ferrous ion chelating potential. The EDTA was taken as standard.

$$\% \text{ inhibition} = Ac - As /Ac \times 100$$

H_2O_2 Scavenging Assay: H_2O_2 radical scavenging activity of the extracts was measured by the method adopted by Awala and Oyetayo [16]. 20 mM H_2O_2 solution was prepared by mixing 226 µl from 30% H_2O_2 in 99.8 ml of 0.1 mM phosphate buffer saline having pH 7.4. In a different sample/standard concentration (2, 4, 6, 8 and 10 µl/ml), 2 ml of 20 mM H_2O_2 solution was added and incubated in the dark for 10 min. The absorbance of the scavenging activity was taken at 230 nm using UV-VIS double

beam spectrophotometer (Systronics). Phosphate buffer saline was used as blank and BHA as positive control. The amount of H_2O_2 inhibited by the extract was calculated from the equation:

$$\% \text{ inhibition} = Ac - As / Ac \times 100$$

Ferric Reducing Antioxidant Assay: Ferric reducing antioxidant power of the RoPt extract was determination by the method of Adebiyi *et al*. [17] The FRAP reagent was freshly prepared by mixing 300 mmol/L sodium acetate buffer (pH 3.6), 10 mmol/L TPTZ solution in 40 mmol/L HCl and 20 mmol/L iron (III) chloride solution in a ratio of 10:1:1 to generate FRAP reaction solution, which should be warmed to 37 °C in a water bath before use. After that, 100 µL of the diluted sample was mixed with 3 mL of the FRAP reaction solution. It was then incubated in the dark for 4 min @ RT, and the absorbance of the reaction mixture and standard was recorded at 593 nm. The standard curve was constructed using $FeSO_4.7H_20$ solution and the results were expressed as µmol Fe(II)/g dry extract of RoPt. All experiments were performed in triplicate.

Gas Chromatography Coupled with Mass Spectrometry Analysis: The analysis of hydro-alcoholic and acetone extracts of RoPt were performed by Perkin Elmer gas chromatography (Clarus 680) coupled with mass spectrometry (Clarus 600 EI) employed with fused silica column and packed with capillary column Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250 µm df). The components were separated using Helium as carrier gas at a constant flow of 1 ml/min. The injector temperature was set at 260 °C during the chromatographic run. The 1 µL of extract sample injected into the instrument the oven temperature was as follows: 60 °C (2 min); followed by 300 °C at the rate of 10 °C min^{-1}; and 300 °C, where it was held for 6 min. The mass detector conditions were: transfer line temperature 240 °C; ion source temperature 240 °C; and ionization mode electron impact at 70 eV, a scan time 0.2 sec and scan interval of 0.1 sec. The fragment size from 40 to 600 Da was scanned. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS NIST (2008) library.

Statistical Analysis: Results of all the experiments were presented as mean ± SD of the triplicate experiment (n=3). Relative significant differences among the means were determined by one-way ANOVA test ($p \leq 0.05$) using Microsoft excels (2007).

RESULTS AND DISCUSSION:

Extract Percent Yield: The dried ethanol root extract of *Phlogacanthus thyrsiflorus* produced a total yield of 12% (w/w) and 9% (w/w) from that of acetone extract.

Phytochemical Screening: Qualitative screening of RoPt showed some differences in the constituents of the ethanol and acetone extracts. The 70% ethanol and acetone extracts are rich in phenols, flavonoids, tannins, resins, terpenoids, alkaloids, glycosides, cardiac glycosides, and steroids except for the reducing sugar and anthraquinones. Saponin showed positive in 70% ethanol extract **Table 1**.

TABLE 1: QUALITATIVE TESTS

Phytochemical Tests	RoPt		Phytochemical Tests	RoPt	
	70% EE	AE		70% EE	AE
Phenols	+	+	Glycosides	+	+
Flavonoids	+	+	Cardiac Glycosides	+	+
Tannins	+	+	Reducing sugar	-	-
Resins	+	+	Anthraquinone	-	-
Terpenoids	+	+	Saponins	+	-
Alkaloids	+	+	Steroids	+	+

(+) Represents the presence and (-) indicates the absence of the phytochemical compound. RoPt-EE: Root of *Phlogacanthus thyrsiflorus* ethanol extract, RoPt-AE: Root of *Phlogacanthus thyrsiflorus* acetone extract.

Total Phenolic and Flavonoid Contents: Result for the total phenolic and flavonoid content (extracts of RoPt) are presented in **Fig. 7**. Phenolic and flavonoid content in the ethanol (70%) and acetone extracts were determined from linear curve of standard gallic acid ($y = 0.0166x + 0.0913$; $R^2 = 0.9959$) and standard quercetin ($y = 0.0123x - 0.0218$; $R^2 = 0.9985$) respectively.

The highest content of TPC and TFC of RoPt was found in ethanol (70%) extract with 101.26 ± 2.52 mg GAE/g and 99.92 ± 0.93 mg QE/g then the acetone extract which showed 84.21 ± 4.82 mg GAE/g and 68.22 ± 2.34 mg QE/g of dried extract respectively.

Total Reducing Power Assay and Total Antioxidant Property: The reducing power activity of the extracts of RoPt was determined from the standard linear curve of BHA ($y = 0.0174x + 0.0771$; $R^2 = 0.9933$).

RoPt ethanol (70%) extract showed somewhat higher reducing power activity with 109.39 ± 3.89 then acetone extract which showed 104.22 ± 4.19 BHAE mg/g of dried extract. Whereas total antioxidant property of the extracts of RoPt was determined from the standard linear curve of ascorbic acid ($y = 0.0143x + 0.0907$; $R^2 = 0.9993$).

From the study, the highest concentration was found in ethanol extract of RoPt having 198.35 ± 3.59 in respect to acetone extract which showed 189.94 ± 9.1 mg AAE/g of the dried extract. The data obtained are presented in **Fig. 7**.

DPPH Radical Scavenging Activity: The highest inhibition of 34.88 ± 1.89% was observed in the 160 µg/ml concentration of 70% ethanol extract of RoPt with IC_{50} value of 265.87 ± 17.58 µg/ml against 30.05 ± 3.56% observed in the 160 µg/ml concentration of acetone extract with IC_{50} value 302.55 ± 35.68 µg/ml **Table 2**. DPPH scavenging activity of ascorbic acid showed 97.62% inhibition at 160 µg/ml concentration **Fig. 1**.

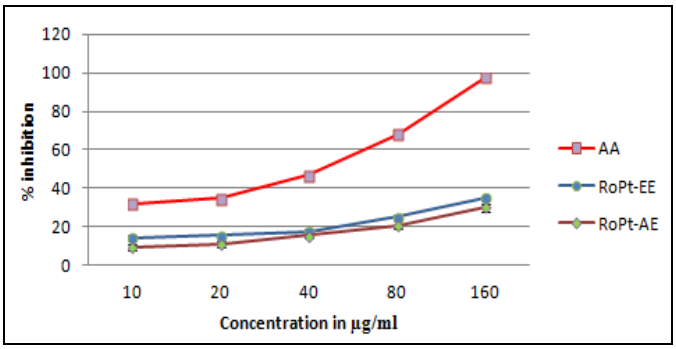


FIG. 1: DPPH- RADICAL SCAVENGING ACTIVITY

Showing an increase in % inhibition of DPPH free radical scavenging activity with an increase in concentration of extracts and ascorbic acid. Result represents mean ± SD value of triplicate experiment. The value of DPPH of Ascorbic acid, RoPt-EE and RoPt-AE are significantly different (P≤0.05).

ABTS Radical Scavenging Activity: The percent inhibition of ABTS free radical scavenging activity was highest in BHT showing scavenging activity at 32 µg/ml concentration, which inhibited 99.72% of ABTS free radicals and having the IC_{50} value of only 7.04 µg/ml **Table 2**.

Whereas the 70% ethanol extract showed best inhibition of 72.82 ± 3.39 percent with the IC_{50} value as low as 17.89 ± 1.18 µg/ml and acetone extract showed % inhibition of 58.97 ± 2.19 with an IC_{50} value of 24 ± 1.61 µg/ml **Fig. 2**.

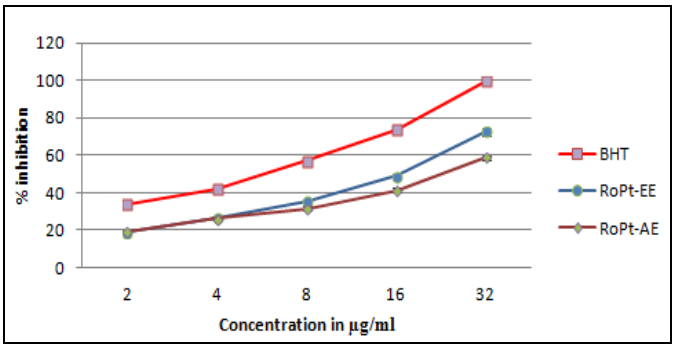


FIG. 2: ABTS- RADICAL SCAVENGING ACTIVITY

Showing % inhibition of ABTS radical scavenging activity at a different concentration by the BHT and extracts of RoPt. The result represents mean ± SD value of triplicate experiment. The value of ABTS of BHT, RoPt-EE, and RoPt-AE are significantly different (P≤0.05).

Iron Chelating Capacity (ICC): The ICC was evaluated taking EDTA as the positive control. The data obtained **Table 2** in the study was presented in **Fig. 3**, which shows the EC_{50} value of hydro-alcoholic extract of RoPt as 535.16 ± 34.82 and acetone extract as 1471.32 ±19.77 µg/ml respectively. The EC_{50} value of EDTA was found to be 63.33 µg/ml.

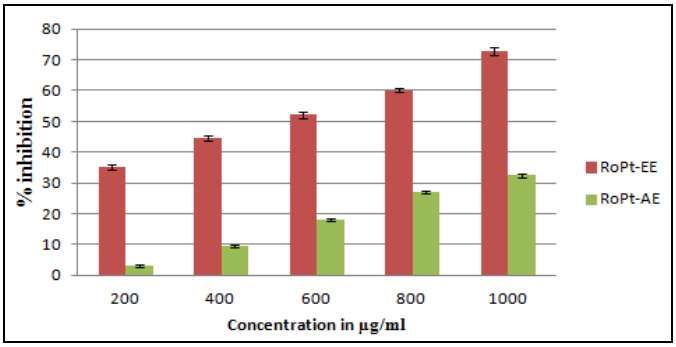


FIG. 3: ICC- IRON CHELATING CAPACITY

Showing % inhibition of Metal chelating activity at a different concentration of RoPt extracts. The result represents mean ± SD value of triplicate experiment. The value obtained are significantly different (P≤0.05).

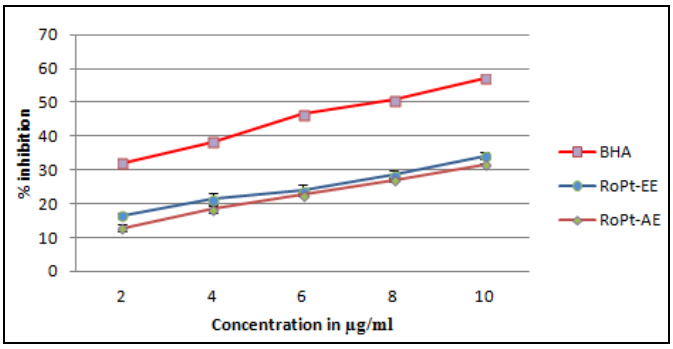


FIG. 4: H_2O_2-RADICAL SCAVENGING ACTIVITY

Showing % inhibition of H_2O_2 radical scavenging activity at a different concentration of BHA and RoPt extracts. The result represents mean ± SD value of triplicate experiment. The value of H_2O_2 of BHA, RoPt-EE, and RoPt-AE are significantly different (P≤0.05)

H_2O_2 Radical Scavenging Activity: The result indicated a concentration-dependent activity in BHA, 70% ethanol and acetone extracts with % inhibition of 57.23, 34.13 ± 1.89 and 31.68 ± 0.54 respectively in only 10 µg/ml concentration. For the same the IC_{50} values of the BHA, 70% ethanol and acetone extracts are found to be 7.59, 17.89 ± 1.05 and 18 ± 1.87 µg/ml respectively **Table 2**. The data obtained from the H_2O_2 radical scavenging is shown in **Fig. 4**.

Ferric Reducing Antioxidant Property: The results of ferric reducing antioxidant property was evaluated from the standard linear curve of $FeSO_4.7H_2O$ (y = 0.0051x - 0.0408; R^2 = 0.9965). The Present study has revealed that acetone extract of RoPt showed better antioxidant capacity with 820 ± 9.1 µmol Fe^{2+}/g than the ethanol extract 751.67 ± 13.88 µmol Fe^{2+}/g. The results of FRAP antioxidant capacity is presented in **Table 2**.

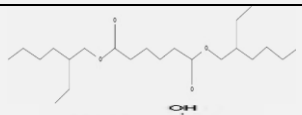
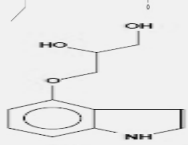
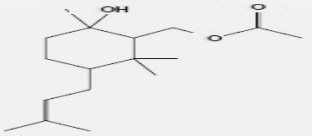
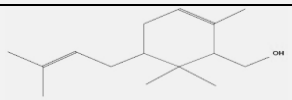
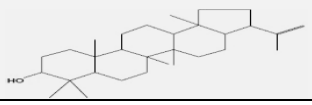
TABLE 2: VALUES OF RADICAL SCAVENGING TESTS

Extracts / Standard	DPPH	ABTS	H_2O_2	Metal chelating	FRAP (µmol/g $FeSO_4.7H_2O$) of dried extract
	IC_{50} values (µg/mg of dried extract)			EC_{50} in µg/mg	
Standards	48.93 (AAE)	7.04 (BHTE)	7.59 (BHAE)	63.33 (EDTAE)	-
RoPt-EE	265.87 ± 17.58	17.89 ± 1.18	17.89 ± 1.05	535.16 ± 34.82	751.67 ± 13.88
RoPt-AE	302.55 ± 35.68	24 ± 1.61	18 ± 1.87	1471.32±19.77	820 ± 9.1

(NB: AAE: Ascorbic acid equivalent; BHTE: Butylated hydroxyltoluene equivalent; BHAE: Butylated hydroxylanisole; EDTAE: Ethylene-diaminetetraacetic acid equivalent). Result represents mean ± SD value of triplicate experiment. The values obtained are significantly different (P≤0.05).

GC-MS Analysis Results: Fig. 5 and **6** represent the analysis of GC-MS chromatogram of hydro-alcoholic root extract of *Phlogacanthus thyrsiflorus* Nees., which showed a set of peaks which indicates the existence of the phytochemical components. The active components, structure, retention time, peak area in percentage, and possible functions are presented in **Table 3**.

TABLE 3: BIOACTIVE COMPOUNDS IDENTIFIED IN THE GC-MS ANALYSIS FROM ROOT EXTRACTS (*PHLOGACANTHUS THYRSIFLORUS* NEES.)

Peak	Compound name (RoPt-EE)	Structure	MW	RT	Area (%)	Function
1	Hexanedioic Acid, Bis (2-Ethylhexyl) Ester (CAS: 103-23-1)		370	21.896	17.168	---
2	1,2,3-Propatriol, 1-Indol-4-Yl (Ether) (CAS: 61212-32-6)		207	26.113	11.589	Antibacterial & antifungal [18]
3	2R-Acetoxymethyl-1,3,3 Trimethyl-4t-(3-Methyl-2-Buten-1-Yl)- 1t-Cyclohexanol (CAS: 900144-12-4)		282	29.354	71.243	Antibacteria & Anticancer [19]
Peak	**Compound name (RoPt-AE)**	**Structure**	**MW**	**RT**	**Area (%)**	**Function**
1	2,4,4-Trimethyl-3-Hydroxymethyl-5A-(3-Methyl-But-2-Enyl)-Cyclohexene (CAS: 900144-10-5)		222	29.174	88.107	Anti-microbial & anti-inflammatory [20,]
2	Hop-22(29)-En-3.Beta.-Ol (CAS: 58801-23-3)		426	29.664	11.893	Antibacteria & anticancer [21]

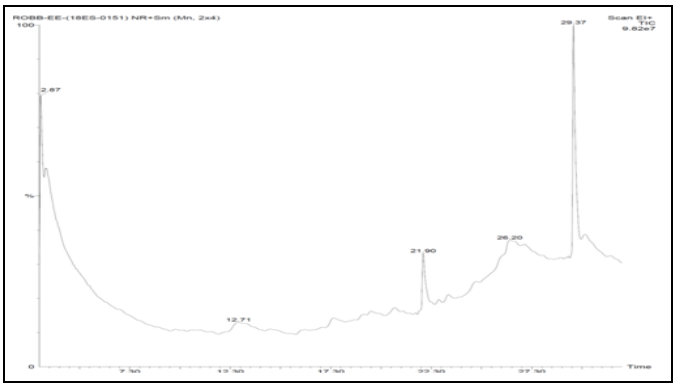


FIG. 5: GC-MS CHROMATOGRAM OF HYDRO-ALCOHOLIC EXTRACTS OF *PHLOGACANTHUS THYRSIFLORUS* NEES-ROOTS

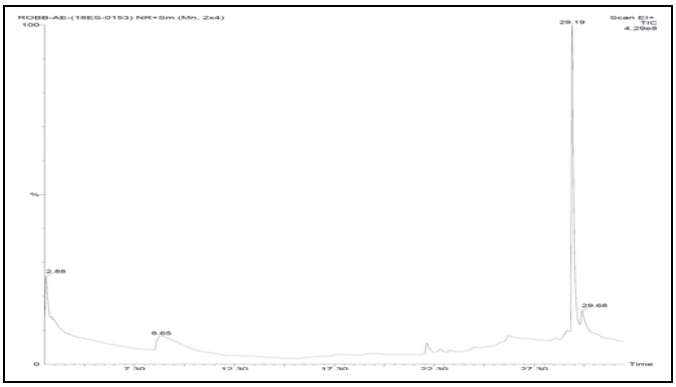


FIG. 6: GC-MS CHROMATOGRAM OF ACETONE EXTRACTS OF *PHLOGACANTHUS THYRSIFLORUS* NEES-ROOTS

DISCUSSION: Root extracts of *Phlogacanthus thyrsiflorus* Nees., showed important phyto-constituents such as Phenolics, flavonoids, and tannins which are a major group of compounds that act as natural antioxidants or free radical scavengers which possess antimicrobial, anti-allergic, anti-mutagenic, anti-inflammatory and anti-carcinogenic properties [22, 23]. In another study, the presence of tannins, flavonoids, saponins, carbohydrates, steroids, alkaloids, reducing sugar, and terpenoids were reported in the leave extracts of *Phlogacanthus thyrsiflorus* [24]. The higher amount of phenolic and flavonoid contents are detected in the present study which corresponds to stronger antioxidant capacity. In the **Fig. 7**, the RoPt-EE showed higher content of phenolic, flavonoid and better reducing power than the RoPt-AE.

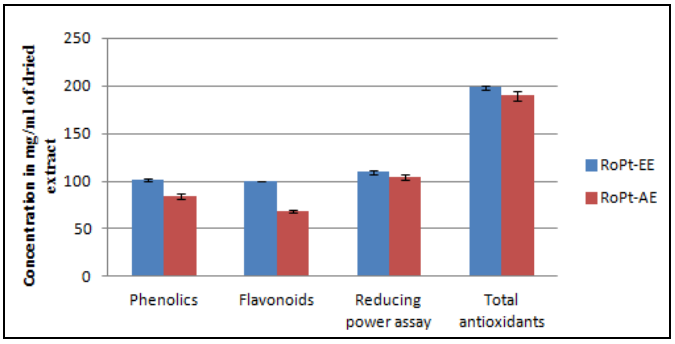


FIG. 7: ANTIOXIDANT TESTS

The figure revealed that the ethanol extract has slightly higher contents of all the antioxidants tests. The result represents mean ± SD value of triplicate experiment. The value of phenolics, flavonoids, reducing power assay and total antioxidants of RoPt-EE are significantly different ($P \leq 0.05$) with RoPt-AE.

Phyto-constituents of the extracts help in reducing power activity by reducing ions or by donating electron but not by radical quenching mechanism. The activity of extracts might be due to the occurrence of flavones hydroxyl, phenolic hydroxyl or methoxyl groups, free carboxylic groups, keto groups, triterpenes and their derivative [25]. Antioxidant capacity can be measured from the absorbance value; higher the absorbance higher will be its antioxidant capacity. The phospho-molybdate assay follows the principle that chemistry of conversion of Mo (IV) to Mo (V) compounds by the reducing agents which result in formation of green phosphate/Mo (V) complex which provide maximum absorbance at 765 nm and the highest concentration was found in *Sida cordata* ethanol extract [13] with 200 µg/ml, which is comparable to that of RoPt-EE (198.35 ± 3.59) of the present study being the highest concentration. The **Fig. 7**, showed that RoPt-EE had high reducing activity and total antioxidants than the RoPt-AE.

DPPH is stable proton free radical, which give characteristic absorption at 517 nm and was studied to see the ability of extract to reduce proton radicals [14]. The RoPt-EE and RoPt-AE showed good DPPH radical scavenging activity. Increase in radical scavenging activity was observed with increased concentration of the extracts and the activity is demonstrated as IC_{50}, a parameter that represents the extract concentration which can inhibit 50% of the DPPH radicals. The highest activity was seen in ascorbic acid > RoPt-EE > RoPt-AE **Table 2**.

ABTS radical scavenging activity uses the same principle as DPPH where antioxidants from the plant extracts react with stable free radical of ABTS. As shown in the **Fig. 2**, best scavengers for ABTS radicals were in the order of Butylated hydroxyl toluene > RoPt-EE > RoPt-AE **Table 2**

which is 8 folds better than the IC_{50} value of 143 ± 0.8 µg/ml reported in methanolic extract of *Sida cordata* whole plant [13].

For the oxygen transport, respiration and many enzyme activities the Iron (II) is very much important. Chelating agents inhibit the process of lipid peroxidation by stabilizing the transition metals. The metal chelating ability of extracts of *K. foetidissima,* the methanolic extract was most effective having EC_{50} value 1.0 mg/ml and least effective was found in petroleum ether extract with EC_{50} value 7.6 mg/ml [15]. The result obtained in the RoPt-EE was better than the *K. foetidissima* methanolic extract reported earlier. The chelating ability of extracts increases with increase in concentration.

The ability of extracts of RoPt to scavenge H_2O_2 is presented in **Fig. 4**. The result of radical scavenging was in the order of BHA > RoPt-EE > RoPt-AE. The hydro-alcoholic extracts of *S. latifolia*, BHT and gallic acid showed percent inhibition of 86.48%, 78.53%, and 96.82% respectively at 0.5 mg/ml concentration. The conversion of H_2O_2 to hydroxyl radical might be toxic to the cells which is extremely reactive free radical formed naturally in the biological system and known to implicate highly destructive among the free radical species. Therefore, inhibition of hydrogen peroxide is very much important [5].

FRAP is an assay which evaluates extract's ability to reduce ferric (III)-TPTZ complex to ferrous (II)-TPTZ. *Dioscorea bulbifera* showed the highest antioxidant capacity of 856.92 µmol Fe^{2+}/g, followed by *Tussilago farfara* with 455.64 µmol Fe^{2+}/g and least antioxidant property in the plant *Sargassum fusiforme* with 0.15 µmol Fe^{2+}/g [26]. The results obtained in the present study showed approximately 2 folds better than *Tussilago farfara* and 5000 folds better than *Sargassum fusiforme.*

GC-MS analysis plays a very important role in the identification and characterization of molecules that are of plant origin. The analysis shows the presence of bioactive compounds which are further confirmed from the library data. Plant materials are generally complexes that make GC-MS best suited for the analysis because of their high sensitivity. Furthermore, it requires a very little volume of the test sample for the analysis to give accurate molecular weight. The main compounds identified are 2R-Acetoxymethyl-1,3,3-trimethyl-4T-(3-methyl -2-buten-1-YL)-1T-cyclohexanol (71.243%); 1,2,3-Propatriol,1-indol- 4- YL (ether) (11.589%); Hexanedioic acid, bis (2-ethyhexyl) ester (17.168 %) from the RoPt-EE and 2, 4, 4-Trimethyl-3-hydroxymethyl- 5A- (3- methyl-but-2-enyl)-cyclohexene (88.1%); HOP-22 (29) -EN-3. Beta-OL (11.89%) from the RoPt-AE, which are having many biological activities such as antibacterial, antifungal, anti-inflammatory, antioxidant and anti-cancerous properties.

CONCLUSION: From the study, it was revealed that hydro-alcoholic extract exhibited a high concentration of phenolics, flavonoids, reducing power assay, total antioxidant activity and better % inhibition activity in DPPH, ABTS, iron chelating ability and H_2O_2 whereas the acetone extract was found better in the FRAP antioxidant capacity. The high antioxidant activity might be attributed to the presence of various phytochemicals in both the extracts of RoPt.

However, positive control of respective radical scavenging test has shown better antioxidant activity than both the extracts. From the literature, it was found that the compounds identified by the GC-MS analysis were having anti-bacterial, anti-oxidants, anti-inflammatory and anticancer properties. Based on this finding we can conclude that the roots of *Phlogacanthus thyrsiflorus* Nees. have a promising source of natural antioxidants and other bioactive compounds and could be useful in pharmaceutical applications.

The Significance of the Finding: The present study validates that the root of *Phlogacanthus thyrsiflorus* have promising antioxidant property and the bioactive compounds that were detected in GC-MS analysis could be useful in pharmaceutical applications.

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