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Original Article

# Phytochemical Extraction, Screening, GCMS Analysis and Antioxidant Properties of *Ophiorrhiza recurvipetala*

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## ABSTRACT

**Background and Objectives:** Most of the people from developing countries depend on plant-based ailments for several diseases due to the occurrence of pharmacological bioactive substances with lowered adverse effects. *Ophiorrhiza recurvipetala* (OR), a newly identified species from the Rubiaceae family, which was unexplored for its bioactive compounds and antioxidant properties. **Materials and Methods:** The whole plant samples were collected, authenticated extracted with ethanol and n-hexane, subjected to phytochemical analysis and analyzed by GC-MS. Antioxidant activity of the extracts were assessed using FRAP, DPPH, ABTS, hydroxyl, and superoxide radical scavenging assays. **Results:** The phytochemical examination explored the presence of several phytochemicals including flavonoids, alkaloids, phenols, steroids, tannins, glycosides, and saponins in the ethanolic (OREE) and n-Hexane (ORNHEX) extracts. GC-MS analysis identified 58 and 81 compounds in OREE and ORNHEX, respectively. Although the results demonstrated that both extracts exhibited significant antioxidant properties, OREE displayed superior radical scavenging activity which was evidenced by $IC_{50}$ values. **Conclusion:** These findings suggest that *O. recurvipetala* has potential bioactive compounds and could serve as a valuable source of natural antioxidants for therapeutic use. This study provides a foundation for further research aimed in isolating and identifying novel compounds with potential applications in medicine and drug development. As far as we understand, this is the first study on this plant species.

**Keywords:** *Ophiorrhiza recurvipetala*, Ethanol, n-Hexane, Phytochemicals, GC-MS Analysis, Antioxidants.

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## INTRODUCTION

About 80% of the people from all the continents depend on plant-based medicine.[1] Around 40,000 plants were utilised in traditional medicines due to the presence of rich ingredients, which is proved to be safe because of their less adverse effect.[2] The genus *Ophiorrhiza* of Rubiaceae family was distributed throughout the world.[3] The term "Ophiorrhiza" has been derived from Greek words 'Ophis' meaning snake and 'rhiza' meaning root.[4] The pharmacologically active metabolites such as terpenoids, anthraquinones, iridoids, indole alkaloids, flavonoids, and several phenolic compound derivatives were found in *Ophiorrhiza* genus.[5-8] In addition, few vital phytochemicals present in most Ophiorrhiza species are camptothecin, luteolin, pumiloside, harman, etc.,[9] These compounds are reported to have anticancer, anti-inflammatory and antimicrobial activities.[5] These bioactive components like flavonoids, terpenoids, tannins, phenols and saponins are the base for allopathic medicines.

Out of 318 species worldwide, 9 varieties and 47 species were found in India, and the comprehensive description, ecological distribution and phenology of a new species *Ophiorrhiza recurvipetala* has been reported recently from Assam, India by Bhuyan *et al.*,[10] Gas Chromatography-Mass Spectroscopy (GC-MS), is a well-suited and mostly used technique for the identification and quantification purpose by comparing the obtained spectra with the reference.[11] Several studies showed that oxidative stress is linked with chronic diseases like cancer, neurodegenerative disorder, ageing and cardiovascular disease. The phenolic and flavonoid compounds found in all parts of the plant are responsible for antioxidant function.[12,13] Therefore, in this study, the whole plant samples were collected, extracted with ethanol and n-hexane, analyzed by GC-MS and *in vitro* antioxidant activities. To the best of our knowledge, no detailed

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study was available for bioactive compounds and antioxidant properties on *O. recurvipetala* whole plant extract.

## MATERIALS AND METHODS

### Plant material

Whole plant of *Ophiorrhiza recurvipetala* was obtained from Western Ghats, Nilgiris, Tamil Nadu, and identified by Plant taxonomist, Dr. K. Madhava Chetty, (IAAT: 337), Botany Department, SV University, Tirupathi, India and a sample with voucher specimen (0899) was placed in the department's Herbarium.

### Chemicals

Ethanol, n-Hexane, $H_2SO_4$, NaOH, $FeCl_3$, HCl, chloroform, sodium bicarbonate, ammonia, acetone and glacial acetic acid were purchased from Himedia, India.

### Solvent extracts

The shaded dried whole plant material (14 g) of *O. recurvipetala* was grounded and extracted with 100 mL of 95% ethanol and n-Hexane by maceration for 8 hr in Soxhlet apparatus to get ethanolic (OREE) and n-hexane (ORXHE) extract of *O. recurvipetala*. The crude liquid extract was filtered (Whatman no. 1 paper) and then filtrate was evaporated to dry at 40°C using rotary evaporator. Dried crude OREE and ORNHEX were kept in air-tight containers and stored in a desiccator for further use.

### Qualitative phytochemical analysis

The screening of OREE and ORNHEX were performed via standard procedures.[14] To the Crude extracts, 2 mL of Benedict's reagent was mixed, heated and the occurrence of carbohydrate is confirmed by the formation of a reddish-brown precipitate. The plant extracts were mixed with 2% $H_2SO_4$ and heated for 2 min, filtered and added with Dragendorff's reagent. The occurrence of alkaloids is confirmed by the presence of reddish-brown precipitate. The crude extracts were added with Millon's reagent, and the occurrence of protein is confirmed by formation of white precipitate. In Ferric chloride test**,** $FeCl_3$ (2%) solution was added with crude extract and the presence of phenols and tannins is confirmed by the formation of blue-green precipitate. In Zinc-HCl reduction test, a pinch of Zinc dust and a few drops of concentrated HCl were mixed with crude extract and the occurrence of flavonoids is confirmed by magenta colour formation. In Salkowski's test, extracts were added with chloroform and conc. $H_2SO_4$ and the occurrence of triterpenes/ steroids is confirmed by golden yellow/ greyish colour formation.

In the Froth test, a few drops of sodium bicarbonate solution were mixed vigorously with extracts and kept for 3 min. The occurrence of saponin is indicated by formation of honey comb like froth. In Keller-Kilani test, extracts were mixed with glacial acetic acid, drops of $FeCl_3$ and conc. $H_2SO_4$. A brown ring formation indicates the presence of glycoside. The extracts were treated with chloroform and ammonia. A pink, red colour formation indicates the presence of anthracene derivatives. The crude extract was mixed with sodium hydroxide and the occurrence of quinone is indicated by red or blue green colour formation (Bontrager's test). In Paper test, a drop of extracts was placed between 2 filter papers in an undisturbed manner. The occurrence of oils and fats were confirmed by oil stain on paper. The extract was mixed with acetone and added to distilled water. The occurrence of resins is confirmed by the formation of turbidity. The chloroform and sodium hydroxide were mixed with crude extracts and the occurrence of coumarins indicated the formation of yellow colour. Crude extracts were added with chloroform, shanked vigorously and filtered. To the filtrate, sulphuric acid was mixed and the blue colour formation in the interface denotes the occurrence of carotenoids.

### GC-MS analysis

The extracts of OREE and ORNHEX were evaluated for the occurrence of several volatile compounds by GC-MS (Agilent, 7890). Sample was prepared and injected in split mode with an injection temperature of 250°C. The injection was performed after three rinses with presolvent, post-solvent, and sample, respectively, using a plunger speed set to high. The column oven was programmed to start at 50°C and ramp to 280°C, with 2 min hold time at final temperature. 16.2 mL/min and 1.2 mL/ min total flow and the column flow respectively was used and maintained at under linear velocity control mode. The MS was configured to scan between 50 and 500 m/z at a speed of 1666 amu/sec and an event duration of 0.30 sec. Temperatures of 200°C for the ion source and 250°C for the interphase were established. After applying a solvent cut time of three and half minutes, data collection started right away. The procedure took 35 min to run in total.

### ABTS radical scavenging assay

Equal volume of two solutions, potassium persulfate (2.4 mM) solution and ABTS (7mM) were mixed and kept at darkness in 37°C for 14 hr. 1 mL of working solution was diluted with methanol and also allowed to react with OREE, ORNHEX and ascorbic acid (12.5. 25, 50, 100, 200 µg/mL) and were measured at 734 nm. The percentage inhibition was calculated.[15] All determinations were performed in triplicate ($n$=3).

### Ferric Reducing Ability of Plasma (FRAP) assay

The FRAP reagent was mixed with OREE, ORNHEX and gallic acid (125, 250, 500, 1000, 2000 µg/mL). The change in OD was determined at 593 nm and Fe II standard solution was tested in parallel.[15]

### Superoxide radical scavenging activity

The sodium phosphate buffer (100 mM, pH 7.4) with NBT (150 mM) and NADH (468 mM) was mixed with OREE, ORNHEX and gallic acid (125, 250, 500, 1000, 2000 µg/mL). Then PMS (60 mM) solution was mixed, kept for 300 sec and OD was calculated at 560 nm.[16]

### DPPH radical scavenging assay

To DPPH (100 mM) solution, OREE, ORNHEX and ascorbic acid (12.5. 25, 50, 100, 200 µg/mL) were mixed, shanked vigorously, incubated for 30 min in darkness and OD was calculated at 517 nm.[17]

### Hydroxyl radical scavenging assay

Different concentrations of OREE, ORNHEX and gallic acid (125, 250, 500, 1000, 2000 µg/mL) were mixed with KH2PO4 buffer, pH 7.4 (0.05 M), containing deoxyribose (2.8 mM), EDTA (0.1 mM), $FeCl_3$ (0.1 mM) and $H_2O_2$ (1 mM). The mixture was incubated for 30 min at room temperature and mixed with trichloroacetic acid (2.8% w/v) and thiobarbituric acid. Then it was incubated in water bath for 30 min, cooled and OD was observed at 532 nm.[18]

## RESULTS

### Preliminary Phytochemical Screening

The results of the phytochemical analysis of OREE and ORNHEX are shown in Table 1. It depicts the occurrence of tannins, proteins and amino acids, alkaloids, phenols, flavonoids, steroids/ terpenoids, saponins, glycosides, quinones, fixed oils, resins, coumarins and carbohydrates in both OREE and ORNHEX.

### GC-MS analysis

The GC-MS chromatograms spectra obtained for OREE and ORNHEX revealed that *O. recurvipetala* is plenteously rich in bioactive compounds. The GC-MS spectrum for OREE and ORNHEX, showed 58 and 81 peaks and indicating 58 and 81 compounds, from which some of the bioactive compounds showing various pharmaceutical activities were shown in Figures 1 and 2. The spectra of these bioactive compounds were matched with NIST library's software of GC-MS. Retention time, Molecular formula, Peak area, molecular weight and nature of the compound were presented in Table 2 for OREE and Table 3 for ORNHEX.

### Evaluation of the antioxidant activity of the extracts

#### *DPPH radical-scavenging assay*

The DPPH assay is primarily investigated for reducing the ability of several extracts/compounds that are based on the occurrence of hydrogen-donating stimulants. The results of DPPH assay are depicted in Figure 3a. The $IC_{50}$ values recorded for the OREE, ORNHEX and AA were 60.477 µg $mL^{-1}$, 166.25 µg $mL^{-1}$ and 30.97 µg $mL^{-1}$, respectively. Although the values of the extracts were not equivalent with standard ascorbic acid, they are present within the range. However, the study revealed that both extracts have the potential to inhibit the production of free radicals.

### ABTS radical-scavenging activity

Outcomes of ABTS radical scavenging assay were depicted in Figure 3b. The OREE and ORNHEX extracts scavenged ABTS radical effectively on a dose-dependent fashion than other extracts. OREE (115.94 µg $mL^{-1}$) showed potent radical activity than ORNHEX (157.99 µg $mL^{-1}$) extract. AA (59.63 µg $mL^{-1}$) showed potent antioxidant activity than other extracts.

### FRAP assay

In the FRAP assay, a potent antioxidant activity was observed in OREE (56.50 µg $mL^{-1}$) as compared to those ORNHEX (84.82 µg $mL^{-1}$), but still lower than gallic acid (31.51 µg $mL^{-1}$) (Figure 3c).

**Table 1: Preliminary phytochemical analysis of OREE and ORNHEX.**

| Sl. No. | Phytochemical test | OREE | ORNHEX |
|---|---|---|---|
| 1 | Proteins and amino acids | + | + |
| 2 | Alkaloids | + | + |
| 3 | Phenols and Tannins | + | + |
| 4 | Saponins | + | + |
| 5 | Steroids/ Terpenoids | + | + |
| 6 | Flavonoids | + | + |
| 7 | Glycosides | + | + |
| 8 | Quinones | + | + |
| 9 | Fixed oils | + | + |
| 10 | Resins | + | + |
| 11 | Coumarins | + | + |
| 12 | Carbohydrates | + | + |

**Table 2: Compounds detected for OREE in GCMS analysis.**

| Peak | Name of the compound | Retention Time | Area% | Molecular formula | Molecular Weight | Nature of the compound |
|---|---|---|---|---|---|---|
| 1 | 5-Keto-D-fructose | 3.761 | 0.49 | $C_6H_{10}O_6$ | 178.05 | Diketone |
| 2 | 2,5-Dihydroxybenzaldehyde, 2TMS derivative | 5.872 | 1.14 | $C_7H_6O_3$ | 138.12 | Phenol |
| 3 | 2,4-Dihydroxy-3-methylbenzaldehyde, 2TMS | 7.198 | 0.75 | $C_8H_8O_3$ | 152.15 | Resorcinol |
| 4 | Octane, 2,3,3-trimethyl- | 7.275 | 0.29 | $C_{11}H_{24}$ | 156.31 | Alkane |
| 5 | Hexadecane, 1,1-bis(dodecyloxy)- | 8.27 | 0.38 | $C_{40}H_{82}O_2$ | 594.63 | Alcohol |
| 6 | 4-Vinylphenol | 9.455 | 3.64 | $C_8H_8O$ | 120.15 | Phenol/Styrene |
| 7 | 5-Amino-1-methyl-1H-pyrazole-4-carboxamide, 3TMS | 9.632 | 4.21 | $C_5H_8N_4O$ | 140.14 | Pyrazole |
| 8 | Cyclopentasiloxane, decamethyl- | 9.818 | 0.69 | $C_{10}H_{30}O_5Si_5$ | 370.77 | Cyclomethicone |
| 9 | Octadecane-1,2-diol, 2TMS derivative | 9.959 | 0.68 | $C_{24}H_{54}O_2Si_2$ | 430.90 | Organo silicone |
| 10 | Cyclohexasiloxane, dodecamethyl- | 11.022 | 1.26 | $C_{12}H_{36}O_6Si_6$ | 444.92 | Silicone |
| 11 | 2-Methoxy-4-vinylphenol | 11.122 | 1.66 | $C_9H_{10}O_2$ | 150.17 | Phenol |
| 12 | Cyclohexasiloxane, dodecamethyl- | 11.192 | 0.78 | $C_{12}H_{36}O_6Si_6$ | 444.92 | Silicone |
| 13 | Butanoic acid, heptyl ester | 11.424 | 0.32 | $C_{11}H_{22}O_2$ | 186.29 | Fatty acid ester |
| 14 | Propanoic acid, 2-methyl-, 3-hydroxy-2,2,4-trimethylpentyl ester | 11.829 | 0.44 | $C_{12}H_{24}O_3$ | 216.32 | Carboxylic ester |
| i | Copaene | 12.159 | 0.24 | $C_{15}H_{24}$ | 204.35 | Sesquiterpenoids |
| 16 | 3,3,5-Triethoxy-1,1,1,7,7,7-hexamethyl-5-(trimethylsilyloxy)tetrasiloxane | 12.265 | 0.55 | $C_{15}H_{42}O_7Si_5$ | 474.91 | Silicone |
| 17 | 2-Amino-N-(4-fluorophenyl) benzamide, 2TBDMS derivative | 12.452 | 0.87 | $C_{13}H_{11}FN_2O$ | 230.24 | Halogenated amines |
| 18 | 3,3,5-Triethoxy-1,1,1,7,7,7-hexamethyl-5-(trimethylsilyloxy)tetrasiloxane | 12.518 | 2.17 | $C_{15}H_{42}O_7Si_5$ | 474.91 | Silicone |
| 19 | Hexasiloxane, tetradecamethyl- | 12.608 | 0.67 | $C_{14}H_{42}O_5Si_6$ | 458.99 | Silicone |
| 20 | 2-[(p-Trimethylsilyloxy) phenyl]-2-[(p-trimethylsilyloxyethylenoxy) phenyl]propane | 13.174 | 0.66 | $C_{18}H_{34}O_4Si_3$ | 398.70 | Silicone |
| 21 | 11-(2-Cyclopenten-1-yl) undecanoic acid, (+)- | 13.824 | 0.86 | $C_{16}H_{28}O_2$ | 252.39 | Fatty acid ester |
| 22 | Cycloheptasiloxane, tetradecamethyl- | 13.958 | 1.53 | $C_{14}H_{42}O_7Si_7$ | 519.08 | Skin care |
| 23 | Silane, trimethyl[2-methylene-1-(4-pentenyl) cyclopropyl]- | 14.611 | 0.34 | $C_{12}H_{22}Si$ | 194.39 | Silane |
| 24 | 2-Amino-N-(4-fluorophenyl) benzamide, 2TBDMS derivative | 15.042 | 0.98 | $C_{13}H_{11}FN_2O$ | 230.24 | Halogenated amines |
| 25 | Bis(heptamethylcyclotetrasiloxy)siloxane | 15.202 | 0.56 | $[(CH_3)_3SiO]_2SiHCH_3$ | 222.50 | Silicone |
| 26 | Octane, 2,6,6-trimethyl- | 15.491 | 1.8 | $C_{11}H_{24}$ | 156.31 | Saponins |
| 27 | Bis(pentamethylcyclotrisiloxy) tetramethyldisiloxane | 15.599 | 1.47 | $C_{14}H_{42}O_9Si_8$ | 579.20 | Silicone |
| 28 | Phenol, 3,4,5-trimethoxy- | 15.733 | 0.32 | $C_9H_{12}O_4$ | 184.19 | Phenol |
| 29 | 1-Deoxy-d-mannitol | 16.713 | 9.13 | $C_6H_{13}NO_7$ | 211.17 | Inorganic |
| 30 | Octadecane, 1-(ethenyloxy)- | 16.908 | 1.97 | $C_{20}H4_0O$ | 296.53 | Saponins |
| 31 | 3-Hexanol, 3,5-dimethyl- | 17.157 | 1.29 | $C_8H_{18}O$ | 130.23 | Alcohol |

| Peak | Name of the compound | Retention Time | Area% | Molecular formula | Molecular Weight | Nature of the compound |
|---|---|---|---|---|---|---|
| 32 | N-(Trifluoroacetyl)-N, O, O', O''-tetrakis(trimethylsilyl)norepinephrine | 17.371 | 0.79 | $C_{22}H_{42}F_3NO_4Si_4$ | 553.9 | Organic Compound |
| 33 | Malonic acid, bis (2-trimethylsilylethyl ester | 17.483 | 1.21 | $C_{13}H_{28}O_4Si_2$ | 304.53 | Malonic acid ester |
| 34 | 6-Methylheptanoic acid | 17.731 | 0.72 | $C_8H_{16}O_2$ | 144.21 | Eponemycin analogue |
| 35 | Decanoic acid, ethyl ester | 18.186 | 0.38 | $C_{12}H_{24}O_2$ | 200.32 | fatty acid ester |
| 36 | 3,4-Dihydroxymandelic acid, 4TMS derivative | 18.489 | 0.27 | $C_{20}H_{40}O_5Si_4$ | 472.87 | L-Dopa |
| 37 | Neophytadiene | 18.756 | 2.59 | $C_{20}H_{38}$ | 278.50 | Diterpene |
| 38 | Oxirane, octyl- | 18.85 | 0.47 | $C_{10}H_{20}O$ | 156.27 | Heterocyclic ether |
| 39 | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | 19.073 | 0.54 | $C_{20}H_{40}O$ | 296.50 | Phytol |
| 42 | d-Mannitol, 1-O-(22-hydroxydocosyl)- | 19.779 | 0.57 | $C_{28}H_{58}O_7$ | 506.80 | Alcohol |
| 43 | n-Hexadecanoic acid | 20.28 | 2.29 | $C_{16}H_{32}O_2$ | 256.42 | Ester |
| 44 | Boric acid, 3TMS derivative | 20.433 | 1.03 | $C_9H_{27}BO_3Si_3$ | 278.38 | Borate |
| 45 | Hexadecanoic acid, ethyl ester | 20.681 | 6.74 | $C_{18}H_{36}O_2$ | 284.48 | Ester |
| 46 | 1-Heptadecyne | 20.817 | 0.3 | $C_{17}H_{32}$ | 236.44 | Alkynes |
| 47 | 1H-Naphtho[2,1-b] pyran, 3-ethenyldodecahydro-3,4a,7,7,10a-pentamethyl-, [3R-(3. alpha.,4a. beta.,6a. alpha., 10a.beta., 10b.alpha.)] | 21.082 | 3.27 | $C_{20}H_{34}O$ | 290.48 | Manolyloxide |
| 50 | cis, cis, cis-7,10,13-Hexadecatrienal | 22.368 | 4.37 | $C_{16}H_{26}O$ | 234.38 | Unsaturated aldehyde |
| 51 | Dichloroacetic acid, tridec-2-ynyl ester | 22.697 | 15.3 | $C_{15}H_{24}Cl_2O_2$ | 307.26 | Ester of dichloroacetic acid |
| 52 | Octadecanoic acid, ethyl ester | 22.968 | 1.61 | $C_{20}H_{40}O_2$ | 312.53 | Ethyl stearate |
| 54 | 2,6,10,14,18-Pentamethyl-2,6,10,14,18-eicosapentaene | 29.579 | 1.18 | $C_{25}H_{42}$ | 342.60 | Ester |
| 55 | Sulfurous acid, pentadecyl 2-propyl ester | 30.394 | 1.23 | $C_{18}H_{38}O_3S$ | 334.60 | Ester |
| 56 | Octadecane, 3-ethyl-5-(2-ethylbutyl)- | 32.784 | 0.78 | $C_{26}H_{54}$ | 366.70 | Ester |
| 57 | ((1R,4S,5R)-1-Methyl-4-(prop-1-en-2-yl) spiro [4.5]dec-7-en-8-yl)methanol | 32.892 | 0.47 | $C_{15}H_{24}O$ | 220.35 | Spiro compound |
| 58 | (22R,23S)-22-Acetoxy-24-methylene-5. Alpha. -lanost-8-ene-3.beta.-22-diol | 34.641 | 0.8 | $C_{28}H_{48}O_3$ | 432.70 | Terpenoid |

The $IC_{50}$ values were present within the range of standard gallic acid.

## Hydroxyl radical scavenging assay

In the OH scavenging assay, a potent antioxidant activity was observed in OREE as compared to those ORNHEX, but still lower than gallic acid (Figure 3d). The $IC_{50}$ values recorded for the OREE and ORNHEX were 74.44 μg $mL^{-1}$ and 107.38 μg $mL^{-1}$, respectively; the values were comparable with standard gallic acid (33.73 μg $mL^{-1}$).

## Superoxide radical scavenging assay

A potent antioxidant activity was observed in OREE as compared to those ORNHEX (Figure 3e), but still lower than gallic acid. The $IC_{50}$ values recorded for the OREE and ORNHEX were 78.13 μg $mL^{-1}$ and 106.83 μg $mL^{-1}$, respectively; the values were comparable with standard gallic acid (66.73 μg $mL^{-1}$).

**Table 3: Compounds detected for ORNHEX in GCMS analysis.**

| Peak | Name | Retention Time | Area% | Molecular formula | Molecular weight | Nature of the compound |
|---|---|---|---|---|---|---|
| 1 | Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)- | 5.071 | 1.44 | $C_{10}H_{16}$ | 136.23 | Terpenoid |
| 2 | Undecane, 4,7-dimethyl- | 6.519 | 0.2 | $C_{13}H_{28}$ | 184.36 | Alkane |
| 3 | Hexane, 3,3-dimethyl- | 10.475 | 0.45 | $C_8H_{18}$ | 114.23 | Alkane |
| 4 | Hexadecanoic acid, (2-pentadecyl-1,3-dioxolan-4-yl)methyl ester | 11.251 | 0.51 | $C_{34}H_{66}O_4$ | 538.88 | Fatty acid derivative |
| 5 | Borane, diethyl(decyloxy)- | 13.836 | 0.27 | $C_{14}H_{33}BO$ | 228.23 | Organoborane compound |
| 6 | Hexane, 3,3-dimethyl- | 13.97 | 1.13 | $C_8H_{18}$ | 114.23 | Alkane |
| 7 | Cyclopropyl methyl carbinol | 14.115 | 0.15 | $C_5H_{10}O$ | 86.13 | Alcohol |
| 8 | Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters | 14.267 | 0.53 | $C_{21}H_{34}O_3$ | 334.49 | Phenolic ester |
| 9 | Benzoic acid, 4-ethoxy-, ethyl ester | 14.485 | 1.59 | $C_{11}H_{14}O_3$ | 194.23 | Aromatic ester |
| 10 | Nonane, 1-iodo- | 14.639 | 0.22 | $C_9H_{19}I$ | 254.15 | Alkyl iodide |
| 11 | Phthalic acid, ethyl 3-methylbutyl ester | 15.523 | 0.27 | $C_{15}H_{22}O_4$ | 266.33 | Phthalate ester |
| 12 | p-Octylacetophenone | 16.018 | 1.85 | $C_{16}H_{24}O$ | 232.36 | Ketone |
| 13 | 1-Hexanol, 5-methyl-2-(1-methylethyl)- | 16.617 | 0.28 | $C_{10}H_{22}O$ | 158.29 | Alcohol |
| 14 | Nonane, 1-iodo- | 16.725 | 0.12 | $C_9H_{19}I$ | 254.15 | Alkyl iodide |
| 15 | Nonane, 1-iodo- | 16.913 | 0.26 | $C_9H_{19}I$ | 254.15 | Alkyl iodide |
| 16 | Dodecane, 5-methyl- | 17.048 | 0.77 | $C_{13}H_{28}$ | 184.36 | Alkane |
| 17 | Nonane, 1-iodo- | 17.165 | 0.21 | $C_9H_{19}I$ | 254.15 | Alkyl iodide |
| 18 | Nonane, 1-iodo- | 17.633 | 0.3 | $C_9H_{19}I$ | 254.15 | Alkyl iodide |
| 19 | Fluoro(methyl)(2,4,6-tri-tert-butylphenyl) silanol | 18.082 | 0.64 | $C_{18}H_{31}FOSi$ | 310.53 | Organosilicon compound |
| 20 | 16-Heptadecenal | 18.759 | 0.38 | $C_{17}H_{32}O$ | 252.44 | Aldehyde |
| 21 | 4-Octanone | 18.857 | 0.24 | $C_8H_{16}O$ | 128.21 | Ketone |
| 22 | Phenol, 2,4,6-tri-tert-butyl- | 18.981 | 0.54 | $C_{18}H_{30}O$ | 262.43 | Phenol derivative |
| 23 | Phthalic acid, cyclobutyl tridecyl ester | 19.204 | 0.22 | $C_{24}H_{38}O_4$ | 390.56 | Phthalate ester |
| 24 | trans-3-Methylcyclohexanol | 19.3 | 0.14 | $C_7H_{14}O$ | 114.19 | Cyclohexanol derivative |
| 25 | 2,2,4,6,6,8-Hexamethyl-4,8-diphenylcyclotetrasiloxane | 19.412 | 0.57 | $C_{20}H_{30}O_4Si_4$ | 450.81 | Organosilicon compound |
| 26 | Nonane, 1-iodo- | 19.507 | 0.13 | $C_9H_{19}I$ | 254.15 | Alkyl iodide |
| 27 | Hexane, 3,3-dimethyl- | 19.725 | 0.23 | $C_8H_{18}$ | 114.23 | Alkane |
| 28 | Pentadecanoic acid, 14-methyl-, methyl ester | 19.858 | 2.13 | $C_{17}H_{34}O_2$ | 270.45 | Fatty acid ester |
| 29 | Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester | 20.129 | 0.56 | $C_{19}H_{28}O_3$ | 304.42 | Aromatic ester |
| 30 | 2-Bromononane | 20.242 | 0.16 | $C_9H_{19}Br$ | 207.15 | Alkyl bromide |
| 31 | Dibutyl phthalate | 20.371 | 7.2 | $C_{16}H_{22}O_4$ | 278.34 | Phthalate ester |
| 32 | Butanoic acid, 2-methyl-, methyl ester | 20.694 | 0.36 | $C_6H_{12}O_2$ | 116.16 | Ester |
| 33 | 2-t-Butyl-5-(dimethoxy-phosphoryl)-3-methyl-4-oxoimidazolidine-1-carboxylic acid, t-butyl ester | 20.792 | 0.13 | $C_{15}H_{28}N_2O_5P$ | 346.37 | Organophosphorus compound |

| Peak | Name | Retention Time | Area% | Molecular formula | Molecular weight | Nature of the compound |
|---|---|---|---|---|---|---|
| 34 | Pentane, 3-(bromomethyl)- | 21.914 | 0.29 | $C_6H_{13}Br$ | 165.07 | Alkyl bromide |
| 35 | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | 22.079 | 3.14 | $C_{20}H_{40}O$ | 296.53 | Terpene alcohol |
| 36 | Tridecanoic acid, methyl ester | 22.224 | 0.74 | $C_{14}H_{28}O_2$ | 228.37 | Fatty acid ester |
| 37 | Sulfurous acid, hexyl octyl ester | 22.314 | 0.49 | $C_{14}H_{30}O_3S$ | 278.46 | Organosulfur compound |
| 38 | Tetrahydrofurfuryl acrylate | 22.433 | 0.22 | $C_9H_{14}O_3$ | 170.21 | Acrylate ester |
| 39 | Pentane, 3-(bromomethyl)- | 22.608 | 0.25 | $C_6H_{13}Br$ | 165.07 | Alkyl bromide |
| 40 | Sulfurous acid, hexyl nonyl ester | 22.684 | 0.22 | $C_{15}H_{32}O_3S$ | 292.48 | Organosulfur compound |
| 41 | Decane, 1-iodo- | 22.779 | 0.3 | $C_{10}H_{21}I$ | 282.18 | Alkyl iodide |
| 42 | Penigequinolone A, 3TMS | 22.863 | 0.5 | $C_{21}H_{28}N_2O_4Si_3$ | 472.71 | Quinolone derivative |
| 43 | Nonane, 1-iodo- | 23.012 | 0.33 | $C_9H_{19}I$ | 254.15 | Alkyl iodide |
| 44 | Hexane, 3,3-dimethyl- | 24.07 | 0.12 | $C_8H_{18}$ | 114.23 | Alkane |
| 45 | 4H,5H-Pyrano(4,3-b)pyran-4,5-dione, 2,3-dihydro-3-.alpha.-hydroxy-2-. beta.-methyl-7-propenyl- | 24.609 | 0.63 | $C_{14}H_{18}O_4$ | 250.29 | Coumarin derivative |
| 46 | Acetaldehyde, 2-butenylhydrazone | 24.733 | 0.34 | $C_6H_{10}N_2$ | 110.16 | Hydrazone |
| 47 | 2-Thiopheneacetic acid, 2-tridecyl ester | 24.945 | 0.53 | $C_{18}H_{30}O_2S$ | 310.5 | Thiophene derivative |
| 48 | Decane, 1-iodo- | 25.089 | 0.2 | $C_{10}H_{21}I$ | 282.18 | Alkyl iodide |
| 49 | (3S,8S,9S,10R,13R,14S,17R)-17-((2R,5R)-5-Ethyl-6-methylhepta n-2-yl)-3-methoxy-10,13-dimet hyl-2,3,4,7,8,9,10,11,12,13,14,15,16,1 | 25.789 | 3.01 | $C_{32}H_{52}O_2$ | 472.76 | Sterol derivative |
| 50 | 9,19-Cyclolanostan-3-ol, acetate, (3.beta.)- | 25.984 | 12.49 | $C_{32}H_{52}O_2$ | 472.76 | Steroid |
| 51 | 2-Propanone, 1-cyclohexyl- | 26.533 | 0.64 | $C_9H_{16}O$ | 140.23 | Ketone |
| 52 | Bis(2-ethylhexyl) phthalate | 26.636 | 2.82 | $C_{24}H_{38}O_4$ | 390.56 | Phthalate ester |
| 53 | Eicosane, 1-iodo- | 27.033 | 1.39 | $C_{20}H_{41}I$ | 396.45 | Alkyl iodide |
| 54 | (1S,2R,5R)-2-Methyl-5-((R)-6-methylhept-5-en-2-yl) bicyclo[3.1.0]hexan-2-ol | 27.144 | 2.29 | $C_{15}H_{26}O$ | 222.37 | Terpene alcohol |
| 55 | 4,6-Bis(4-ethoxybenzylthio)-5-ni tropyrimidine | 27.425 | 0.84 | $C_{22}H_{24}N_2O_4S_2$ | 460.57 | Nitropyrimidine derivative |
| 56 | (Z)-6-Methyl-2-(tricos-14-en-1-yl)-2H-pyran-4(3H)-one | 27.6 | 0.7 | $C_{24}H_{40}O_2$ | 360.57 | Pyranone derivative |
| 57 | cis-Thujopsene | 27.747 | 1.31 | $C_{15}H_{24}$ | 204.36 | Terpene |
| 58 | Cyclohexene, 1,5,5-trimethyl-6-acetylmethyl- | 27.899 | 0.66 | $C_{12}H_{20}O$ | 180.29 | Terpene |
| 59 | Octadecane, 1-chloro- | 27.994 | 1.42 | $C_{18}H_{37}Cl$ | 288.94 | Alkyl chloride |
| 60 | 9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, acetate, (3.beta.,4.alpha.,5. alpha.)- | 28.356 | 11.97 | $C_{30}H_{50}O_2$ | 442.72 | Sterol derivative |
| 61 | 2(1H)Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)- | 28.754 | 2.22 | $C_{15}H_{22}O$ | 218.34 | Naphthalenone derivative |
| 62 | 9-Octadecenamide, (Z)- | 28.979 | 2.58 | $C_{18}H_{35}NO$ | 281.48 | Fatty acid amide |

| Peak | Name | Retention Time | Area% | Molecular formula | Molecular weight | Nature of the compound |
|---|---|---|---|---|---|---|
| 63 | Sulfurous acid, 2-propyl tridecyl ester | 29.133 | 1.41 | $C_{16}H_{34}O_3S$ | 306.5 | Organosulfur compound |
| 64 | Ergost-25-ene-6,12-dione, 3,5-dihydroxy-, (3.beta.,5.alpha.)- | 29.492 | 1.24 | $C_{28}H_{44}O_4$ | 460.65 | Steroid |
| 65 | Squalene | 29.582 | 2.14 | $C_{30}H_{50}$ | 410.72 | Terpene |
| 66 | Sulfurous acid, 2-propyl tridecyl ester | 30.399 | 0.63 | $C_{16}H_{34}O_3S$ | 306.5 | Organosulfur compound |
| 67 | Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1) | 31.096 | 0.88 | $C_{54}H_{81}O_3P$ | 806.17 | Phosphite ester |
| 68 | 1,4:3,6:5,7-Tribenzal-.beta.-mannoheptitol | 31.299 | 0.19 | $C_{27}H_{28}O_7$ | 464.51 | Sugar derivative |
| 69 | Decane, 2,3,5,8-tetramethyl- | 31.616 | 0.48 | $C_{14}H_{30}$ | 198.39 | Alkane |
| 70 | Milbemycin b, 5-O-demethyl-28-deoxy-6,28-epoxy-25-(1-methylethyl)-13-(phenylthio)-, (6R,13R,25R)- | 31.818 | 0.24 | $C_{32}H_{46}O_7S$ | 574.78 | Macrolide antibiotic |
| 71 | 2,4a,5,8a-Tetramethyl-1,2,3,4,4a,7,8,8 a-octahydronaphthalen-1-ol | 32.481 | 0.37 | $C_{14}H_{24}O$ | 208.34 | Terpene alcohol |
| 72 | Diethylmalonic acid, monochloride, 3,5-dimethylphenyl ester | 32.643 | 0.22 | $C_{13}H_{15}ClO_4$ | 270.71 | Ester |
| 73 | 2-Methyltetracosane | 32.791 | 0.87 | $C_{25}H_{52}$ | 352.68 | Alkane |
| 74 | 3.alpha.,7.beta.-Dihydroxy-5.beta.,6. beta.-epoxycholestane | 32.889 | 1.41 | $C_{27}H_{46}O_3$ | 418.65 | Steroid |
| 75 | 8-Hexadecenal, 14-methyl-, (Z)- | 33.172 | 1.08 | $C_{17}H_{32}O$ | 252.44 | Fatty aldehyde |
| 76 | dl-.alpha.-Tocopherol | 33.349 | 1.64 | $C_{29}H_{50}O_2$ | 430.71 | Vitamin E derivative |
| 77 | Nerolidol 2 | 33.542 | 0.51 | $C_{15}H_{26}O$ | 222.37 | Sesquiterpene |
| 78 | Uvidin C | 33.765 | 2.71 | $C_{19}H_{26}O_7$ | 362.41 | Coumarin derivative |
| 79 | Heptacosane, 1-chloro- | 34.08 | 1.2 | $C_{27}H_{55}Cl$ | 415.18 | Alkyl chloride |
| 80 | Neophytadiene | 34.5 | 1.52 | $C_{20}H_{38}$ | 278.52 | Diterpene |
| 81 | 9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, acetate, (3.beta.,4.alpha.,5. alpha.)- | 34.74 | 4.47 | $C_{30}H_{50}O_2$ | 442.72 | Sterol derivative |

## DISCUSSION

Phytochemical screening is aimed to identify the availability of bioactive compounds that could be helpful in the production of therapeutic agents.[19] The whole plant extract of ethanol and n-hexane yielded proteins and amino acids, alkaloids, tannins and phenols, flavonoids, steroids/ terpenoids, saponins, glycosides, quinones, fixed oils, resins, coumarins and carbohydrates as per our current results (Table 1). These findings are supported by Madhavan *et al.*,[20] who demonstrated the presence of tannins, alkaloids, glycosides, sugars, phenols, phytosterols and flavonoids in alcoholic extract of *Ophiorrhiza mungos* leaves through phytochemical analysis. The pharmacological activities of plants are attributed by the synergistic effects of these phytochemicals[21] that are produced by several physiological processes. Through several biological mechanisms, plant extracts perform a vital effect in preventing chronic diseases like neurodegenerative diseases, cancer and cardiovascular diseases.[22] GC-MS analysis is the commonly used technique for quantifying the active principles present in plants involved in cosmetics, food and pharmaceutical industries.[23] GC-MS chromatogram analysis of the ethanolic and n-hexane whole plant extract of *O. recurvipetala* (Figures 1 and 2) showed various peaks indicating the presence of different phytochemical constituents (Tables 2 and 3).

The main chemical substances found in *O. recurvipetala* crude extracts are phenols, steroids, terpenoids, saponins, alkaloids, coumarins, quinolone, napthalone, fatty acid derivatives,

vitamin E, Pyridine, phthalate, alkanes, esters and organosilicon compounds. Regardless of the high or low concentration, some compounds are reported to possess several pharmacological activities. Most of the identified compounds are reported to display antioxidant and antimicrobial activities.[24,25]

As suggested by studies,[26,27] the ABTS and DPPH assays are employed to analyse the free radicals quenching action of antioxidants, but FRAP assay is used to analyse reduction ability of antioxidants.[28] DPPH assay is performed to examine the radical neutralizing ability of OREE and ORXHEX, and we found a concentration dependent scavenging effect of DPPH radical. DPPH method is a basic, inexpensive and fast assay with reproducible results and used to quantify the radical scavenging ability of single or combined antioxidant compounds.[29] The result is similar to previous experiments in scavenging DPPH assay[9,25] by other species of *Ophiorrhiza*, suggesting that OREE has effective antioxidant function. ABTS radical assay is often used for accessing the total antioxidant capacity of crude extracts.[30,31]

Although superoxide anion is considered as a poor oxidant, it forms powerful and hazardous oxidants like hydroxyl radicals and singlet oxygen. Superoxide anion radical reacted with hydrogen peroxide radical and formed singlet oxygen and OH radicals that are the potent reactive oxygen species. The dose-dependent rise in the scavenging ability of the OREE, ORNHEX and ascorbic acid for superoxide radical suggested that the OREE is having scavenging activity as like that of ascorbic acid. It has the ability to initiate auto-oxidation, polymerization and fragmentation reactions in various biomolecules by inducing double bond addition, electron transfer and radical formation, hydrogen withdrawal etc.[32]

Hydroxyl radical is a powerful ROS that persuades irreversible alterations such as peroxidation of lipids of biological membranes, oxidative DNA lesions like changes the base and sugar, strand breaking and DNA-protein crosslinking by attacking purine, pyrimidine and deoxyribose sugar backbone in DNA, forming different oxidation products by attacking several amino acids in proteins (for ex. lysine forms α-aminoadipic semialdehyde, leucine and valine into hydroxyl leucine and

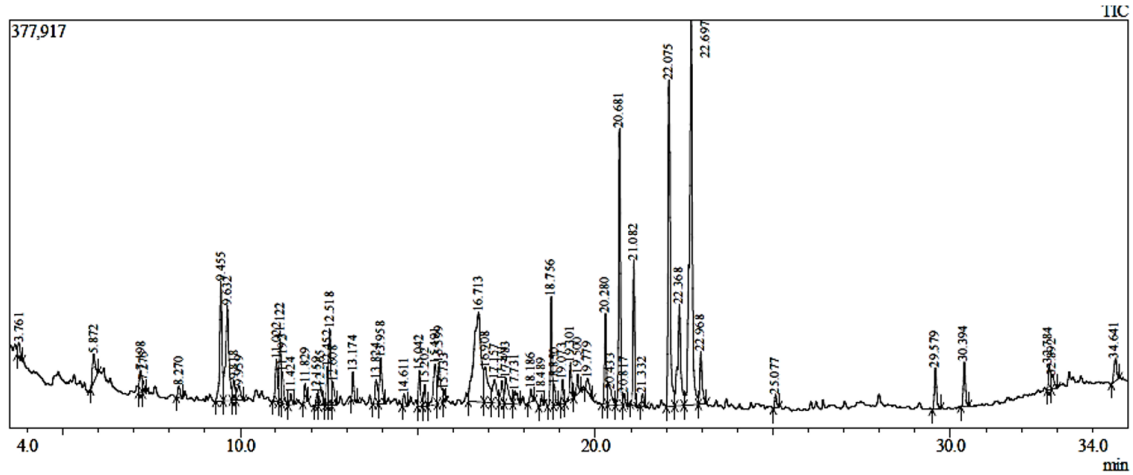



**Figure 1:** GC-MS chromatogram of OREE.

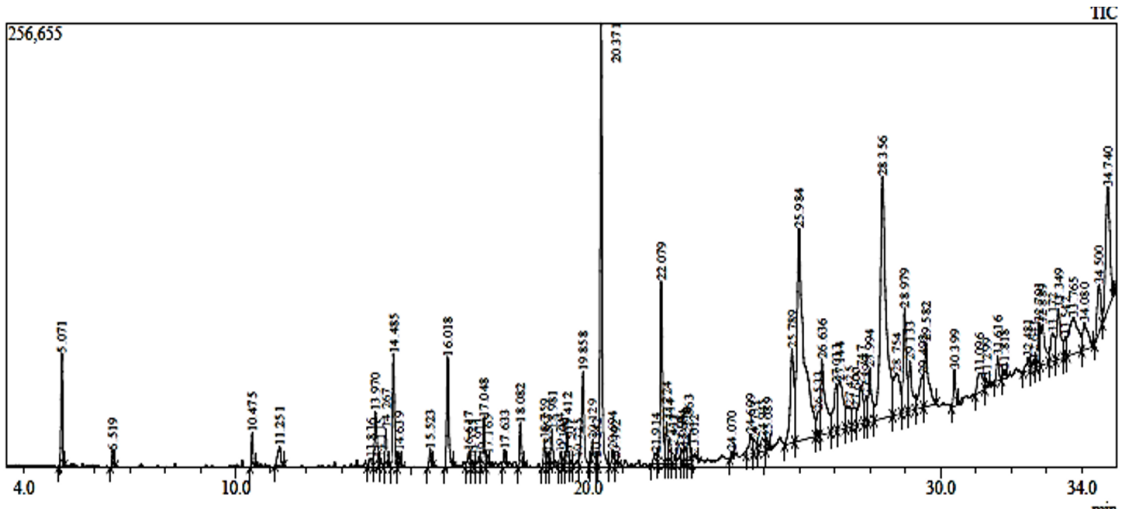
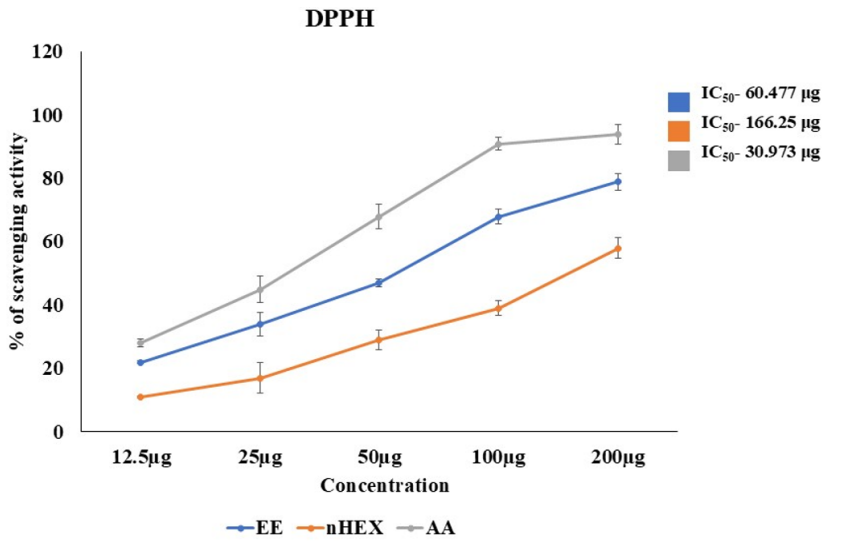
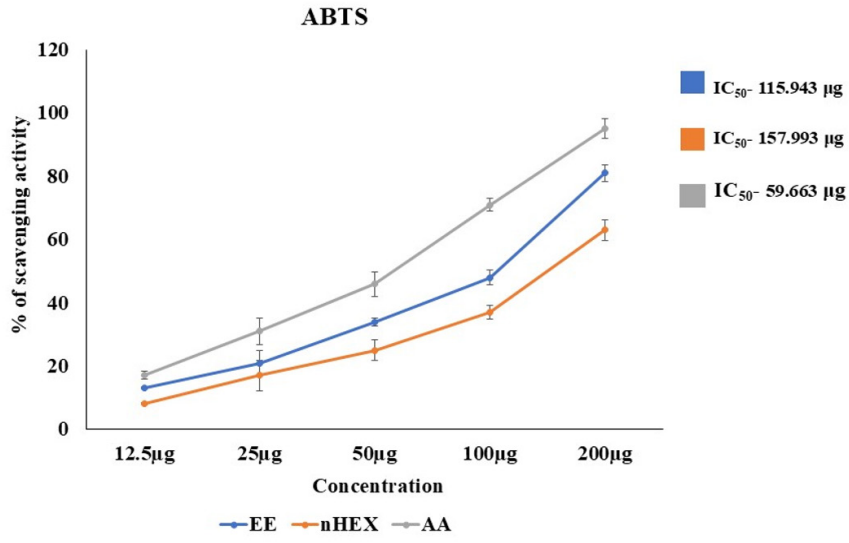


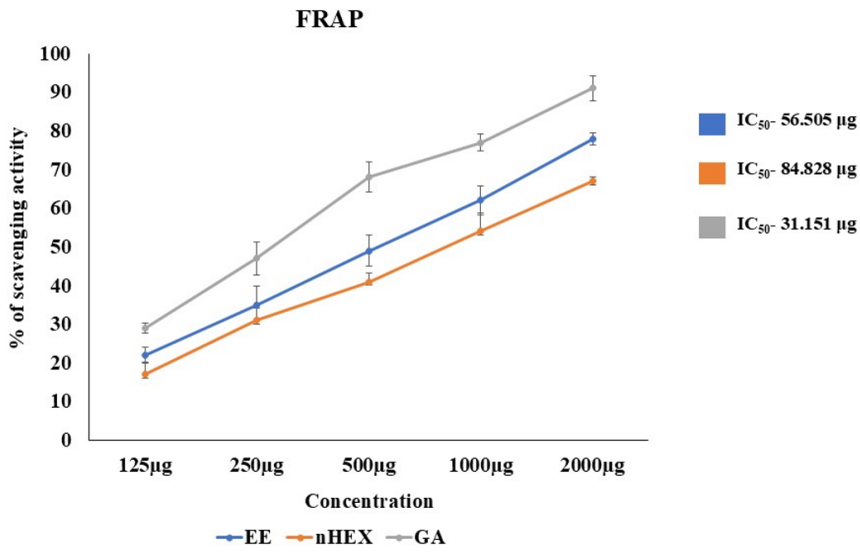

**Figure 2:** GC-MS chromatogram of ORNHEX.


DPPH
% of scavenging activity
120
100
80
60
40
20
0
12.5µg
25µg
50µg
100µg
200µg
Concentration
EE
nHEX
AA
IC50- 60.477 µg
IC50- 166.25 µg
IC50- 30.973 µg

a


ABTS
% of scavenging activity
120
100
80
60
40
20
0
12.5µg
25µg
50µg
100µg
200µg
Concentration
EE
nHEX
AA
IC50- 115.943 µg
IC50- 157.993 µg
IC50- 59.663 µg

b


FRAP
% of scavenging activity
100
90
80
70
60
50
40
30
20
10
0
125µg
250µg
500µg
1000µg
2000µg
Concentration
EE
nHEX
GA
IC50- 56.505 µg
IC50- 84.828 µg
IC50- 31.151 µg

c

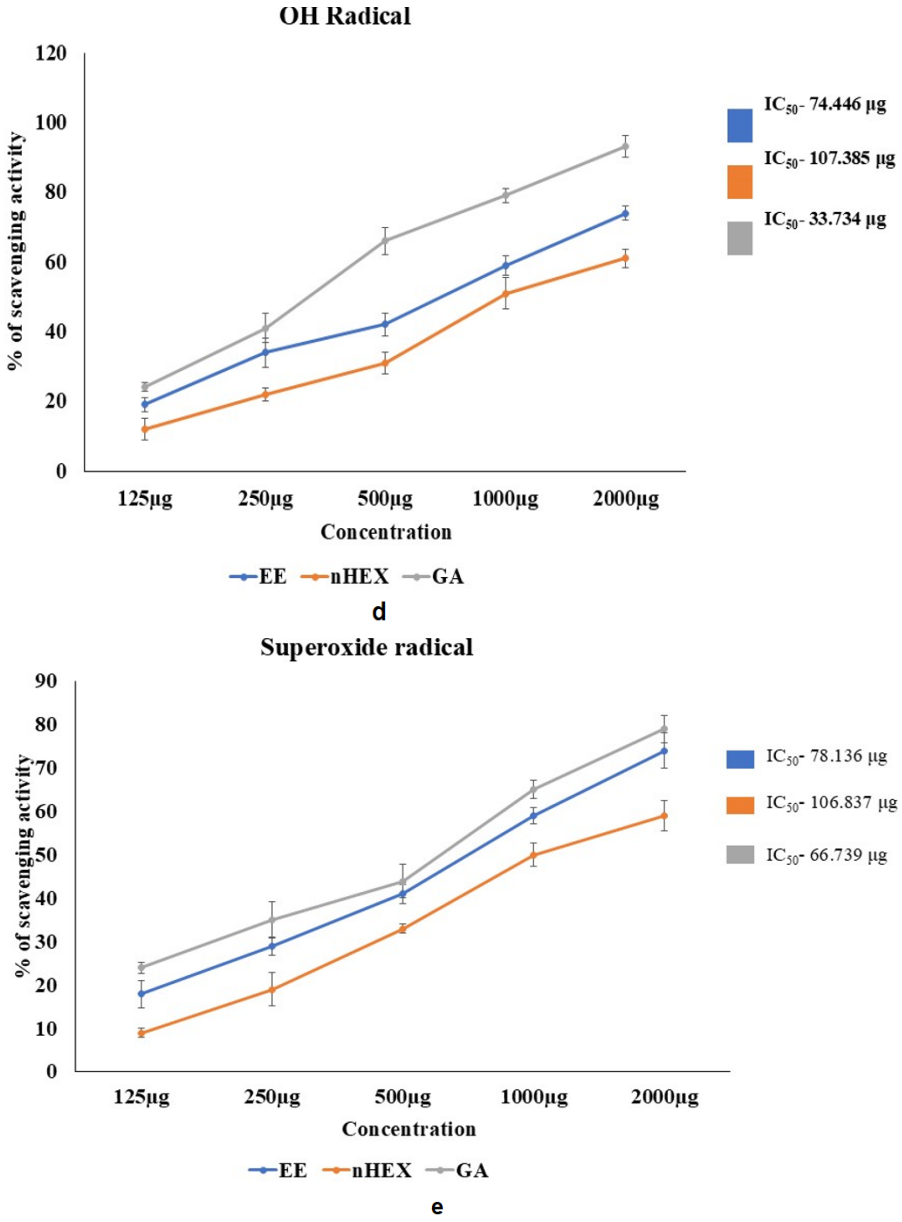



**Figure 3:** (a-e) The *in vitro* Antioxidant Activity of OREE and ORNHEX. Data are mean±SEM (Each experiment has triplicate values). EE-Ethanolic extract; nHEX-n Hexane extract; AA-Ascorbic acid; GA-Gallic acid.

valine and tryptophan converted into kynurenine.[33] The results demonstrated the exposure of OREE to the reactant eliminates OH radicals and averts further injury. Hence, hydroxyl radical scavenging is vital for the protection of living system.[34] In ferric ion reducing power assay,[35] the reducing ability of a substance connects with the probable antioxidant activity. The results of the present study indicated the ferric reducing abilities of OREE, ORNHEX and ascorbic acid increased in a dose-dependent manner. The reduction potential is a measure of the ability of a substance to donate electrons,[36] OREE should be a better free radical scavenger than ORNHEX.

## CONCLUSION

In the present study, phytochemical and GCMS analysis indicated the occurrence of various active components in ethanolic and n-hexane extracts of *O. recurvipetala.* They were reported to have anti-microbial, anti-diabetic, anti-inflammatory and anti-cancer properties, thereby revealing immense medicinal potential of the plant. Further, the results of *in vitro* anti-oxidant assays explored their potent radical scavenging activities. More *in vivo* trials and other antioxidant mechanisms shall explore the capacity of the plant towards its usefulness in drug discovery.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of Interest.

## ABBREVIATIONS

**GC-MS:** Gas Chromatography-Mass Spectrometry; **NIST:** National Institute of Science and Technology; **OREE:** Ethanolic extract of *Ophiorrhiza recurvipetala*; **ORNHEX:** n hexane extract of *Ophiorrhiza recurvipetala*; **ABTS:** 2,2-Azino-Bis-3-Ethylbenzothiazoline-6-sulphonic acid; **DPPH:** 2,2-Diphenyl-1-Picrylhydrazyl; **FRAP:** Ferric reducing antioxidant power.

## SUMMARY

The whole plant ethanolic and n-Hexane extracts of *Ophiorrhiza recurvipetala* were analysed for finding the existence of phytochemicals by qualitative investigational methods and bioactive compounds by GC-MS. *In vitro* antioxidant activity of the extracts was measured using FRAP, DPPH, ABTS, hydroxyl, and superoxide radical scavenging assays. The phytochemical examination explored the presence of flavonoids, alkaloids, phenols, steroids, tannins, glycosides, and saponins in the ethanolic and n-Hexane extracts. The results of *in vitro* anti-oxidant assays explored their potent radical scavenging activities which may be due to the harmonious activity of these bioactive substances.

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