



**ANTIOXIDANT AND GC-MS ANALYSIS OF LEAVES EXTRACT OF
STROBILANTHES HEYNEANUS NEES**

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ABSTRACT

Strobilanthes heyneanus (*Nilgirianthus heyneanus*) is the Acanthaceae family containing numerous species with an expected variety of treatments. Also, it is called Karun kurinji in Tamil and is mostly found in the Western Ghats of India. The folklore of this species is generally used to treat rheumatic diseases, sprain problems, and hernia. The study of the research work is to investigate the phytochemical, antioxidant, and GC-MS analysis of the leaves extract of *Strobilanthes heyneanus*. The DPPH[·] radical and hydroxyl (OH[·]) radical scavenging assays along with Fe³⁺ reducing power assays phosphomolybdenum reduction and were determined for the leaves extract. The highest DPPH[·] radical scavenging activity was 89.69±1.15 at 120 µg/mL concentration and the calculated IC₅₀ was 53.16 µg/mL concentration. The highest OH[·] radical scavenging activity was 45.16±0.62 at 120 µg/mL concentration and the calculated IC₅₀ was 131.69 µg/mL concentration. The highest ABTS^{·+} radical scavenging activity was 97.41±0.62 at 60 µg/mL concentration and the calculated IC₅₀ was 18.40 µg/mL concentration. The highest phosphomolybdenum reduction was 87.65±1.01 at 120 µg/mL concentration and the calculated RC₅₀ was 24.74 µg/mL

concentration. The highest Fe^{3+} reduction was 68.65 ± 0.91 at $120 \mu\text{g/mL}$ concentration and the calculated RC_{50} was $58.37 \mu\text{g/mL}$ concentration.

Keywords: *Strobilanthes heyneanus*, phytochemical analysis, DPPH[•] radical, Antioxidant, GC-MS analysis

INTRODUCTION

Karun Kurinji is a bushy shrub that is about a meter high, and with the grooved stem, covered with hairs. Unequal, oppositely arranged, ovate leaves are hairy and have a margin. Blue urn-shaped flowers 1-1.2 cm long, occurs in axillary spikes. Five sepals are joint at the base. The flower tube is enlarged in the middle and has five, rounded petals. The swollen part is white. Stamens four, filaments are long-haired at the base. Capsule 6-8 mm long, square, 4-seeded. Seeds winged. Karun kurinji is mostly found in the South-West parts of India and believed to be a type of Kurinji. Like Kurinji, it flowers a long flowering cycle. Flowering during the September-October [1]. *S. heyneanus* leaves are used to treat leprosy, to control blood sugar, jaundice, inflammation, urinal problems, and excess menses [2]. The genus *Strobilanthes* are used as a diuretic, anti-diabetic, laxative, and potent antimicrobial agents [3].



Figure 1 *Strobilanthes heyneanus* Nees

MATERIALS AND METHODS

Preparation of the extract

S. heyneanus were collected from Wayanad, Kerala, India. The leaves were separated, washed, and dried for ten days in shadow. Then the leaves were powdered and soaked in methanol for 3 days (72 h) at room temperature. The clear supernatant above the plant powder was drained out through the filter paper and condensed by the rotary evaporator at 50°C , which yields green viscous extract.

Phytochemical investigation

The leaves extract of *S. heyneanus* was tested for phytochemical analysis for different categories of secondary metabolites of phytochemicals using particular chemical reagents [4, 5].

Total phenols Content

Folin-Ciocalteu reagent method was used to determine the total phenolic content (TPC) with slight modifications [6]. Hundred μL of the leaves extract stock concentration (1mg/mL) of *S. heyneanus* was mixed with $900 \mu\text{L}$ of methanol and 1 mL of 1:10 diluted, Folin Ciocalteu reagent and then 1 mL of 20% (w/v) Na_2CO_3 solution was added and shaken well vigorously. The reaction mixture was

incubated in the dark for 30 minutes at room temperature. Using the spectrophotometer at 765 nm the absorbance was measured and the total phenolic content was calculated as Gallic acid equivalent ($\mu\text{g}/\text{mg}$ of extract).

Total flavonoids Content

The total flavonoid content of the leaves extract of *S. heyneanus* was assessed by the aluminium chloride reagent method with minor modifications [7]. Five hundred μL of extract (1 mg/mL) was mixed with 500 μL of methanol and to this 0.5 mL of 5% (w/v) sodium nitrite solution was added. And then 0.5 mL of aluminium chloride solution (10%, w/v) was added followed by 50 μL of 1 Molar NaOH solution was added and shaken well. And the reaction mixture was incubated for 30 minutes at room temperature and the absorbance was measured at 510 nm using a spectrophotometer. The flavonoid content was calculated as quercetin equivalent ($\mu\text{g}/\text{mg}$ of extract).

DPPH[•] radical scavenging activity

The antioxidant activity of the leaves extracts of *S. heyneanus* was measured by DPPH (1, 1-diphenyl 2-picrylhydrazyl) radical scavenging assay method [8]. One mL of the leaves extract with different concentrations (20-120 $\mu\text{g}/\text{mL}$) was mixed with 1 mL of 0.1 mM DPPH solution in methanol. One mL of DPPH solution mixed with 1 mL of methanol was used as

the control. The mixture was incubated in the dark for 30 minutes and then the absorbance measured at 517 nm. The standard ascorbic acid was used. The percentage of inhibition was calculated as:

$$\% \text{ of DPPH}^{\bullet} \text{ radical inhibition} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100$$

Hydroxyl radical scavenging assay

The hydroxyl radical scavenging activity was analyzed by the salicylic acid method with small modifications [9]. To 1 mL of various concentrations of the extract, 300 μL of FeSO_4 (8 mM) solution and 250 μL of H_2O_2 (20 mM) was added and shaken well. To start the reaction, 250 μL of salicylic acid in methanol (3 mM) was added and then, the reaction mixture was incubated at 37°C for 30 min. Then, 200 μL of distilled water was added and the mixture was centrifuged at 5000 rpm for 5 min. At 510 nm the absorbance of the supernatant was measured and the percentage of OH^{\bullet} radical scavenging activity was calculated as:

$$\% \text{ of OH}^{\bullet} \text{ radical inhibition} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100$$

ABTS^{•+} radical cation scavenging activity

The antioxidant ability of the leaves extract of *S. heyneanus* was evaluated by the ABTS^{•+} radical cation scavenging assay method with slight modifications [10]. ABTS^{•+} radical cation was obtained by 7 mM of ABTS reacted with 2.45 mM of potassium persulfate in 200 mM of PBS

(pH 7.4) and incubated for 12-16 h in dark, before use. The ABTS^{•+} radical cation solution was diluted with PBS and set an absorbance of 0.70±0.02 at 734 nm. Diluted ABTS^{•+} radical cation solution was prepared freshly every day for this assay. Different concentrations (5-30 µg/mL) of the leaves extract was mixed with 500 µL of diluted ABTS^{•+} radical cation solution and after 5 min, the absorbance was measured at 734 nm. Ascorbic acid was used as the standard reference. The ABTS^{•+} radical cation scavenging activity was expressed as

$$\% \text{ of ABTS}^{\bullet+} \text{ radical cation inhibition} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100$$

Phosphomolybdenum reduction assay

The antioxidant capacity of the leaves extract of *S. heyneanus* was assessed by phosphomolybdenum reduction assay method [11]. One mL of various concentrations of the leaves extract (20-120 µg/mL) was mixed with 1 mL of reagent solution including ammonium molybdate (4 mM), sodium phosphate (28 mM), and sulphuric acid (600 mM). The mixture of the reaction was incubated in a hot water bath at 95°C for 90 minutes and the absorbance of the coloured compound was measured at 695 nm. Ascorbic acid was used as the standard. The percentage of Mo⁶⁺ reduction was calculated as:

$$\% \text{ of Mo}^{6+} \text{ reduction} = \frac{\text{Sample} - \text{Control}}{\text{Sample}} \times 100$$

Ferric (Fe³⁺) reducing power assay

The reducing power assay of the leaves extract of *S. heyneanus* was determined by the potassium ferricyanide assay method with minor modifications [12]. One mL of the leaves extract of different concentrations (20 - 120 µg/mL) was mixed with 1 mL of potassium ferricyanide [K₃Fe (CN)₆] (1%, w/v) solution and 1 mL of phosphate buffer (0.2 M, pH 6.6) solution. Then, the mixture was incubated at 50°C in a water bath for 20 min. Five hundred µL of trichloroacetic acid (10% w/v) was added to each mixture followed by 100 µL of freshly prepared FeCl₃ (0.1%, w/v) solution was added and shaken well. The absorbance was measured at 700 nm. Ascorbic acid was used as the standard. The percentage of Fe³⁺ reduction was calculated as:

$$\% \text{ of Fe}^{3+} \text{ reduction} = \frac{\text{Sample} - \text{Control}}{\text{Sample}} \times 100$$

GC-MS Analysis

The methanol leaves extract of *S. heyneanus* was injected into an HP-5 column (30 m X 0.25 mm i.d with 0.25 µm film thickness), Agilent technologies 6890 N JEOL GC Mate II GC-MS model. The chromatographic conditions were helium as the carrier gas, the flow rate of one mL/min; and the injector was operated at 200°C and column oven temperature was programmed as 50-250°C at a rate of 10°C/min injection mode. The MS

conditions were ionization voltage of 70 eV; ion source temperature of 250°C; interface temperature of 250°C; the mass range of 50-600 mass units [13]. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST (National Institute of Standards and Technology) library.

Statistical analysis

All values acquired are cumulative of three replicates, and it was represented as a mean \pm standard deviation.

RESULTS AND DISCUSSION

The methanol leaves extract of *S. heyneanus* was tested for phytochemical compounds, which showed the presence of phenols, flavonoids, tannins, alkaloids, steroids, terpenoids, saponins, glycosides, and carbohydrate (Table 1).

Total Phenols and Flavonoids

Plant phenolic and flavonoids compounds are the most common phyto-compounds and extensively distributed occurring in all parts of the plant, mostly the photosynthesizing plant cells. The major coloring element of flowering plants are flavonoids and important part of human and animal nourishment [14]. Free radicals are molecules with an unpaired electron and steal electrons from other molecules to get stability which leads to the deterioration of molecules such as lipids, proteins, or DNA and gets oxidized [15, 16]. These oxidized

proteins are usually recognized and degraded in the cells, but some of them can accumulate over time and lead to cellular dysfunction. Antioxidant activity of phenolic compounds found in nature are flavones, isoflavones, anthocyanins, flavanones, and proanthocyanidins act as reducing agents to free radicals [17]. Phenolic compounds have also been anti-inflammatory properties depend on their number and position of hydroxyl groups. Flavonoids possess more hydroxyl groups thus present higher antioxidant activity and it is strong enough evidence that polyphenols can interact with nutrients, reactive metabolites, activated carcinogens, and mutagens [18]. It also can modulate the activity of key proteins involved in cell cycle progression [19] and many genes associated with cancer. Green tea flavanols reported in animal models have great effects and anti-cancer properties on human cell lines and in human intervention studies [20].

The total phenolic content of methanol leaves extract was 132.96 ± 2.22 $\mu\text{g}/\text{mg}$ of GAE of extract and the flavonoid content of methanol leaves extract was 42.24 ± 2.10 $\mu\text{g}/\text{mg}$ of QE of extract (Table 2).

DPPH[•] radical scavenging activity

The radical scavenging activity of the leaves extract of *S. heyneanus* was carried out by DPPH[•] (1, 1-diphenyl-2-

picrylhydrazyl) radical scavenging assay method. In this method, the 1, 1-diphenyl-2-picrylhydrazyl radical becomes 1,1-diphenyl-2-picrylhydrazine as non-radical, after the addition of antioxidant compounds from the extract [21]. It reflects the colour changes from purple to yellow. The highest DPPH[•] radical scavenging activity was 89.69±1.15% at 120 µg/mL concentration and the IC₅₀ was 53.16 µg/mL concentration (Table 3 Figure 2). The radical scavenging activity increases with increasing concentration of the extract and the IC₅₀ was compared with the standard quercetin (IC₅₀ = 3.11 µg/mL concentration).

OH[•] radical scavenging activity

Hydroxyl radicals are highly reactive species and attack most of the organic molecules. They are highly oxidizing molecules in nature which is attributed to their oxidation potential and are the principal-agent causing base damage to nucleosides and nucleotides [22]. Hydroxyl radicals attack the cell membrane, causing membrane damage and destroying sugar groups and DNA base sequences, inducing the disintegration of the double-helix structure, even causing cell death and mutations. Therefore, the scavenging activity of the hydroxyl radical is commonly used to evaluate the ability of the antioxidant substance. The highest OH[•] radical scavenging activity of leaves extract

was 45.56±0.62% at 120 µg/mL concentration and the IC₅₀ was 131.69 µg/mL concentration (Table 3, Figure 2). The IC₅₀ was compared with the standard quercetin (IC₅₀ = 4.21 µg/mL concentration).

ABTS^{•+} radical cation scavenging activity

ABTS^{•+} radical cation is a stable blue-green chromophore that is reduced to ABTS by the leaves extract of *S. heyneanus* to colourless [23]. The leaves extract showed the highest ABTS^{•+} radical cation scavenging activity of 97.41±1.02% at 60 µg/mL concentration and the IC₅₀ was 18.40 µg/mL concentration (Table 4, Figure 3). The ABTS^{•+} radical cation scavenging activity increases with increasing concentration of the leaves extract and the IC₅₀ was compared with the standard quercetin (IC₅₀ = 4.21 µg/mL concentration).

Phosphomolybdenum reduction activity

The reduction capacity of *S. heyneanus* leaves extract was determined by the phosphomolybdenum reduction assay method. The leaves extract reduces Mo (VI) to Mo (V) and blue or green phosphate/Mo (V) complex developed at acidic pH during the reaction [24]. The highest phosphomolybdenum reduction of leaves extract was 87.65±1.00% at 120 µg/mL concentration and the RC₅₀ was 24.67 µg/mL concentration (Table 5,

Figure 4). The RC_{50} was compared with the standard quercetin ($RC_{50} = 6.34 \mu\text{g/mL}$ concentration).

Ferric (Fe^{3+}) reducing power assay

The reducing power of the leaves extract of *S. heyneanus* was measured by the potassium ferricyanide reduction assay method. In this method, the Fe^{3+} ion reduced to Fe^{2+} by the electron-donating compounds present in the leaves extracts of *S. heyneanus* and formation of the Ferrous-ferric complex [25]. The highest Fe^{3+} reduction was $68.65 \pm 0.91\%$ at $120 \mu\text{g/mL}$ concentration and the RC_{50} was 58.37

$\mu\text{g/mL}$ concentration (Table 5, Figure 4). The reduction capacity was dose-dependent and the RC_{50} was compared with the standard quercetin ($RC_{50} = 7.72 \mu\text{g/mL}$ concentration).

GC-MS analysis of the leaves extract of *S. heyneanus*

The GC-MS analysis showed the presence of antioxidant compounds such as Flavone, 2H-1-Benzopyran-4,7-diol,3,4-dihydro-2-phenyl [26]. Hexadecanoic acid, methyl ester [27] Oleic acid, methyl ester [28] in the methanol leaves extract of *S. heyneanus* (Table 6, Figure 5).

Table 1: Phytochemical analysis of leaves extract of *S. heyneanus*

Phytochemicals	Test	Inference	Result
Phenols	FeCl ₃ test: To the extract, a little drop of 5% FeCl ₃ solution was added and shaken well.	Violet colour appears	+
Flavonoids	Alkaline Reagent test: To the extract, a little drop of 2% NaOH solution was added and shaken well.	Yellow colour appears	+
Tannins	Lead acetate test: To the extract, a little drops of 5% Pb(CH ₃ COO) ₂ solution was added and shaken well.	White colour appears	+
Alkaloids	Hager's test: To the extract, a saturated aqueous solution of picric acid was added and shaken well.	yellow precipitate appears	+
Steroids	Libermann-Burchard's test: The extract was mixed with 1 mL of acetic anhydride and shaken well. To this, few drops of Conc.H ₂ SO ₄ were added along the sides of the test tube.	The dark violet colour appears	+
Terpenoids	Salkowski test: To the extract, chloroform was added and mixed well. Then, little drops of Conc.H ₂ SO ₄ were added along the edges of the test tube.	The red ring appears.	+
Saponins	Foam test: To the extract, 3 mL of distilled water was added and shaken vigorously.	Foam appears	+
Glycosides	Legal's test: To the extract, little drops of pyridine and few drops of alkaline sodium nitroprusside solution was added and shaken well.	Blood red colour appears.	+
Carbohydrate	Molisch test: To the extract, two drops of alcoholic α -naphthol solution was added and shaken well. To this, a few drops of Concentrated H ₂ SO ₄ was added.	Violet ring appears	+

Note: (+) = present; (-) = absent

Table 2: Phenols and flavonoids estimation of leaves extract of *S. heyneanus*

Phytochemicals	Amount ($\mu\text{g/mg}$)
Phenols	132.96 ± 2.22 GAE
Flavonoids	42.24 ± 2.10 QE

Table 3: DPPH[•] radical and OH[•] radical scavenging activities of the leaves extract of *S. heyneanus*

S. No	Concentration (µg/mL)	Leaves extract	
		% of inhibition	
		DPPH [•] radical	OH [•] radical
1	20	23.42±1.07	5.52±0.55
2	40	38.37±1.02	12.76±0.78
3	60	56.43±1.17	20.52±0.80
4	80	71.53±1.24	28.38±0.55
5	100	85.32±1.11	35.35±0.67
6	120	89.68±1.15	45.56±0.62

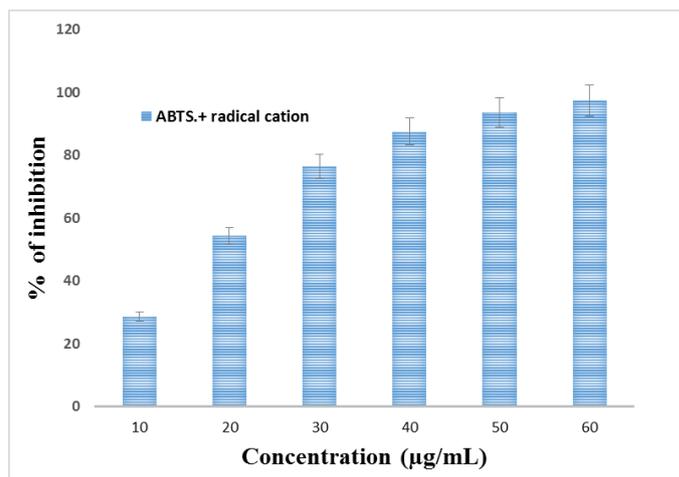


Figure 2: DPPH[•] radical and OH[•] radical scavenging activities of the leaves extract of *S. heyneanus*

Table 4: ABTS^{•+} radical cation scavenging activity of leaves extract of *S. heyneanus*

S. No	Concentration (µg/mL)	Leaves extract
		% of inhibition
		ABTS ^{•+} radical
1	10	28.61±0.69
2	20	54.34±0.99
3	30	76.41±1.24
4	40	87.59±0.85
5	50	93.57±0.84
6	60	97.41±1.02

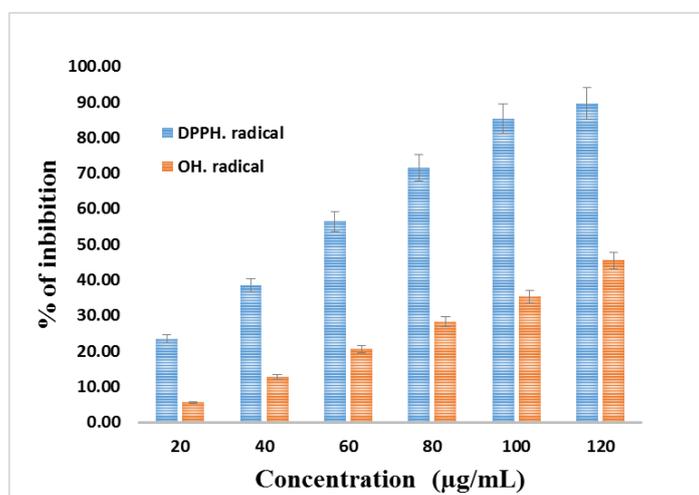
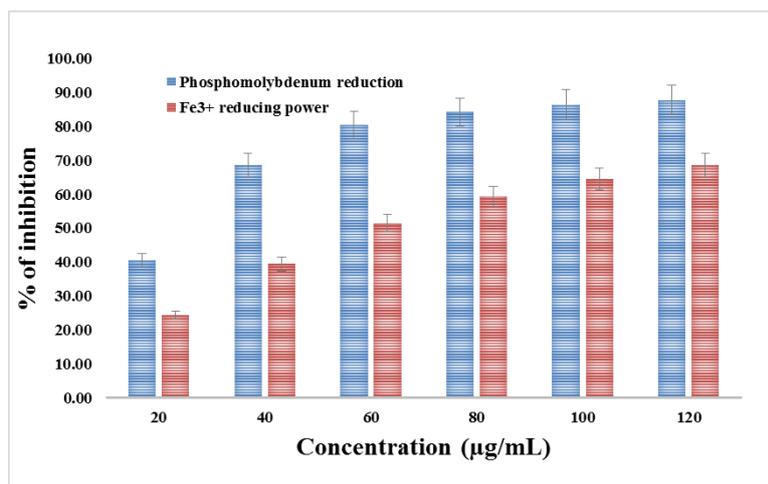


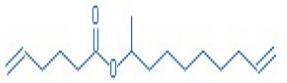
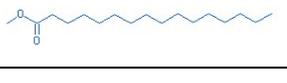
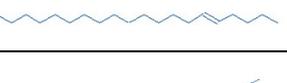
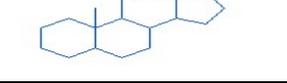
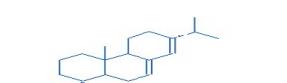
Figure 3: ABTS^{•+} radical cation scavenging activity of leaves extract of *S.heyneanus*

Table 5: Phosphomolybdenum reduction and Fe³⁺ reducing power activity of leaves extract of *S.heyneanus*

S. No	Concentration (µg/mL)	Leaves extract	
		% of reduction	
		Mo ⁶⁺ reduction	Fe ³⁺ reduction
1	20	40.53±0.61	24.30±0.90
2	40	68.50±0.75	39.40±0.65
3	60	80.37±0.73	51.39±1.38
4	80	84.15±1.08	59.42±0.75
5	100	86.43±1.14	64.37±1.18
6	120	87.65±1.00	68.65±0.91

Figure 4: Phosphomolybdenum reduction and Fe³⁺ reducing power activity of the leaves extract of *S. heyneanus*Table 6: GC-MS analysis of the leaves extract of *S. heyneanus*

S. No	Compound name	R.T	Compound structure	Mol. weight g/mol	Mol. formula	Peak Area %
1.	4-Hydroxybenzamide	12.72		137.14	C ₇ H ₇ NO ₂	5.45
2.	Coumarin 4,7-dimethoxy (citrופן)	13.48		206.00	C ₁₁ H ₁₀ O ₄	5.24
3.	Flavone	15.07		222.00	C ₁₅ H ₁₀ O ₂	6.85
4.	Dodecanoic acid,11-oxo, Methyl ester	15.78		228.33	C ₁₃ H ₂₄ O ₃	5.86
5.	2H-1-Benzopyran-4,7-diol,3,4-dihydro-2-phenyl	16.38		242.00	C ₁₅ H ₁₄ O ₃	6.07

6.	5-Hexenoic acid, (9-decen-2yl) ester	16.6		252.00	C ₁₆ H ₂₈	6.04
7.	11- Hexadecenoic acid, methyl ester	17.03		268.00	C ₁₇ H ₃₂ O ₂	9.61
8.	Hexadecanoic acid, methyl ester	17.23		270.00	C ₁₇ H ₃₄ O ₂	10.12
9.	5-Eicosene,[E]	17.88		280.5	C ₂₀ H ₄₀	6.70
10.	Oleic acid, methyl ester	19.00		296.00	C ₁₉ H ₃₆ O ₂	8.58
11	Octadecanoic acid, methyl ester	19.22		298.00	C ₁₉ H ₃₈ O ₂	8.61
12	Pregnan-20-one[5a,17a]	19.8		302.00	C ₂₁ H ₃₄ O	6.29
13	Methyl abietate	20.55		316.5	C ₂₁ H ₃₂ O ₂	8.19
14	1-Tetradecene,2-decyl	21.60		336.00	C ₂₄ H ₄₈	6.40

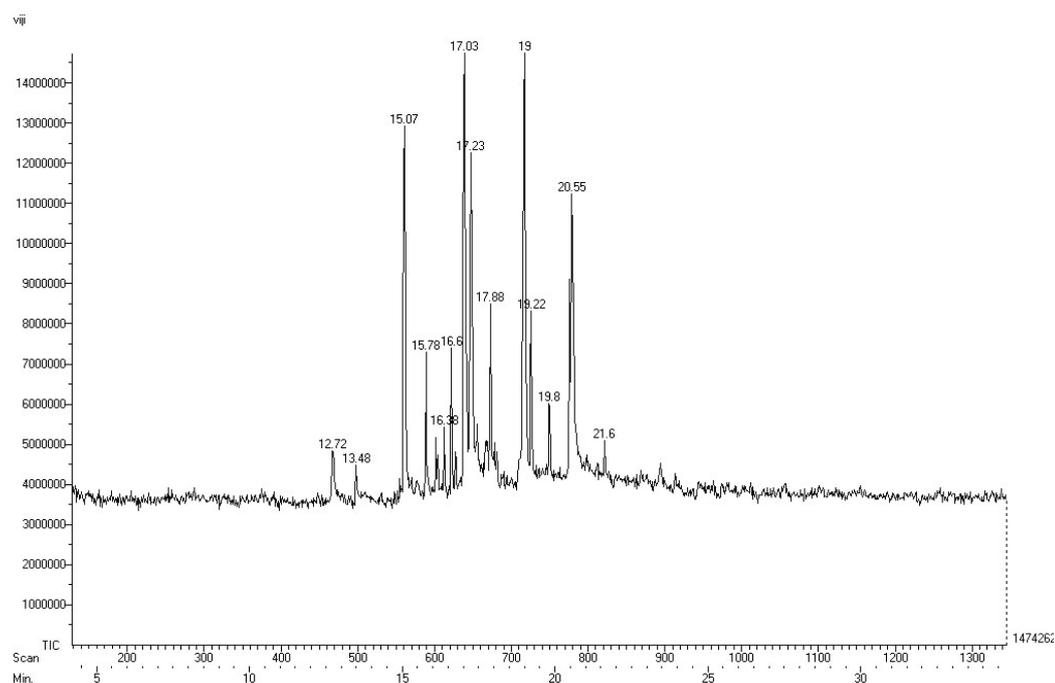


Figure 5: Gas chromatogram of the leaves extract of *S. heyneanus*

CONCLUSION

The process of oxidation in the human body damages cell membranes including cellular proteins, lipids and DNA. When oxygen is metabolised, it creates unstable molecules called 'free radicals', which damage to DNA and other cells. The body can manage with some free radicals and needs them to function efficiently. However, the damage caused by an overload of free radicals over time may become irreversible and lead to certain diseases including heart and liver disease and some cancers such as oral, oesophageal, stomach and bowel cancers. Antioxidants are substances that may protect your cells against free radicals and are found in certain foods and may prevent some of the damage caused by free radicals by neutralising them. The study reveals that the leaves extract of *S. heyneanus* has significant antioxidant properties and GCMS analysis also revealed that the leaves extract has some antioxidant compounds which are useful to counteract harmful free radicals.

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