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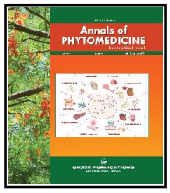

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# Phytochemical screening and GC-MS analysis of *Cryptocoryne retrospiralis* (Roxb.) Kunth

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**Abstract**

*Cryptocoryne retrospiralis* (Roxb.) Kunth is a marshy herb of the family Araceae. Rhizome and leaves of *C. retrospiralis* have been selected for phytochemical screening to identify different metabolite classes and quantitative estimation of phenolics, flavonoids, and starch. GC-MS analysis was also done to determine the bioactive metabolite of both leaves and rhizomes. Phytochemical screening revealed the presence of carbohydrates, proteins, phenolics, flavonoids, saponins, glycosides, sterols, alkaloids, fixed oils, and fats. Quantitative phytochemical analysis showed that leaf extracts are richer in phenolics and flavonoids than rhizome extracts are richer in starch content than leaf extracts. Major bioactive metabolites identified by GC-MS analysis include eicosane, phytol, neophytidiene, hexadecanoic acid, pentadecanoicacid, cycloeucalenyl acetate, squalene, linoleic acid, and tetradecane.

## 1. Introduction

Bioactive compounds derived from plants have recently become of great interest due to their various application in different fields of the modern world. The medicinal significance of plants primarily stems from the presence of bioactive compounds such as alkaloids, glycosides, volatile oils, tannins, phenolics, flavonoids, and other phytochemicals. These compounds are synthesized as the product of the primary or secondary metabolism of plants (Yadav *et al*., 2011). Phytochemicals have various functions like medicinal, antioxidant, antimicrobial, *etc*., other than as a source of nutrients. Systematic screening of various plant species to determine various bioactive compounds is an important research in modern laboratories across the world (Parekh *et al*., 2006). Around 60-80% world's population still depends on phytomedicine for curing various diseases. Even though, the traditional Indian system of medicine has a long history of treatment, it lacks enough scientific knowledge to evaluate the potential effects of bioactive compounds (Shrivastava *et al*., 2010). Through improved phytochemical screening methods, one can detect the various bioactive compounds, which could be used as the base of modern drugs (Sheikh *et al*., 2013).

*C. retrospiralis* is typically located at the edges of ditches, in sandy river soils, and across open fields on plateaus. Traditional healers utilize the rhizomes of *C. spiralis* and *C. retrospiralis* for treating conditions such as diarrhea, fever, jaundice, burns, and boils (Kamble *et al*., 2010; Divaka *et al*., 2013). The rhizomes of *C. spiralis* serve as a substitute for *Aconitum heterophyllum* Wall. in the treatment of diarrhea, as well as for cough, abdominal issues, fever, and vomiting in infants (Prasad *et al*., 2014). The rhizome of *C. retrospiralis*, known as mala in Malayalam, was consumed as food by prehistoric humans (Te Beet, 1998). The current study aims to qualitatively and quantitatively analyze various phytochemicals and to identify different bioactive compounds through GC-MS analysis.

## 2. Materials and Methods

### 2.1 Collection and preparation of plant extracts

Rhizome and leaves of *C. retrospiralis* were collected from its natural locality, the bank of Kuppam River, Chapparappadavu, Kannur District, Kerala, India. The plant species was identified and authenticated by specimens available at Calicut University Herbarium (CALI) and relevant data have been recorded. Descriptions were made based on fresh specimens collected as well as on the herbarium specimen examined.The rhizomes and leaves were harvested, shade-dried, and processed into a fine powder with the aid of a mechanical grinder. This powdered substance underwent a series of extractions with hexane, chloroform, methanol, and water utilizing a Soxhlet apparatus. The extracts obtained were concentrated to a volume of 50 ml through the use of a rotary evaporator under reduced pressure. Subsequently, these concentrated extracts were employed for phytochemical screening and GC-MS analysis.

### 2.2 Morphological description of *C. retrospiralis*

Rhizome stout and upright, long and highly branched contractile roots. Leaves; 13-33 cm long, lanceolate. Petiole 6-13 cm long, lamina

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6-20 cm long, and 0.8-1.1 cm broad entire and undulate in submerged forms, midrib pronounced. Peduncle 2.5-3.5 cm long, spathe 12-22 cm long, kettle 1-2 cm long and 0.6-0.8 cm wide, 2-3 times broader than basal part of the tube. Vulvule; white with purplish red spots. Upper tube 7.1-14.4 cm long and 0.25-0.4 cm wide, white with red spots inside, greyish blue on the outside. Limb 3-6.6 cm long and 0.4-0.7 cm wide at the base, many times spirally twisted without a collar. Spadix 1.1-2 cm long, 0.2-5 cm long female portion, 0.7-1.5 cm long naked interstice, 0.2-0.3 cm long male portion with more than 200 stamens, and a short 0.02-0.05 cm long sterile appendix. Female flowers 5, white, stigma covered with small hairs and a depression in the middle; each pistil with 10-25 ovules, 4-6 olfactory bodies placed above the female flowers. Infructascence;syncarp having 1.2-1.5 long and 0.8-1 cm wide with many seeds.


**Figure 1:** ***C. retrospiralis*** **(a) habit (b) dried leaves (c) dried rhizomes.**

### 2.3 Preliminary phytochemical screening

The process of examination of various extracts derived from both the rhizome and leaves of *C. retrospiralis* to determine their composition of different classes of compounds, employing established methodologies (Kokate, 2009; Raman, 2006).

### 2.4 Quantitative estimation of phytochemicals

Total phenolic content was determined by the method proposed by Singleton *et al.* (1965) and the results were presented in Table 2. Total flavonoid content was measured by aluminium chloride calorimetric assay (Zhishen *et al.*, 1999). The result of total flavonoid content and the ratio of total flavonoid/total phenolics are presented in Table 2. Total starch content was estimated by the method developed by Haris (2007) and the results were presented in Table 2.

### 2.5 GC-MS analysis

GC-MS analysis was conducted utilizing a Thermo Scientific Trace 1300 gas chromatograph, which was fitted with a TG-5MS column (30 m × 0.25 mm ID × 0.25 µm) and connected to an ISQ-QD mass spectrometer (Perkin-Elmer GC Clarus 500). The detection process employed an electron ionization (EI) system operating at an ionizing energy of 70 eV. Helium gas (99.999%) served as the carrier gas, maintained at a constant flow rate of 1 ml/min, with a 1 µl injection volume. The temperature of the injection port was held at 280°C, while the ion source temperature was set to 200°C. The temperature program for the oven commenced at 60°C for 3 min, followed by a ramping rate of 50°C/min until reaching 240°C, concluding with a final hold of 5 min. The scanning interval was established at 0.2 sc, and the mass range was configured from 40 to 550 amu. The overall runtime for the GC analysis was 35 min. Component identification was accomplished by comparing their relative retention times and mass spectra against data from the Wiley NIST 17N library. Additionally, confirmation of the compounds was reinforced by analyzing the elution profiles and their corresponding indices on non-polar phases in conjunction with existing literature references.

### 2.6 Statistical analysis

The statistical analysis was conducted utilizing Microsoft Excel. Each data set reflects the average of three replicates. The results are presented as a mean ± standard deviation.

## 3. Results

### 3.1 Preliminary phytochemical screening

The results of preliminary phytochemical screening of water, methanol, hexane, and chloroform extracts of rhizome and leaf of *C. retrospiralis* were presented in Table 1. Carbohydrates, proteins, glycosides, alkaloids, sterols, phenolics, tannins, flavonoids, fixed oils, and fats were the major phytochemicals found in the leaves and rhizome of *C. retrospiralis.* The water extract contains carbohydrates, proteins, phenolics, tannins, glycosides, saponins, alkaloids, and flavonoids. Methanol extract contains carbohydrates, proteins, alkaloids, phenolics, tannins, flavonoids, glycosides, saponins and sterols. Hexane and chloroform extracts contain fixed oils and fats.

### 3.2 Quantitative estimation of phytochemicals

The total phenolic content derived from the methanol extracts of the leaves and rhizomes of *C.retrospiralis* is presented in Table 2. A standard curve for determining total phenolic content was established using various concentrations of gallic acid equivalent (GAE), with the corresponding optical densities illustrated in Figure 2. The leaf extract exhibited a higher phenolic content, while the rhizome extracts showed lower values. The concentration of phenolic compounds varies across different parts of the plant, significantly contributing to their medicinal properties. Additionally, the total flavonoid content from the methanol extracts of both the leaf and rhizome of *C. retrospiralis* is also detailed in Table 2. The standard curve for total flavonoid content was created using different concentrations of quercetin equivalent (QUE), with the respective optical densities displayed in Figure 3. A higher flavonoid content was noted in the leaf compared to the rhizome. The leaf demonstrated a significant flavonoid to phenolic (F/P) ratio of 1.06, indicating its richness in flavonoids. Furthermore, the total starch content from the leaf and rhizome of *C. retrospiralis* is shown in Table 2. The standard curve for total starch content was prepared using varying concentrations of dextrose equivalent (DE), with theoptical densities represented in Figure 4. It was found that the rhizome contained a higher starch content (272.06 ± 0.53) compared to the leaf (127.94 ± 0.65).

**Table 1: Priliminary phytochemical screening of leaf and rhizome of *C. retrospiralis***

| S.No. | *C.retrospiralis* Name of the compound | Name of the test | Leaf | | | | Rhizome | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | WE | ME | HE | CE | WE | ME | HE | CE |
| 1 | Carbohydrate | Fehling's test | + | + | - | - | + | + | - | - |
| | | Molisch's test | + | + | - | - | + | + | - | - |
| | | Benedict's test | + | + | - | - | + | + | - | - |
| 2 | Proteins and amino acids | Millions test | + | + | - | - | + | + | - | - |
| | | Biuret test | + | + | - | - | + | + | - | - |
| | | Ninhydrine test | + | + | - | - | + | + | - | - |
| 3 | Alkaloids | Mayer's test | + | + | - | - | + | + | - | - |
| | | Drangendroff test | + | + | - | - | + | + | - | - |
| | | Wagner's test | + | + | - | - | + | + | - | - |
| 4 | Phenolics and tanins | Ferric chloride test | + | + | - | - | + | + | - | - |
| | | Alkaline reagent test | + | + | - | - | + | + | - | - |
| | | Vanillin hydro chloride test | + | + | - | - | + | + | - | - |
| 5 | Flavonoids | Aqueous sodium hydroxide test | + | + | - | - | + | + | - | - |
| | | Conc.sulphuric acid test | + | + | - | - | + | + | - | - |
| | | Shinoda test | + | + | - | - | + | + | - | - |
| 6 | Glycosides | Borntrager's test | + | + | - | - | + | + | - | - |
| | | Legal test | + | + | - | - | + | + | - | - |
| 7 | Phytosterols | Libermann-Buchard test | - | + | - | - | - | + | - | - |
| | | Libermann sterol test | - | + | - | - | - | + | - | - |
| | | Salkowski test | - | + | - | - | - | + | - | - |
| 8 | Saponins | Foam test | + | + | - | - | + | + | - | - |
| | | Haemolysis test | + | + | - | - | + | + | - | - |
| 9 | Fixed oils and fats | Spot test | - | - | + | + | - | - | + | + |
| | | Saponification test | - | - | + | + | - | - | + | + |

**WE**= Water extract, **ME**= Methanol extract, **HE**- Hexane extract, **CE**= Chloroform extract, + = presence, - = Absence

**Table 2: Estimation of total phenolics , flavonoids and starch content in leaves and rhizomes of *C. retrospiralis***

| Total flavonoid content mg QE/100 g | | Total phenolic content mg GAE/100 g | | Ratio of TF/TP | | Total starch content mg DE/100 g | |
|---|---|---|---|---|---|---|---|
| Leaf | Rhizome | Leaf | Rhizome | Leaf | Rhizome | Leaf | Rhizome |
| 35.47 ± 0.85 | 4.32 ± 0.60 | 33.40 ± 0.39 | 12.64 ± 0.02 | 1.06 | 0.34 | 127.94 ± 0.65 | 272.06 ± 0.53 |

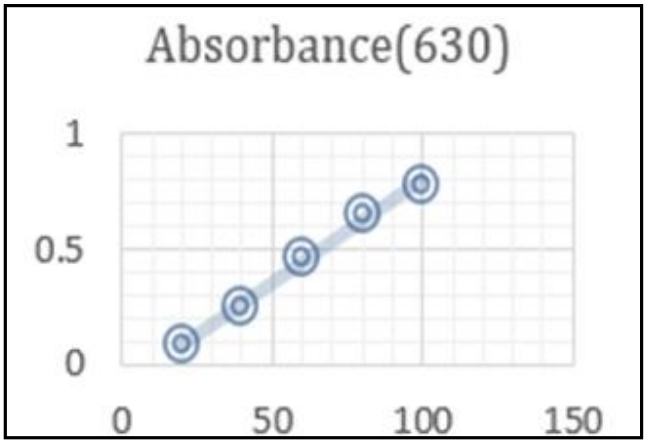


**Figure 3: Standard calibration curve for quercetin**

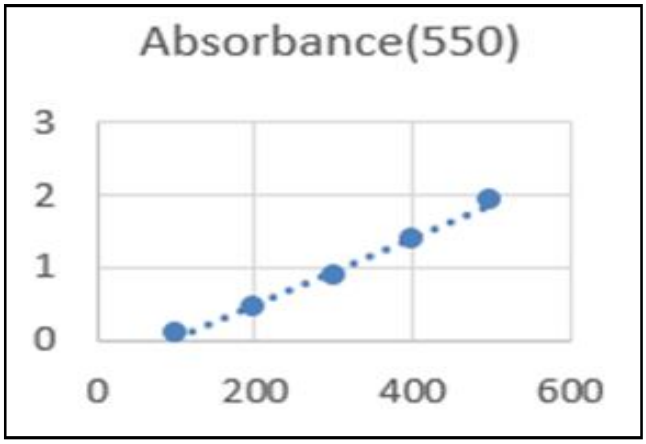


**Figure 2: Standard calibration curve for gallic acid**

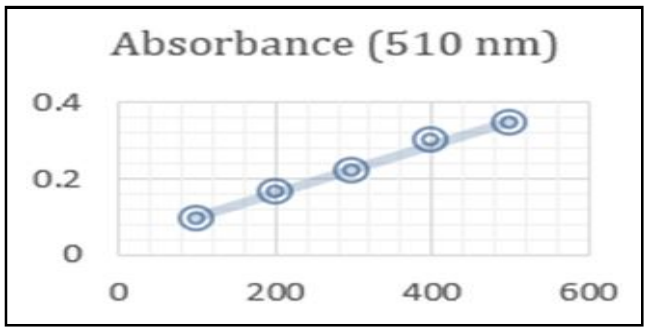


**Figure 4: Standard calibration curve for dextrose.**

### 3.3 GC-MS analysis

GC-MS analysis of leaf and rhizome of *C. retrospiralis* as done by using methanol extracts. The chromatogram displayed multiple peaks that correspond to the bioactive compounds present. GC-MS chromatogram of methanol extracts of leaf is shown in Figure 5 and the compound identified is presented in Table 3.The predominant compounds in this fraction are beta sitosterols and stigmasta-5, 22-dien-3-ol 33.78% and 32.32%. Neophytadiene, phytol, cycloeucalenyl, 1,2- benzene dicarboxylic acid, and 1,2-benzenedicarboxylic acid bis (2-methylpropyl) ester are ranked as moderately abundant compounds with their peak area from 108 to 14.07. GC-MS chromatogram of the methanol extract of the rhizome is presented in Figure 6. Twenty compounds were identified in the hexane extracts of the rhizome (Table 4). Major compounds of this fraction include squalene, vitamin E, 1-tetradecene, tetradecane, E-15-heptadecenal, hexadecanal, hexadecanoic acid methyl ester, hexadecanoic acid, 10-heneicosene,9,12-octadecadienoic acid (ZZ)- methyl ester, 9-octadecenoic acid (Z) methyl ester, octadecanoic acid methyl ester, trans-4-methylcyclo hexanemethanol and trifluoroacetoxyhexadecane.

**Table 3: Compound identified in the methanol extract of rhizome of *C. retrospiralis***

| Peaks | RT | Name of the compound | Molecular formula | Molecular weight | Area% |
|---|---|---|---|---|---|
| 1 | 26.491 | Neophytadiene | $C_{20}H_{38}$ | 278 | 1.08 |
| 2 | 27.271 | 1,2-Benzenedicarboxylic acid bis (2-methylpropyl)ester | $C_{16}H_{22}O_4$ | 278.34 | 14.07 |
| 3 | 31.843 | Phytol | $C_{20}H_{40}O$ | 296.5 | 8.16 |
| 4 | 35.050 | Stigmasta-5,22-dien-3-ol | | | 32.32 |
| 5 | 39.075 | 1,2- benzene dicarboxylic acid | $C_8H_6O_4$ | 166.3 | 2.17 |
| 6 | 39.806 | Stigmasta-5-en-3-ol(3 beta,24S)( beta- sitosterol) | $C_{29}H_{50}O$ | 414.71 | 33.78 |
| 7 | 45.315 | Cycloeucalenyl acetate | $C_{32}H_{52}O_2$ | 468.8 | 8.42 |

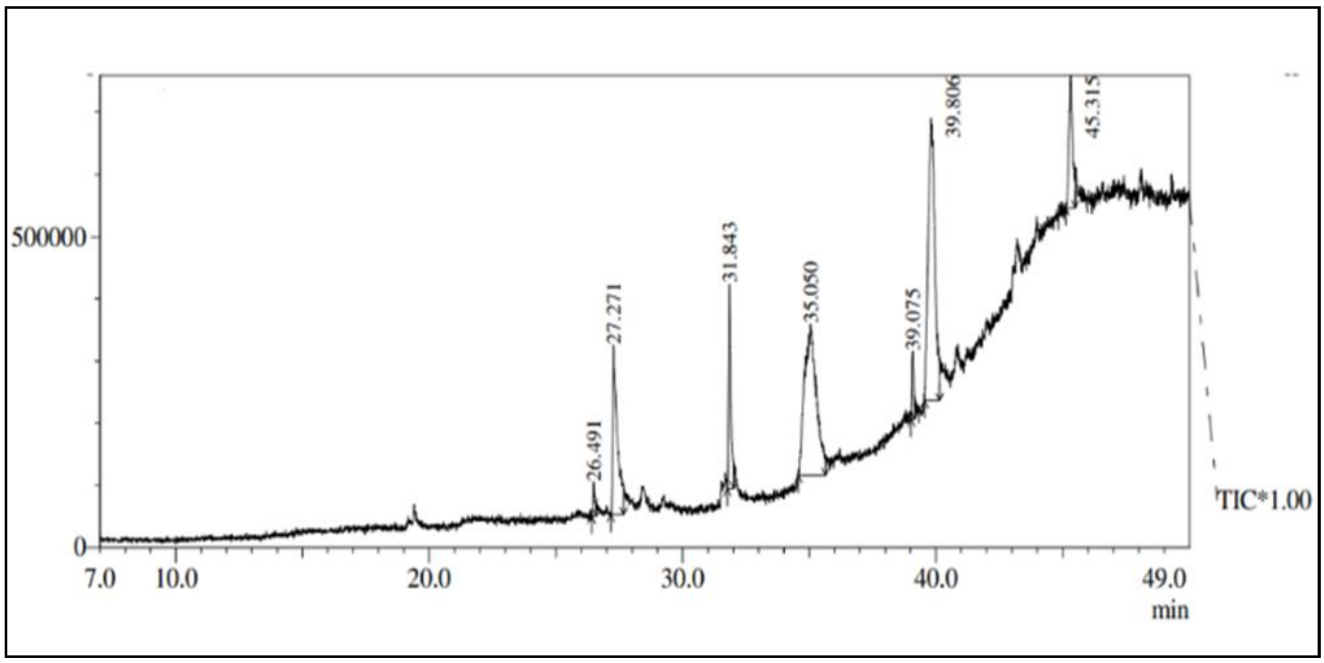


**Figure 5: GC-MS chromatogram of methanol extract of leaves of *C. retrospiralis*.**

**Table 4: Compound identified in the methanol extract of rhizome of *C.retrospiralis***

| Peaks | RT | Name of the compound | Molecular formula | Molecular weight | Area% |
|---|---|---|---|---|---|
| 1 | 12.423 | 5- hydroxymethylfurfuryl | $C_6H_6O_3$ | 126.11 | 53.52 |
| 2 | 16.111 | 1-tetradecene | $C_{14}H_{28}$ | 196.37 | 1.37 |
| 3 | 16.310 | Tetradecane | $C_{14}H_{30}$ | 198.39 | 1.16 |
| 4 | 16.574 | Dimethyl(bis[(2Z)-pent-2-en-1-yloxy])silane | $C_{12}H_{24}O_2Si.$ | 228.40 | 2.46 |
| 5 | 21.058 | E-14-hexadecenal | $C_{16}H_{30}O$ | 238.41 | 2.33 |
| 6 | 25.483 | E-15-heptadecenal | $C_{17}H_{32}O$ | 252.4 | 1.85 |
| 7 | 26.042 | Hexadecanal | $C_{16}H_{32}O$ | 240.42 | 0.92 |
| 8 | 27.199 | 1,2-Benzenedicarboxylic acid bis(2-methylpropyl)ester | $C_{16}H_{22}O_4$ | 278.34 | 2.68 |
| 9 | 28.227 | Hexadecanoic acid methyl ester(palmitic acid methyl ester) | $C_{17}H_{34}O_2$ | 270.5 | 4.51 |
| 10 | 29.095 | Hexadecanoic acid(palmitic acid) | $C_{16}H_{32}O_2$ | 256.42 | 7.72 |
| 11 | 29.484 | 10-heneicosene | $C_{21}H_{42}$ | 254.6 | 1.82 |
| 12 | 31.445 | 9,12-octadecadienoic acid (ZZ)- methyl ester(á linoleic acid) | $C_{19}H_{34}O_2$ | 280.4 | 7.41 |
| 13 | 31.549 | 9-octadecenoic acid (Z) methyl ester | $C_{18}H_{34}O_2$ | 282.5 | 2.35 |
| 14 | 32.000 | Octadecanoic acid, methyl ester(methyl stearate). | $C_{19}H_{38}O$ | 298.5 | 1.45 |
| 15 | 32.324 | Trans-4-methylcyclo hexane methanol | $C_8H_{16}O$ | 128.21 | 3.26 |
| 16 | 33.140 | Trifluoroacetoxyhexadecane | $C_{18}H_{33}F_3O_2$ | 338.4 | 0.71 |
| 17 | 39.043 | 1,2- benzene dicarboxylic acid | $C_8H_6O_4$ | 166.3 | 0.88 |
| 18 | 42.494 | 9-octadecenamide | $C_{18}H_{35}NO$ | 281.5 | 0.87 |
| 19 | 43.016 | Squalene | $C_{30}H_{50}$ | 410.7 | 1.54 |
| 20 | 48.044 | Vitamin E | $C_{29}H_{50}O_2$ | 430.71 | 1.19 |

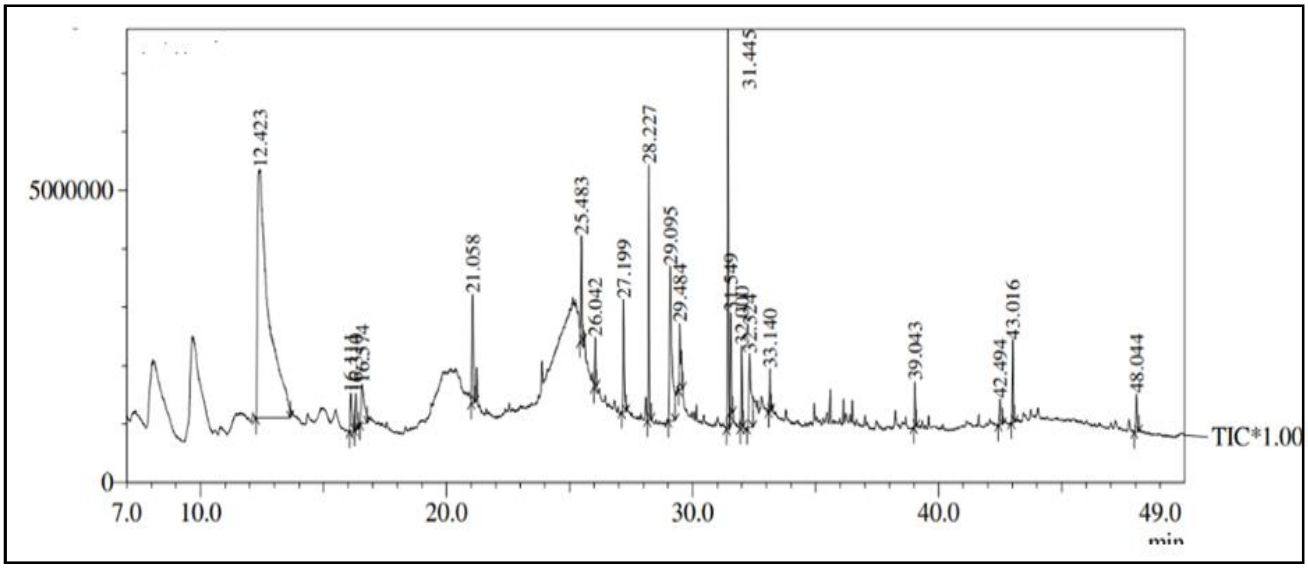


**Figure 6: GC-MS chromatogram of hexane extract of rhizome of *C. retrospiralis*.**

## 4. Discussion

*C. retrospiralis* contains a variety of bioactive compounds with diverse biological activities. The findings of this study revealed the presence of various components in different solvent extracts, confirming the existence of multiple classes of metabolites, including alkaloids, flavonoids, terpenoids, and saponins. Wadkar *et al*. (2017) reported the presence of alkaloids, flavonoids, glycosides, saponins, sterols, and tannins in *C. retrospiralis.* In this study, significant amounts of phenolics and flavonoids were observed. A positive correlation between phenolic content and free radical scavenging activity has been established (Oki *et al*., 2002). Phenolic compounds are known to possess antioxidant, anticancer, and mutagenic properties (Mohanlal *et al*., 2013). Flavonoids, which are polyphenolic compounds, exhibit a range of bioactivities, including antioxidant, antibacterial, antifungal, antiviral, anti-inflammatory, anti-allergic, and anticancer effects (Montero *et al*., 2005). The substantial starch content observed in the rhizomes suggests that *C. retrospiralis* may have been used as a food source by prehistoric humans.

The presence of various bioactive compounds detected through GC-MS analysis supports the pharmacological uses of *C. retrospiralis*. Wadkar *et al*. (2017) identified compounds such as santalol, cis-α-santalol, and cyclohexasiloxane in the ethanol extract of the rhizome of *C. retrospiralis*. The majority of the identified compounds demonstrate significant biological activities. Neophytadiene has been shown to possess antioxidant and anti-inflammatory properties (Kazemi, 2015). Linolenic acid esters exhibit antiarthritic, antiandrogenic, antihistaminic, and hypocholesterolemic effects (Duke, 1996). Phytol, a diterpene compound, has demonstrated antimicrobial, anti-inflammatory, anticancer, antidiuretic, and antiarthritic activities (Ogunlesi *et al*., 2009). The compound 9,12-octadecadienoic acid methyl ester, a polyunsaturated fatty acid, is known for its antihistaminic, hepatoprotective, and hypocholesterolemic activities (Wu *et al*., 2010). Hexadecanoic acid has been found to exhibit antioxidant, hypocholesterolemic, nematicidal, and 5-alpha-reductase inhibitory activities (Jagadeeswari *et al*., 2012). Squalene, a triterpene, is recognized for its potent antioxidant activity (Pham *et al*., 2015). Heneicosane has been shown to possess anti-inflammatory, analgesic, and antipyretic properties. These findings collectively highlight the pharmacological potential of *C. retrospiralis* in various therapeutic applications.

## 5. Conclusion

Phytochemical screening reveals that maximum classes of phytochemicals are present in the leaf and rhizome of *C. retrospiralis*. The present study ensures that the leaves and rhizome extracts of the plant contain various bioactive phytochemicals. GC-MS analysis of leaf and rhizome revealed the presence of various bioactive phytochemicals with some potent biological and pharmacological activities including antioxidant, anticancerous, antimicrobial, and hypercholesterolemic properties. Further isolation and purification of such bioactive chemicals may open the door for the potential source in applied pharmacology.

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## Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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