*Section A-Research paper*

# Microwave Assisted Extraction of *Citrus limetta* peel extract and exploration of its Bio-Actives Using FTIR and GC-MS

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**Abstract**

The Sweet lime (*Citrus limetta)* is one of the world's most important commercial fruits. Several bioactive and volatile components can be found in sweet lime. The current work investigated a methanolic extract of Sweet lime peel retrieved using Microwave Assisted extraction (MAE) to assess bioactive components, different phytochemicals, and antioxidant activity, revealing that total phenolic content (TPC) is 16.66 mg GAE/g. Total flavonoid content (TFC) was found to be mg 9.59 QE/g, respectively, *and in vitro* free radical scavenging activity was found 77.62% The existence of 34 components was shown by GC-MS analysis, 12 of which were phytochemical components having antibacterial, antioxidant, and anticancer activity. The current results of this analysis demonstrate the existence of crucial phytocompounds in SLP and are useful for further in-depth research into generating functional foods from SLP to avoid a variety of ailments. The current research is targeted at identifying all-natural therapeutics for a broad range of illnesses and conditions.

**Key-words** Sweet lime peel, GC-MS, TPC, TFC, Bioactive components, Microwave Assisted extraction (MAE)

## Introduction

*Citrus limetta*, widely known as sweet lime or mosambi, is a citrus fruit rich in vitamins, minerals, dietary fibers, and bioactive secondary metabolites. These bioactive chemicals include, among other elements, flavonoids, volatile oils, limonoids, coumarins, alkaloids, sterols, and carotenoids (Favela *et al.,* 2016). Citrus flavonoids demonstrated antioxidant properties by scavenging free radicals (Zou *et al.,* 2016). Hesperetin had a significant antibacterial effect against *Salmonella typhi* and *S. typhimurium* (Kawaguchi *et al.,* 2004). The mechanisms through which limonene exerts its anticancer effects appear to be apoptosis, anti-proliferative activity, and selective cytotoxicity (Ke *et al.,* 2015). Although all biological systems have innate ant oxidative defense mechanisms that remove damaged molecules, these processes can be insufficient. To protect cells from free radical damage, antioxidants must be absorbed through food (Rahman *et al.,* 2015). The benefits of plant-derived phenolics in disease prevention have

recently drawn increasing attention to phenolic compounds, a large group of secondary metabolites found in plants (Soto *et al.,* 2012).

The current study tried to investigate the phytoconstituents found in SLP extracted by novel MAE technique, seeking natural cures for a variety of diseases and disorders, using GC-MS and FTIR analysis, which could be further utilized in different food products as it possessed nutraceutical activities.

## Materials and Methods

### Chemicals

Methanol, FolinCiocalteu reagent, 2, 2-diphenyl-1-picrylhydrazil were procured from Sigma-Aldrich, all the chemicals and reagents used for the research were of analytical grade and were procured from the reputed manufacturers.

### Raw material collection and Preparation of extract

Sweet lime peel (SLP) var. *mosambi* is a waste product that was collected from various regions of Aurangabad City and treated with a $Na_2CO_3$ (5%) solution before being tray-dried at 60 ° C till it reaches to constant moisture content below 10% and pulverized into powder and sieved through 60 mesh size sieve and were used for extraction. The powder (100 g) was extracted in methanol (1:10 Solid-Solvent ratio) by microwave extractor for 20 minutes at 60 ° C, (Make Microsynth) at 750 W powers. The mixture was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated using a rotary evaporator at low pressure to produce an orange-yellow semi-solid extract. The extract was then kept at 4 °C for further assessment. The yield percentage was calculated as proposed by Adam *et al.,* 2019 as follows:

$$\%Yield = (Weight\ of\ extract\ /\ weight\ of\ powder\ ) \times 100$$

### Preliminary phytochemical analysis

The presence of many kinds of secondary chemicals, such as alkaloids, phenolics, flavonoids, tannins, saponins, and terpenes, was detected in the sweet lime peel extract using phytochemical analysis (Khandelwal, 2008).

### Determination of free radical scavenging activity

The total antioxidant activity of MESLP was measured in terms of the percentage of radical scavenging activity using 2, 2-diphenyl-1-picrylhydrazil. A DPPH solution (1 mg/mL) was made by dissolving DPPH in methanol. The DPPH solution was diluted to 5 mL and the absorbance was calculated at 517 in a UV-Spectrophotometer (Gogavekar *et al.*, 2012).

The following formula was used to determine the antioxidant activity.

$$\%\ Free\ radical\ scavengin = \frac{(Abs.of\ control - Abs.of\ sample)}{Abs.of\ control \times 100}$$

**Determination of Total Phenolics Content**

The Folin Ciocalteu reagent (FC Reagent) was used to calculate the total phenolics content. The standard for the calibration curve was Gallic acid. The total phenolic content was expressed as Gallic acid equivalents (mg/g) (Chun *et al.*, 2003).

**Determination of Total Flavonoids Content**

The total flavonoid concentration was assessed using a colorimetric assay .The standard for the calibration curve was Qurecitin. Per g of material, the total flavonoids in the extract were estimated as mg/g of Qurecitin equivalents (Zhishen *et al.*, 1999).

**Gas chromatography-mass spectrometry (GC-MS) analysis**

Shimadzu GCMS QP2020 gas chromatograph and mass spectrophotometer equipped with an SH-RXI-5Sil MS fused silica column (5% phenyl methyl siloxane 30.0 m 250 m, film thickness 0.25 m) was employed for the GC-MS analysis. Computerized mass spectra corresponded to measures to achieve in mass spectrum libraries. The carrier gas was helium, and the column velocity flow rate was 1.28 mL/min, the purge flow rate was 3.0 mL/min, and the pressure was set to 100 kPa. Other GC-MS settings include an ion-source temperature of 200 °C, an interface temperature of 290 °C, a pressure of 100 kPa, an out time of 1.8 mm, and a 1 µL injector in split mode with a split ratio of 1:10 and an injection temperature of 280 °C, purge flow of 3mL/min, and linear velocity of 43 cm/sec. The overall elution time was 35.03 minutes. Each component's relative percent amount was computed by comparing its average peak area to total areas. The supplier's MS solution software was utilized to control the system and collect data.

**Identification of compounds**

The retention indices of the components were used to identify them, and the mass spectrum was interpreted using the NIST Standard Reference Database library.

**FTIR analysis of SLP**

To examine FTIR (IRAffinity 1S), Shimadzu spectrometers were deployed. 2.0 mg of sample was mixed with 200 mg of spectroscopic grade KBr before being compacted into a disc at 10 MPa for 3 minutes. The resulting spectra were recorded in the 4000-400/cmrange with a resolution of 4/cm. The acquired peaks and their functional groups were identified. The FTIR peak values were recorded.

**Statistical analysis**

To compute the means and standard deviations of the data measured for each sample, statistical analysis was performed using the software SPSS 16 for Windows.

**Results and Discussion**

**Phytochemical screening of methanolic extract of MESLP**

As shown in Table 1, the presence of various phytoconstituents was discovered during a

| Sr No. | Phytoconstituents | Test | Observations | Result |
|---|---|---|---|---|
| 1 | Alkaloids | Dragendorff 's test | Orange-red precipitate | + |
| | | Mayer's test | White precipitate | + |
| | | Hager's test | Yellow precipitate | + |
| 2 | Flavonoids | Alkaline reagent test | Deep yellow turns colorless | + |
| | | Shinod's test | Deep pink color | + |
| 3 | Phenolic compounds and tannins | Ferric chloride test | Deep blue color | + |
| | | Lead tetra acetic acid test | Precipitate | + |

qualitative preliminary phytoconstituents screening of the *MESLP* that indicated the presence of alkaloids, flavonoids, Phenolic compounds, tannins, steroids, glycosides and terpenoids, saponins, Alkaloids have been observed to hinder cell division, making them an essential plant tenet that may be used as a cancer treatment (Noble, 1990).

Table 1.Phytoconstituents of methanolic extract of Sweetlime peel

| | | | | |
|---|---|---|---|---|
| 4 | Glycosides | Keller Killiani test | Deep blue color | + |
| 5 | Saponins | Foam Test | Foam appears | + |
| 6 | Terpenoids | Horizon test | Red precipitate | + |
| 7 | Steroids | Salkowski test. | Red color | + |

**Extraction yield**

Furthermore, plant components might be polar or non-polar in nature. Because phenolic compounds are more soluble in polar organic solvents due to the presence of a hydroxyl group, methanol was chosen as the extraction solvent (Wang and Curtis, 2006).The extraction yield of crude methanolic extract of *SLP* was approximately 12.91% as showed in Table 2, which was due to the migration of targeted compounds from the matrices of the peel cells to the surroundings at a faster rate due to microwave energy and solvent selection, MAE generates direct heat, which results in faster diffusion rate of molecules into solvent (Dhanani *et al.*, 2017), (Spigno *et al.*, 2009), and (Terigar *et al.*, 2011).

**TPC and TFC of MESLP**

*MESLP* has a fair amount of phenolic and flavonoid components, according to the TPC and TFC values. Table 2, demonstrates that the proportion of TPC and TFC in the extract was 16.66 mg GAE/g and 9.59 mg QE/g, respectively. According to the evidence, genetic variety, biological, environmental, seasonal, and periodic changes all have a significant impact on the flavonoid content of vegetables (Kumar and Roy, 2018).

**DPPH scavenging assay**

Plant extracts contain hydroxyl groups, which aid in free radical scavenging. *MESLP*'s potential *in vitro* antioxidant activity was evaluated based on its ability to neutralize stable free radicals by donating an electron or hydrogen (Sasikumar and Kalaisezhiyen, 2014). Table 2 shows that *MESLP* has 77.62% free radical scavenging activity. Total polyphenol content and antioxidant activity against free radicals are highly related (Huang *et al.*, 2005), and a linear association between total phenolic and flavonoid concentration and antioxidant capability has been discovered (Shrestha and Dhillion, 2006).

Table 2. % Extraction yield, TPC, TFC and in vitro free radical scavenging activity of methanolic extract of Sweet lime peel

| **MESLP** | **% Extraction yield** | **TPC mg GAE/g** | **TFC mg QE/g** | **% Free radical scavenging activity** |
|---|---|---|---|---|
| MAE | 12.91±0.04 | 16.66± 0.05 | 9.59± 0.08 | 77.62±0.05 |

(Results are mean ± SD of 3 determinations)

**FT-IR analysis of SLP**

FTIR analysis confirmed the existence of bioactive compounds that were suggested by GCMS investigations. The functional groups included in the methanol extract of *SLP* were identified using a range of peaks discovered by FTIR analysis, as illustrated in Figure 1. The functional group of the active components was measured using the FTIR spectrum based on the peak value in the infrared radiation band (Skoog *et al.*, 2017).

The FTIR analysis of SLP methanolic extract reveals unique peaks at 3317.56 due to the presence of O-H stretching of polyphenolic alcohols, a peak at 2943.37 due to C-H stretching of Alkanes, and C-C stretching at 2121.7 due to Alkyne. Because of the existence of Imine and Oxime (C=N stretching), the peak at 1635.64, peak at 1452.4, and peak at 1514.12 are created due to the presence of Nitro compound at, N-O stretching. Carboxylic acid O-H bending is indicated by a peak at 1411.89. The presence of Halo compounds is suggested by the peaks at 1016.49,1053.13, 1103.28,1265.3 C-O stretching of Alcohol, Ether, Ester, Carboxylic Acid, and Anhydride, as well as the peak at 584.43 (C-Br stretching).

Presence of these functional groups in the MESLP analyzed signifies the presence of phytochemicals such, Hesperidins, α-carotene, rutin, Quercitin, Phloretic acid (Kennepohl *et al.*, 2020).Table 3 indicated the present functional group in the MESLP. Fig 1 Shows FTIR graph.

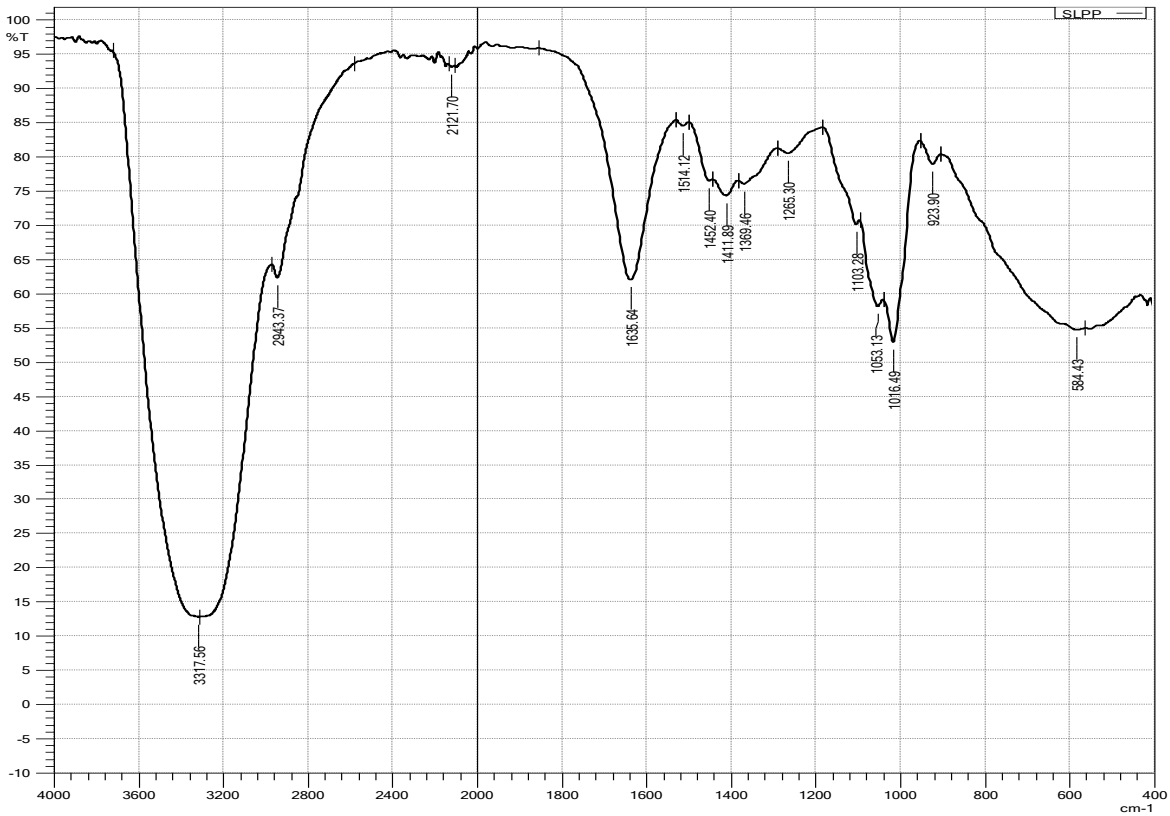


Fig 1.FTIR Spectra of MESLP

Table 3. FTIR spectroscopy of methanolic extract of SLP

| **Sr.No.** | **Peak** | **Area** | **Bond** | **Functional Group** | **Frequency in cm−1 (Intensity*)** |
|---|---|---|---|---|---|
| 1 | 584.43 | 12078.15 | C-Br stretching | Halo compound | 690-515 (s) |
| 2 | 923.9 | 952.07 | C=C bending | Alkenes | 1000-650 (s) |
| 3 | 1016.49 | 2980.00 | C-O stretching | Alcohol, Ether, Ester, Carboxylic Acid, Anhydride | 1300–1000 (s) |
| 4 | 1053.13 | 2106.87 | | | |
| 5 | 1103.28 | 2055.97 | | | |
| 6 | 1265.3 | 1884.60 | | | |
| 7 | 1369.46 | 2027.38 | S=O stretching | Sulfonamide | 1370-1335 (s) |
| 8 | 1411.89 | 1469.73 | O-H bending | Carboxylic acid | 1450 and 1375 (m) |
| 9 | 1452.4 | 1102.91 | N-O stretching | Nitro compound | 1550 and 1350 (s) |
| 10 | 1514.12 | 467.54 | | | |
| 11 | 1635.64 | 5642.94 | C=N stretching | Imine and Oxime | 1690–1640 (w-s) |
| 12 | 2121.7 | 195.24 | C≡C stretching | Alkyne | 2140-2100 (w-m) |
| 13 | 2943.37 | 7377.03 | C–H stretching | Alkane | 3000–2850 (s) |
| 14 | 3317.56 | 22985.60 | O-H stretching | PolyHydroxy compounds | 3400–3200 (m-s) |

(s = strong; m = medium; w = weak)

**GC-MS analysis of methanolic extract of SLP**

The GC-MS analysis of a methanolic extract of sweetlime peel yielded a total of 34 components, The presence of steroids, alkaloids, glycosides, tannin, phenols, saponins, flavonoids, and terpenoids in 12 of them resulted in different phytochemical activity. Figure 1 depicts the chromatogram, and Table 4, summarizes the chemical components in the MESLP as well as their retention time (RT), molecular formula, molecular weight (MW), and concentration (%).The following bioactive compounds were present in the GC-MS analysis carried on methanolic extract of sweet lime peel D-Limonene, n-Hexadecanoic acid, Hexadecanoic acid, ethyl ester , 9,12-Octadecadienoic acid (Z,Z)-, Oleic Acid ,(R)-(-)-14-Methyl-8-hexadecyn-1-ol,

Octadecanoic acid,24-Noroleana-3,12-diene,Squalene, beta-Sitosterol acetate, Vitamin E. (Figure 3-14 depicts the mass spectra of an identified chemical from a methanolic extract of sweet lime peel).

Table 4. Bioactive compounds found in methanolic extract of Sweetlime peel using GC-MS

| Sr No | R.Time (Min) | % Area | Molecular formula | Molecular Weight | Name |
|---|---|---|---|---|---|
| 1 | 13.96 | 5.2394 | $C_{16}H_{32}O$ | 256.4 | n-Hexadecanoic acid |
| 2 | 14.35 | 0.104 | $C_{18}H_{36}O_2$ | 284.47 | Hexadecanoic acid, ethyl ester |
| 3 | 16.4 | 1.68 | $C_{18}H_{32}O$ | 280.44 | 9,12-Octadecadienoic acid (Z,Z)- |
| 4 | 16.48 | 9.6207 | $C_{18}H_{34}O_2$ | 282.5 | Oleic Acid |
| 5 | 16.74 | 0.6094 | $C_{17}H_{32}O$ | 252.2 | (R)-(-)-14-Methyl-8-hexadecyn-1-ol |
| 6 | 16.83 | 1.6295 | $C_{18}H_{36}O_2$ | 284.48 | Octadecanoic acid |
| 7 | 20.64 | 0.4953 | $C_{29}H_{46}$ | 394.67 | 24-Noroleana-3,12-diene |
| 8 | 22.35 | 0.4297 | $C_{24}H_{38}O_4$ | 390.55 | Bis(2-ethylhexyl) phthalate |
| 9 | 26.44 | 0.2193 | $C_{30}H_{50}$ | 410.73 | Squalene |
| 10 | 28.67 | 0.477 | $C_31H_{52}O_2$ | 456.74 | beta.-Sitosterol acetate |
| 11 | 31.84 | 1.747 | $C_{29}H_{50}O_2$ | 430.7 | Vitamin E (*alpha-Tocopherol*) |
| 12 | 2.60 | 4.7085 | $C_{10}H_{16}$ | 136.23 | D-Limonene |

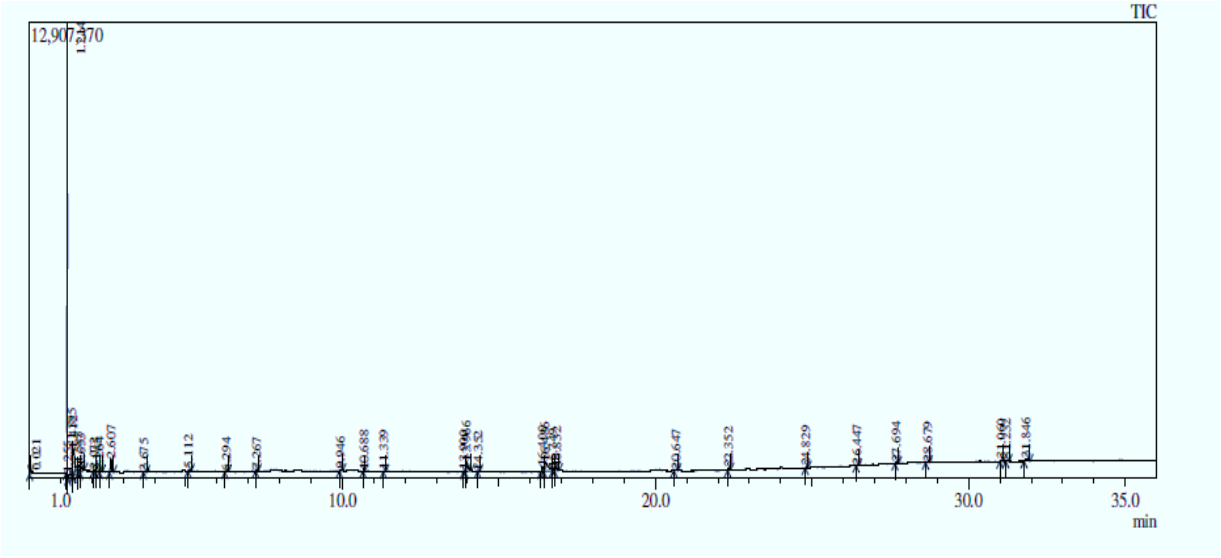


Fig 2. GC-MS chromatogram of methanolic extract of Sweet lime peel

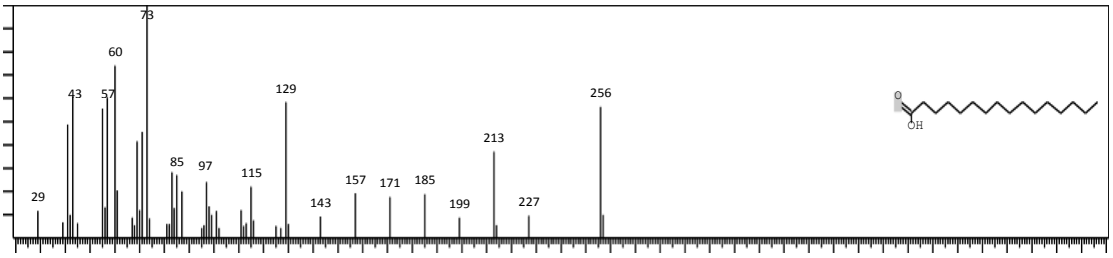


Figure *3. n-Hexadecanoic acid*

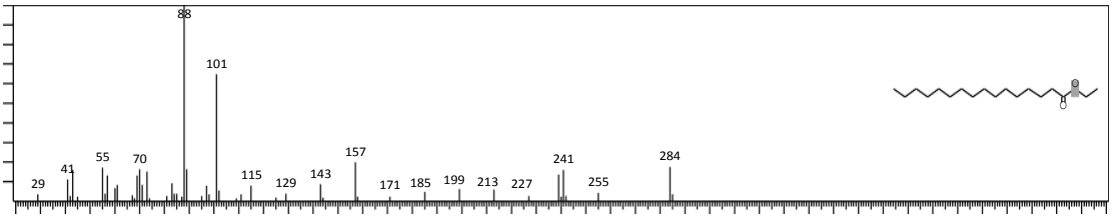


Figure 4. Hexadecanoic acid, ethyl ester

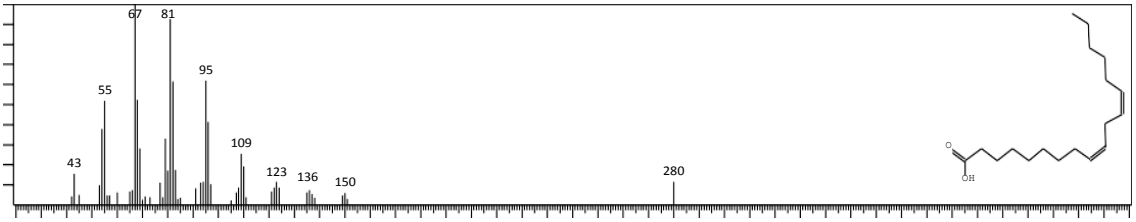


Figure 5. 9, 12-Octadecadienoic acid (Z, Z)-

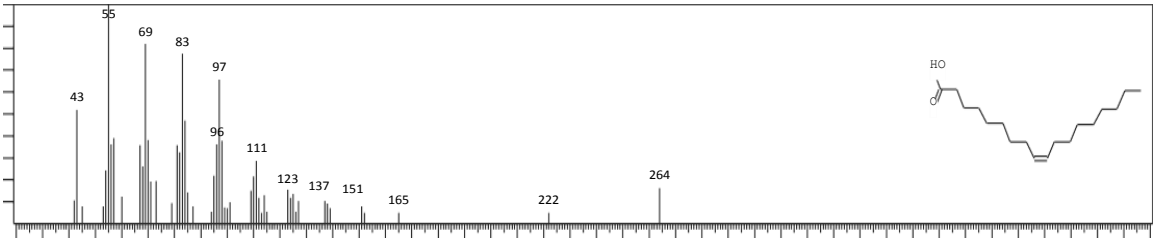


Figure 6. Oleic Acid

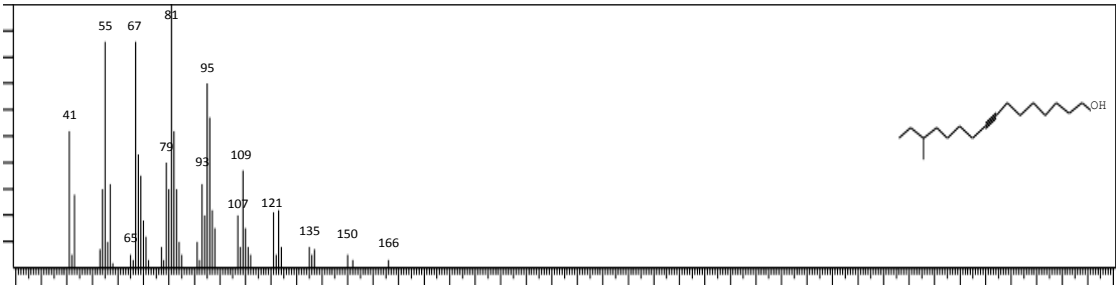


Figure 7. (R)- (-)-14-Methyl-8-hexadecyn-1-ol

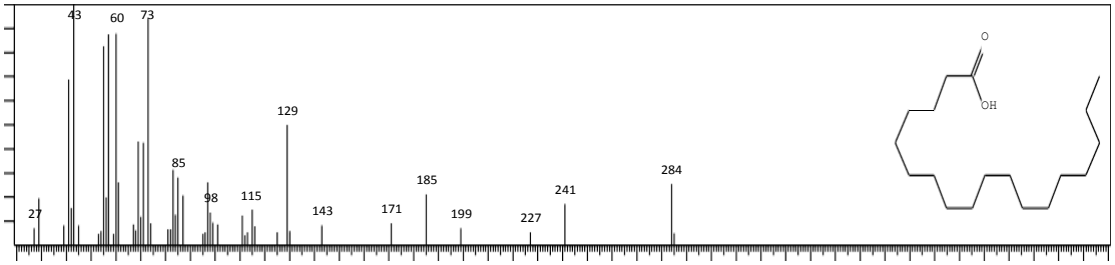


Figure 8. Octadecanoic acid

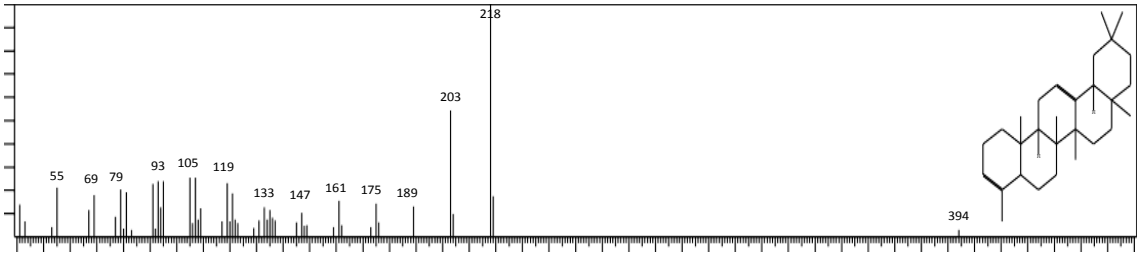


Figure 9. 24-Noroleana-3,12-diene

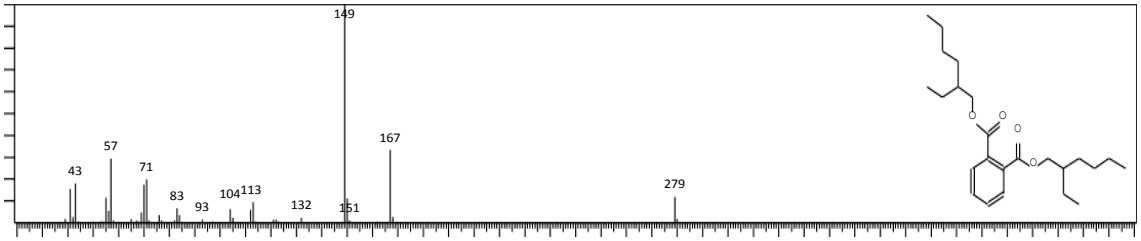


Figure 10. Bis(2-ethylhexyl) phthalate

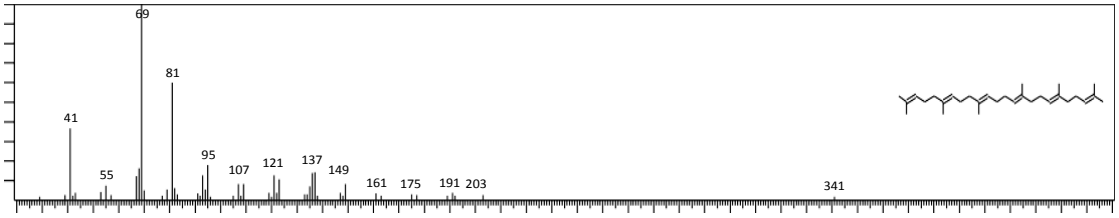


Figure 11. Squalene

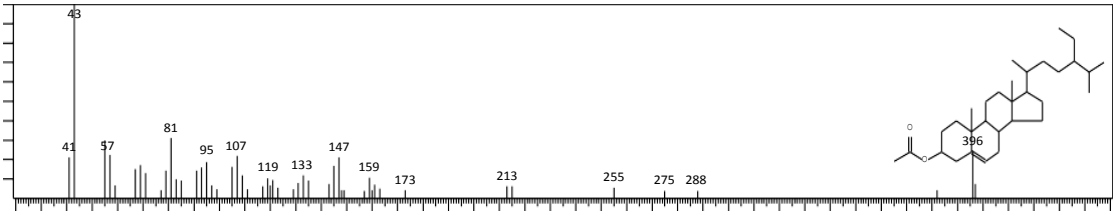


Figure 12. beta.-Sitosterol acetate

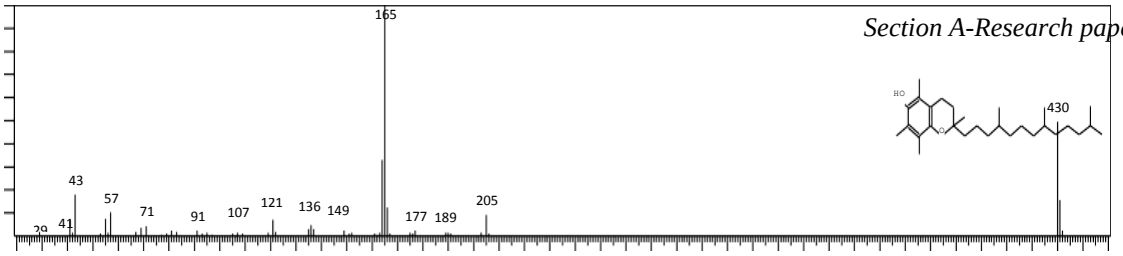


Figure 13. Vitamin E (alpha-Tocopherol)

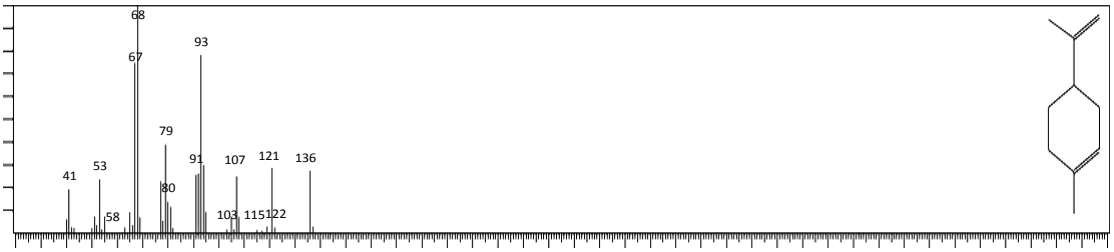


Figure 14. D-Limonene

The majority of phenolic compounds found in plants belong to a varied group with a variety of biological activities, such as gene expression control, anti-inflammatory, antiviral, anti-bacterial, antioxidant, and anti-mutagenic capabilities. There has also been a focus on its role in lowering the risks of a variety of ailments, particularly coronary heart disease, various cancer forms, and pulmonary ailments (Frydoonfar *et al*., 2003) and (Queiroz *et al.*, 2012).

The preponderance of the chemicals discovered has antioxidant, antibacterial, anticancer, and anti-inflammatory properties. Based on these discoveries, D-limonene has been used therapeutically to dissolve cholesterol-containing gallstones because it is excellent cholesterol solvent. It has also been used to treat heartburn due to its ability to neutralize stomach acid and encourage healthy peristalsis. It is well known that d-limonene has chemo preventive properties against a wide range of cancers. A phase-I clinical research found that three patients with colon cancer maintained stable sickness for more than six months, while one patient with breast cancer had a partial response (Sun, 2007). Oleic acid is commonly used to prevent heart disease and lower cholesterol. It's also utilized to keep cancer and other disorders at bay (López *et al.*, 2010). Dietary squalene supplementation lowers cholesterol and triglyceride levels (Kelly, 1999). Beta-sitosterol may help decrease cholesterol levels by limiting the amount of cholesterol that enters the body. It can also help reduce swelling in the prostate and other tissues. Beta-sitosterol is most commonly used to lower cholesterol and alleviate symptoms of an enlarged prostate (BPH) (Watson *et al.*, 2006). N-hexadecanoic acid may aid in the development of particular inhibitors of phospholipase A (2) as anti-inflammatory drugs (Aparna *et al.*, 2012). Hexadecanoic acid and its ethyl ester have antifungal, antitumor, and antibacterial properties (Tyagi, and Agarwal, 2017). 9, 12-Octadecadienoic acid aids in the prevention of cardiovascular disease (Krishnaveni *et al.*, 2014). (R)- (-)-14-Methyl-8-hexadecyn-1-ol has antibacterial activity (Beschi *et al*., 2021). Jasim *et al*. (2015) investigated the antibacterial activity of octadecanoic acid. Antibacterial and antioxidant activities were found in 24-Noroleana-3, 12-diene (Ullah *et al.*, 2022).

## Conclusion

This study discovered that sweet lime peel contains a number of secondary metabolites with varied pharmacological effects, one of which is antioxidant activity. The existence of 12 phytochemical components with antibacterial, antioxidant, anticancer, hypercholesterolemic, anti-inflammatory, and other properties was revealed by GC-MS analysis. As a result, phytochemicals must be held accountable for the therapeutic effects they provide. More study is needed to create novel treatments based on some of the bioactive components identified in sweet lime peel extract, which also has the potential to be valued as a functional food.

## Conflicts of interest

All authors declare that they have no conflicts of interest.

## Funding

This research received no specific grant from any funding agency.

## Abbreviation

FTIR: Fourier-transform infrared spectroscopy

GC-MS: Gas chromatography and Mass Spectroscopy

MAE: Microwave Assisted Extraction

MESLP: Methanolic Extract of Sweetlime peel

TFC: Total Flavanoid content

TPC: Total Phenolic content

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