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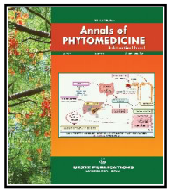

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# Phytochemical analysis and identification of different metabolites profiling in oil extracted from ginger (*Zingiber officinale* Rosc.) using gas chromatography and mass spectroscopy technique

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**Abstract**

The present investigation on the effect of various organic amendments to enhance the quality of ginger (*Zingiber officinale* Rosc.) was conducted in a farmer field at Kailasapatti near Periyakulam in Tamil Nadu during 2022-2023. The experiment was laid out in a randomized block design with three replications using ten treatments (organic amendments and their combinations), *viz*., farm yard manure, vermicompost, poultry manure, goat manure, microbial consortium and recommended dose of fertilizers as control. The results revealed that T8 (application of poultry manure, goat manure, microbial consortium) produced higher essential oil content (1.68%), oleoresin (6.37%) and crude fibre (4.21%), respectively. Major components present in *Z. officinale* essential oil of T8 were 2-methyl-6-propylpyridine (20.87%), 2,6-pyridinedicarboxaldehyde (13.23%), cedrol methyl ether (9.03%) and predominant compounds screened in T9-goat manure + microbial consortium (1%) were 1,1,2-trimethyl-3,5-bis(prop-1-en-2-yl)cyclohexane (10.69%), N-methoxy-N-methyl-1-methylsulfonyl azetidine-3-carboxamide (6.63%), 2-ethyl-3,5-dimethylpyrazine (5.43%). Thus, the combined application of poultry manure and microbial consortium is considered as the best and most effective organic amendment for improving *Z. officinale* phytochemical compound under coconut-based cropping system.

## 1. Introduction

*Zingiber officinale* Rosc. is a perennial herbaceous monocotyledon plant that belongs to the Zingiberaceae family and is economically cultivated as an annual crop for its rhizomes. Since the beginning of time, the country has grown this significant commercial spice crop (Gohain *et al.,* 2020). It grows in tropical and subtropical climates and is a highly prized tropical rhizomatous spice crop. Although, it is grown all throughout the world, its optimal growth occurs in tropical, humid environments. *Z. officinale* has a history dating back more than 5000 years. It originated in South East Asia and over time expanded to other continents, including Africa (Nair, 2019).

In India, *Z.officinale* is used for a variety of things, including pickles, culinary additives, confections, and conventional stomach discomfort cures. It has been used to alleviate all types of nausea, including that caused by motion sickness, pregnancy, surgery, and nausea after chemotherapy (Prasad and Tyagi, 2015). It is a potent antioxidant. *Z. officinale* is a common raw element in both conventional and modern medicines (Soeparjono, 2013). This is because *Z.officinale* contains volatile oleoresin and volatile oil (Aidin *et al.,* 2016).

*Z.officinale* growers that practise organic cultivation should anticipate seeing a considerable return on their investment because to the increased global demand for organic products. Because of its higher quality, stronger market demand, and commitment to environmental protection, organic has drawn increasing attention. Compared to non-organic sources, *Z.officinale* from organic sources has a substantially better capacity for phytochemicals and antioxidants (Ozkur *et al.,* 2022).

In agriculture, metabolites are pivotal for plant growth, development, and adaptation to environmental stresses. They serve as building blocks for cellular structures, energy sources, and signalling molecules essential for orchestrating physiological processes. Furthermore, recent research highlights the critical role of metabolites in shaping plant-microbe interactions, nutrient cycling, and stress responses, underscoring their importance for sustainable crop production (Daraban *et al.,* 2021).

Metabolites derived from dietary sources modulate host metabolism, immune function, and gut microbiota composition, influencing susceptibility to chronic diseases and metabolic disorders (Devi *et al.,* 2020). *Z. officinale* is renowned not only for its culinary allure but also for its rich array of bioactive compounds that underpin its medicinal properties. Among the major active constituents of *Z. officinale* are: gingerols, shogaols, and paradols (Rani *et al.,* 2023). When it comes to treating oxidative stress, inflammation, bacteria, fungal infections, cancer, and other conditions, *Z. officinale* is the most potent medicinal plant in India (Attri *et al.,* 2023). A great source of bioactive compounds and vitamins and minerals such as phosphorus, calcium and vitamin B and pharmacological properties such as antimicrobial, anti-inflammatory and antineoplastic effect and it is beneficial against various diseases and enhance the immune

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system (Parnami and Lakhawat, 2022). *Z.officinale* is known for its medicinal properties, including being a carminative, cholagogue, and antiemetic (Gupta and Sarwat, 2022). It also has promising potential in improving hyperlipidemia and exerting antinephrotoxic effects (Bharathi *et al.,* 2021).

*Z.officinale* can be successfully cultivated in coconut's light shade without degrading the performance of the primary crop. The *Z.officinale* crop grows well in the coconut garden's moderate shade, has a respectable market demand, is simple to process, and has a long shelf-life. In order to determine how organic changes to the coconut ecosystem on quality of *Z.officinale* was carried out in this research.

## 2. Materials and Methods

A field experiment with *Z. officinale* (IISR Varada) was laid out in a randomized block design with three replications. The treatment details as follows: $T_1$-farm yard manure (30 tonnes/ha); $T_2$-vermicompost (5 tonnes/ha); $T_3$-poultry manure (3 tonnes/ha); $T_4$-goat manure (2 tonnes/ha); $T_5$- microbial consortium (1%); $T_6$-$T_1$ + microbial consortium (1%); $T_7$ -$T_2$+ microbial consortium (1%); $T_8$-$T_3$ + microbial consortium (1%);$T_9$-$T_4$ + microbial consortium (1%); $T_{10}$ -control (recommended dose of fertilizers-75:50:25 kg/ha).

The essential oil content of rhizomes of *Z. officinale* was analysed using the techniques recommended by (Datta *et al.,* 2018). A few glass beads were used to distribute 25 to 30 g of *Z. officinale* powder into the flask in order to prevent foaming. The clevenger's apparatus, a device for extracting essential oils, was then attached to the flask. On a heating mantle with a thermostat, the distillation was conducted. 90°C was maintained until boiling, and then 70°C was used for 3 h of the distillation. The distillate was allowed to settle until the oil layer was clear after being cooled to room temperature. Using the formula listed below, the volume was measured and the oil content was determined. The essential oil content was expressed as percentage:

$$\text{Essential oil (\%) (V/W)} = \frac{\text{Volume of oil (ml)}}{\text{Weight of sample (mg)}} \times 100$$

### 2.1 Oleoresin content (%)

According to the association of official analytical chemists protocol (Horwitz *et al.,* 1980), the rhizomes of *Z. officinale* samples were dried and ground into powder to determine the oleoresin content, which was then assessed in per cent recovery:

$$\text{Oleoresin content (\%)} = \frac{\text{Weight of beaker with residue(g)} - \text{Weight of empty beaker(g)}}{\text{Volume of sample taken (g)}} \times 100$$

### 2.2 Crude fibre content (%)

The acid-alkali digestion (ashing) method was used to estimate the crude fibre content using a 5 g sample as described by (Sankaram, 1966). The mixture was heated for 30 min with 200 ml of $H_2SO_4$ and then again with NaOH solution. After thorough drying, the contents underwent 500°C for ashing, and the percentage of crude fibre was measured.

### 2.3 Extraction of sample for analysis

The rhizomes were dug up and washed carefully with tap water to remove any remaining soil. After that, they were carefully dried and rinsed with distilled water. The rhizomes were then cut into smaller pieces to help with drying and kept in the shade for a period of fourteen days. The rhizomes were pulverised finely in a blender after they had dried. The powder that resulted was then put into conical flasks and directly extracted using hexane at a weight/volume ratio of 1:10. This combination was swirled at 125 rpm for a whole day on an orbital shaker. Following the extraction process, the extract was run through Whatman filter paper No. 42 (125 mm) in order to isolate the solvent layer. After allowing the solvent to drain, a filtrate fit for additional analysis was left behind.

### 2.4 Gas-chromatography mass-spectrometry (GC-MS) analysis

After measuring and pouring the required quantity of *Z.officinale* powder into a sealed flask, it was given a methanol treatment to facilitate infusion. The resultant extract was filtered and dried using a vacuum distillation apparatus after being left for 24 h. The residue that was left over was then examined using GC-MS. Thermo GC ultra Clarus 500 system was employed for the GC-MS analysis of these extracts. The system consists of a gas chromatograph linked to a mass spectrometer (GC-MS) that is outfitted with an Elite-I fused RMS 5 silica capillary column made entirely of dimethyl polysiloxane. An electron ionisation device with an ionising energy of 70 eV was used for the detection. An electron ionisation device with an ionising energy of 70 eV was used for the detection. The sample injection volume was 1µl with a sample split ratio of 10:1, and the carrier gas was helium (99.9%) at a constant flow rate of 1 ml/min. The temperatures of the ion source and injector were adjusted to 250°C and 260°C, respectively. The oven was set to begin at 110°C and rise by 5°C every minute until it reached 260°C, where it was isothermal for three minutes. Mass spectra covering fragments ranging from 50 to 650 Da were acquired, using a 0.5-second scan interval. Turbo mass software was used to manage mass spectra and chromatograms, and the average peak area of each component was compared to the overall area to calculate its percentage composition.

### 2.5 Identification of components

The National Institute of Standards and Technology (NIST) database, which has retention values for over 90,000 compounds, was consulted in order to analyse and interpret the mass spectrum from GC-MS (https://www.nist.gov/srd/nist-standard-reference-database1a-v14). Spectra from the NIST and Wiley libraries were used to compare unknown components with known ones. The identification, molecular weight, and composition of the test materials were ascertained by means of this comparison.

### 2.6 Prediction of biological activity of substances

We generated predictions based on the structural formulas of the compounds using PASS (Prediction of Activity Spectra for Biologically Active Substances) in order to investigate the biological impacts of the compounds. As described in the PASS online database (Filimonov *et al.,* 2014), this involved predicting a variety of pharmacological effects, specific toxicities, and putative modes of action linked with the substances.

## 3. Results

### 3.1 Dry recovery per cent (%)

With a mean of 19.45%, dry recovery percentage varied significantly from 16.71 to 21.64%. $T_6$ – farm yard amnure + microbial consortium had the highest dry recovery at 21.64 per cent, followed by $T_9$ - goat manure + microbial consortium (21.56 %), which was on par with $T_8$ - poultry manure + microbial consortium at 21.32 %. Lower dry recovery of 16.71% was produced by $T_{10}$ - control.

### 3.2 Essential oil content (%)

The results of the ten treatments examined varied greatly in terms of essential oil concentration. The range for the essential oil content was 1.25% to 1.68% (Figure 1a). The treatment $T_8$ - poultry manure + microbial consortium produced higher essential oil content (1.68%), followed by $T_9$ - goat manure + microbial consortium (1.63%) and both are on par for the essential oil content. A lower percentage of essential oil (1.25%) was found in the control treatment.

### 3.3 Oleoresin content (%)

The oleoresin content was significantly influenced by various organic amendments. The range of oleoresin content is 4.86% to 6.37% (Figure 1b). The treatment $T_8$ - poultry manure + microbial consortium recorded higher oleoresin content (6.37%), followed by the equally effective $T_9$ - goat manure + microbial consortium (6.24%). In control plot $T_{10}$ less oleoresin (4.86%) was observed.

### 3.4 Crude fibre content (%)

The results on the crude fibre content revealed a distinct difference between the various organic amendments, as shown in Figure 1c. The crude fibre content ranges from 3.19 % to 4.21 %. $T_8$ - poultry manure + microbial consortia had the highest crude fibre content (4.21%), followed by $T_6$-farm yard manure + microbial consortium (4.16%) and $T_9$ - goat manure + microbial consortium (4.08 %). Less crude fibre was found in $T_{10}$ at (3.19 %).

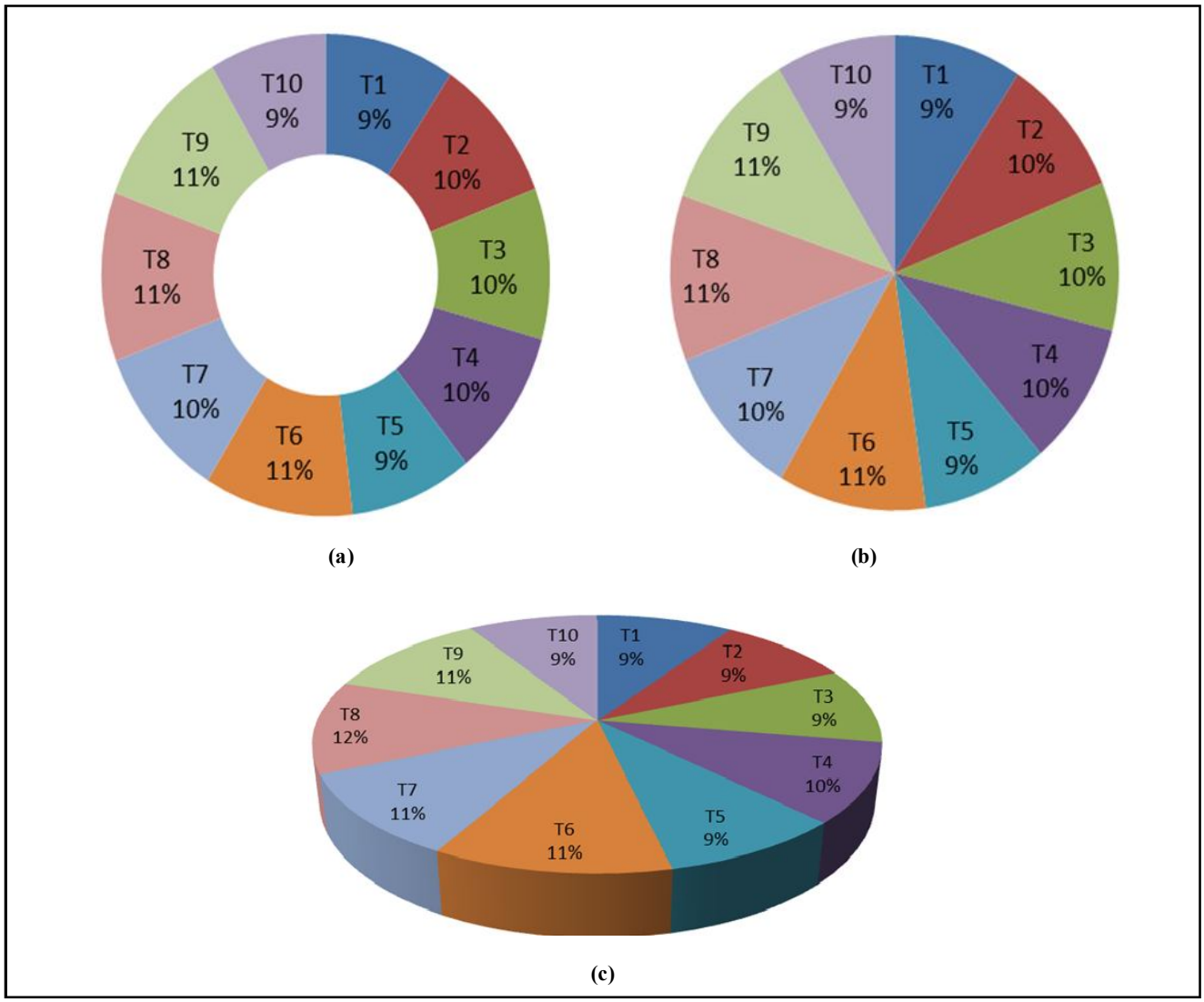


**Figure 1: Effect of organic amendments on quality characters of *Z.officinale*. (a) Essential oil (%) (b) Oleoresin (%) (c) Crude fiber (%).**

### 3.5 Metabolite profiling using GC-MS analysis

GC-MS was used to identify the bioactive compounds found in *Z. officinale* essential oil. The chromatogram (Figure 2) shows that the essential oil from $T_8$ comprises various components. The identified compounds were shown in Table 1 along with their retention time (RT), peak area (%) and molecular formula. Major components present in *Z. officinale* essential oil of $T_8$ - poultry manure + microbial consortium have significant oil compounds identified in the oil profile, including 2-methyl-6-propylpyridine (20.87%), 2,6-pyridinedi carboxaldehyde (13.23%), cedrol methyl ether (9.03 %), 3-methyl-2-aminopyridine (8.25%) and pyridine-2,6-dicarbaldehyde (4.73%). Minor oil components such as santalol (1.01%), 8,14-cedranoxide (0.68%), 7-octylidenebicycloheptane (0.31%), 1,1,2-trimethyl-3,5-bis(prop-1-en-2-yl), cyclohexane (0.24%) were also found. The GC-MS analysis shows that the predominant compounds screened in *Z.officinale* essential oil of $T_9$ were: 1,1,2-trimethyl-3,5-bis(prop-1-en-2-yl) cyclohexane (10.69%), N-methoxy-N-methyl-1methyl sulfonyl azetidine-3-carboxamide (6.63%), 2-ethyl-3,5-dimethylpyrazine (5.43%), 2-methyl-5oxo cyclopentene-1-carbonitrile (3.36%),4-oxo-N-(pyrrolidin-2-ylmethyl)-3-(2,2,2 trifluoroethyl) imidazolidine-1-carboxamide (3.03%). The minor compounds screened are: 4-(3-oxobutyl) phenyl acetate (1.29%), 1,2,3,6-tetramethylbicyclo oct-2-ene (1.06%), Pyridine-2,6-dicarbaldehyde (0.98%), N-methyl-2-(trifluoromethyl) pyridin-4-amine (0.26%).

**Table 1: Different metabolites present in the oil of *Z. officinale* with their retention time (RT), peak area (%) and molecular formula**

| S. No | Compound | Rt (min) | Peak area (%) | Molecular formula |
|---|---|---|---|---|
| 1. | 2-methyl-6 propylpyridine | 9.391 | 20.87 | $C_9H_{13}N$ |
| 2. | 2,6-pyridinedicarboxaldehyde | 9.466 | 13.23 | $C_7H_5NO_2$ |
| 3. | Cedrol methyl ether | 9.359 | 9.03 | $C_{16}H_{28}O$ |
| 4. | 3-methyl-2-aminopyridine | 4.913 | 8.25 | $C_6H_8N_2$ |
| 5. | 1,3-dichloro-2-propanol | 9.864 | 2.69 | $C_3H_6C_{l2}O$ |
| 6. | Methyleneisophorone | 4.868 | 2.24 | $C_{10}H_{14}O$ |
| 7. | Santalol | 10.150 | 1.01 | $C_{15}H_{24}O$ |
| 8. | 8,14 - cedranoxide | 11.263 | 0.68 | $C_{15}H_{24}O$ |
| 9. | 1,1,2-trimethyl-3,5-bis(prop-1-en-2-yl)cyclohexane | 4.687 | 10.69 | $C_{15}H_{26}$ |
| 10. | N-methoxy-N-methyl-1-methylsulfonylazetidine-3-carboxamide | 9.899 | 6.63 | $C_7H_{14}N_2O_4S$ |
| 11. | 2-ethyl-3,5-dimethylpyrazine | 8.513 | 5.43 | $C_8H_{12}N_2$ |
| 12. | 2-methyl-5-oxocyclopentene-1-carbonitrile | 6.989 | 3.36 | $C_7H_7NO$ |
| 13. | 4-oxo-N-(pyrrolidin-2-ylmethyl)-3-(2,2,2-trifluoroethyl) imidazolidine-1-carboxamide | 10.596 | 3.03 | $C_{11}H_{17}F_3N_4O_2$ |
| 14. | 2-methylphenol | 10.775 | 2.79 | $C_7H_8O$ |
| 15. | 3-[N-(4,5-dihydro-1H-imidazol-2-ylmethyl)-4-methylanilino] phenol | 10.362 | 2.15 | $C_{17}H_{19}N_3O$ |
| 16. | Phenyl methanol | 7.532 | 2.02 | $C_7H_8O$ |
| 17. | 2-prop-2-enylfuran | 6.073 | 1.33 | $C_7H_8O$ |
| 18. | 4-(3-oxobutyl)phenyl acetate | 9.351 | 1.29 | $C_{12}H_{14}O_3$ |
| 19. | 1,2,3,6-tetramethylbicyclo oct-2-ene | 10.745 | 1.06 | $C_{12}H_{20}$ |
| 20. | N-methyl-2-(trifluoromethyl)pyridin-4-amine | 5.814 | 1.15 | $C_7H_7F_3N_2$ |

**Table 2: Different bioactive compounds in the oil of *Z. officinale* with their molecular weight and pharmacological activity**

| S.No. | Compound | Molecular weight (g/mol) | Pharmacological activity |
|---|---|---|---|
| 1. | 2-Methyl-6 propylpyridine | 135.21 | Used to treat nausea, neuralgia and vomiting. |
| 2. | 2,6-Pyridinedicarboxaldehyde | 135.12 | Used to treat neutropenia, severe chronic, AIDS-related kaposi sarcoma and hepatitis B, chronic. |
| 3. | Cedrol methyl ether | 236.39 | Used for hyperhidrosis and vaginal discharge treatments. Used in the fragrance of perfume. |
| 4. | 3-Methyl-2-aminopyridine | 108.14 | Treat central nervous system diseases, metabolic diseases and depressive disorder. |
| 5. | 1,3-dichloro-2-propanol | 128.979 | Useful for the treatment of water intoxication, hypercholester olemia and liver injury. |

| | | | |
|---|---|---|---|
| 6. | Santalol | 220.35 | Anticancer, antitumor activities, used for urinary tract infections and act as central nervous system depressant effects such as sedation. |
| 7. | 2-ethyl-3,5-dimethylpyrazine | 136.19 | Used as a flavouring ingredient, treat dysgeusia, paresthesia and halitosis. |
| 8. | 2-methylphenol | 108.14 | Treat photosensitivity disorders, haemorrhage and epilepsy. |
| 9. | 3-[N-(4,5-dihydro-1H-imidazol-2-ylmethyl)-4-methyl-anilino]phenol | 281.35 | Treat sexual dysfunction and conjunctive diseases. |
| 10. | Phenylmethanol | 108.14 | Used to inflammation, neoplasms and skin diseases. |
| 11. | 2-prop-2-enylfuran | 108.14 | Treat liver cirrhosis, dysgeusia and diabetes mellitus, type 2. |
| 12. | 4-(3-oxobutyl)phenyl acetate | 206.24 | Treat malaria, neurotoxicity syndromes and infertility. |
| 13. | Pyridine-2,6-dicarbaldehyde | 135.12 | Treat neutropenia, severe chronic and hepatitis B, chronic. |
| 14. | N-methyl-2-(trifluoromethyl)pyridin-4-amine | 176.14 | Used for the synthesis of APIs in cancer treatment and ligands for chemical reactions. |

## 4. Discussion

Different organic amendments have a positive impact on the quality of *Z.officinale* rhizome. The quality of the rhizome was significantly improved over the control by the addition of poultry manure and a microbial consortium since it had the highest levels of oleoresin, essential oil and crude fibre content. Similar findings were also reported by Jyotsna *et al.* (2013) and Lepcha *et al.* (2019) in *Z.officinale*. All of the organic nutrient sources improved all of the qualitative characters of *Z.officinale* rhizome in a way that was beneficial. The microclimate, specifically the leaf temperature, relative humidity, and inherent fertility condition of the soil, has a significant impact on the synthesis and composition of quality metrics. These facts have a positive effect on the development of enzymes and associated biochemical processes. A central cylinder and cortex with more clusters of specialised cells that carry oleoresin will be favourable morphologically. Oleoresin and essential oil synthesis may have resulted from physiological and biochemical processes caused by a promotive or inhibitory influence at various stages. The potential reason for the lower oleoresin concentration may be due to excessive drying and loss during the desolvenation process. This might be due to variation in genetic makeup and conducive climatological parameters favouring the biological processes, *viz.,* physiological and biochemical reactions influenced by the enzyme activities. The probable reason for such higher crude fibre content could also be ascribed to higher leaf area and efficient translocation of photosynthates to the developing fingers, as reported by Ajithkumar *et al.* (2002) in *Z.officinale*.

Essential oil was distilled from green *Z.officinale* rhizomes from ten various treatments. Among all the other organic amendments, $T_8$– poultry manure + microbial consortium had the maximum oil yield. Therefore, essential oil from best treatments was collected for GC-MS analysis. Various metabolites have been found in the oil profile of $T_8$ were depicted in GC-MS chromatogram and represented in Figure 2. The different bioactive compounds in the essential oil of *Z.officinale* with their molecular weight and pharmacological activity were clearly listed in Table 2.

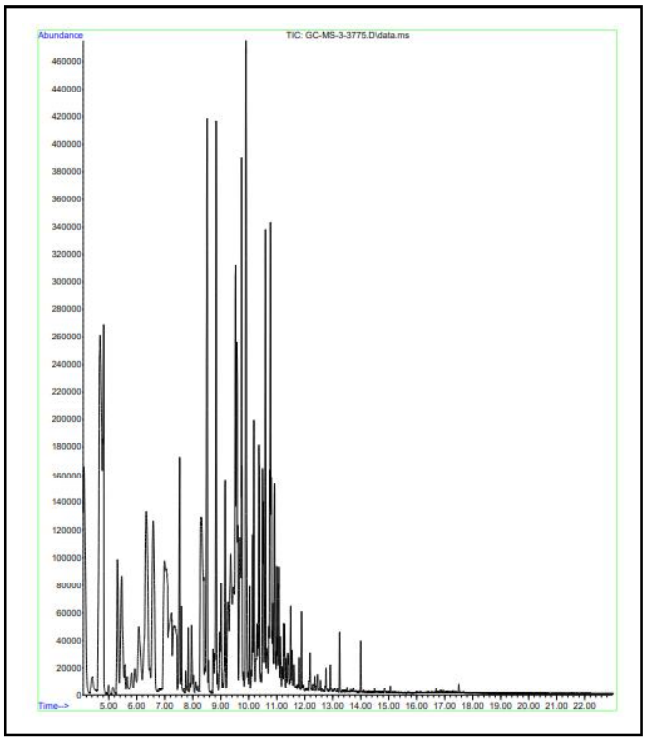


**Figure 2: GC-MS chromatogram of bioactive compounds in the oil of *Z.officinale.***

## 5. Conclusion

According to the findings of the study, it is concluded that the quality characteristics of *Z.officinale* rhizome were increased with the combined application of two organic nutrient sources such as poultry manure and microbial consortium. Among the various treatment combinations, $T_8$ - poultry manure + microbial consortium gave the best response to all the quality characters of *Z.officinale*. As interest in plant-based therapies increases dramatically on a global scale, studies must be carried out on the agricultural produce to look into how they interact with other medicinal and therapeutic activities.

## Conflict of interest

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

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