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Effect of indigenous extraction solvents on the phytochemical, GC-MS profile and antioxidant activities of *Ocimum gratissimum* extracts

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Abstract

This study compared the effects of Ethanol and indigenous extractants (Aqueous, 2% NaCl solution, and 40% v/v Lime juice) on the bioactive properties of *Ocimum gratissimum* extract. Phytochemical, GC-MS, and antioxidant analysis of the Ethanol (EtOHE), Aqueous (AqE), NaCl solution (NaClE), and Lime juice (LjE) extracts were carried out. All the extracts contained saponins, tannins, flavonoids, phenols, steroids, and alkaloids, with Ethanol and Aqueous extracts showing higher concentrations of these compounds compared with NaCl and Lime juice extracts ($p < 0.05$). GC-MS characterization identified twenty-one bioactive compounds in the Ethanol extract, while only fifteen compounds were found in the Aqueous, NaCl, and Lime juice extracts, respectively. The Ethanol extract had higher Total Antioxidant Capacity (TAC) (26.08 ± 0.76 μg/ml) compared with Aqueous (7.79 ± 0.61 μg/ml), NaCl (7.82 ± 0.81 μg/ml), and Lime juice (3.49 ± 0.48 μg/ml) extracts ($p < 0.05$). The Aqueous extract had the strongest Ferric reducing antioxidant power, with a lower IC_{50} value of 117.37 μg/ml, compared with NaCl (228.94 μg/ml) and Lime juice (166.84 μg/ml) extracts, while Ethanol extracts had the lowest IC_{50} value (60.39 μg/ml) for ABTS radicals compared with NaCl (66.75 μg/ml) and Lime juice (78.52 μg/ml) extracts. In conclusion, all the extracts exhibited potential medicinal and antioxidant properties; however, the bioactive and antioxidant properties of the extracts demonstrate a dependence on the extraction methods and solvents. It is therefore important to always ascertain which extraction solvent and method is appropriate for the preparation of safe medicinal herbs.

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1. Introduction

The global increase in the preferential use of herbal medicines over pharmaceutical drugs is due to: increasing drug resistance to antibiotics, shortage or poor availability of orthodox medicines, especially in developing countries, the efficacies of the active

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principles present in herbal medicine, as well as their availability, accessibility, affordability, and acclaimed less-toxic effects [1]. Furthermore, taking into consideration the relative ratios of herbal medicine practitioners and university-trained doctors in relation to the whole population in African countries, traditional practitioners and herbal medicines made from plants play an important role in the health of millions of people [2]. To date, naturally occurring bioactive components of several plants are used in traditional medicine preparation due to their availability and low cost. This has led to the rapid increase in the use of these herbal remedies, particularly in the management of different diseases, with several of them being incorporated into orthodox medicine [3].

In addition, there is increasing attention on herbal remedies, including herbal concoctions of infusion, because health-conscious consumers consider medicinal plants as an alternative for the treatment of several diseases and as a source of dietary polyphenols/dietary antioxidants [4]. Herbal medicines are usually prepared from the extraction of different parts (leaves, fruit, seed, stem, flower, root, bark, or mixture) of various medicinal plants using different extraction solvents (water, alcohol, honey, natural oil) or methods (infusion, decoction, maceration). However, some quality and pharmacological aspects of herbs used in the production of these herbal products are taken for granted by the herbal practitioners, and this can place the consumer's health and safety at risk [4]. One major concern is issues with pre- and post-harvest contaminations of medicinal plants from heavy metals, pesticides, or herbicides. Another concern is the methods of extraction, duration of extraction [5], potential herbal ingredient toxicity, methods of preservation, and the safe dosage of the herbal tea/mixture/concoction of infusion [4]. Hence, there is a need for the investigation, evaluation, and validation of appropriate and safe extraction solvents and methods used by traditional medicine providers, to ensure that the quality and pharmacological properties of medicinal plants are preserved.

Ocimum gratissimum L., also known as scent leaf, is indigenous to coastal, savannah, and tropical areas [6], such as West Africa, Asia, Brazil, and India. It is naturally used in the treatment of different diseases, which include: upper respiratory tract infections, diarrhea, headache, conjunctivitis, skin disease, pneumonia, and fever [7]. These ethnomedicinal properties have been associated with its flowers and leaves being rich in polyphenols and other bioactive compounds such as peptides, alkaloids, essential oils, phenols, and flavonoids, which are phytochemical components in the plants [8]. In addition, previous studies have documented its antimicrobial, antidiarrheal, anti-inflammatory, antihypertensive, antidiabetic, hypolipidemic, hepatoprotective, antioxidant, and immunostimulatory effects [9]. Studies on the effects of common extraction solvents on the phytochemicals and bioactivity of plants have been reported by previous studies. A study by Onyebuchi and Kavaz [6] has reported the effects of different common extraction solvents (methanol, ethanol, and water) and temperature on the phytochemicals, bioactivity, and antioxidant properties of *Ocimum gratissimum*. However, comparative studies on the effect of indigenous extractants on the phytochemicals, bioactive composition, and antioxidant properties of *Ocimum gratissimum* are yet to be carried out. Although the use of lime juice and table salt solution for the preparation of *Ocimum gratissimum* extract and other herbal mixtures is a common traditional method used by traditional medicine practitioners to increase the efficacy of herbs, the mechanisms of action of these extractants on the phytochemical components of the extracts are not well clarified. Hence, this study aims to assess and compare the effects of using indigenous extractants (aqueous, NaCl solution, and lime juice) with ethanol (a common extraction solvent) on the phytochemical composition, bioactive, and antioxidant properties of *O. gratissimum* leaves.

2. Materials and methods

2.1. Plant material

Fresh scent leaves were collected from Arudi's farm in Osara town, Kogi State, Nigeria, in April 2024. A Botanist (Mr. Akanni) of the Department of Botany, Federal University Lokoja, Kogi State, Nigeria, identified and confirmed the plant with voucher number *Ocimum gratissimum* L. (FULH0216).

2.2. Preparation of ethanol extract of Ocimum gratissimum (EtOHE)

The collected *Ocimum gratissimum* leaves were air-dried indoors for 2 days and crushed into smaller sizes; the samples were pulverized into powder with a dry blender. For the ethanol extraction, 50 g of the *Ocimum gratissimum* leaves were soaked in 500 ml of 70% ethanol for 72 hours. The extract was filtered and concentrated in a water bath at 60°C to a constant weight as described by Ujah *et al.* [10]. The use of 70% ethanol for 72 hours as an extraction solvent is a common method used for the extraction of *Ocimum gratissimum* leaves as documented in previous studies [9, 10]. However, the use of indigenous solvents or extraction methods such as decoction, compressing, infusion, and steeping usually involves a mixture of herbs or additives and a shorter period of time.

2.3. Preparation of aqueous NaCl solution and lime juice extracts

The extracts were prepared following the methods used by local folks from Alade Idanre town of Idanre Local Government Area, Ondo State, Nigeria, as described by Makinwa *et al.* [9] with modifications.

2.3.1. Aqueous extract (AqE)

For the aqueous extract, 50 g of freshly hand-picked *Ocimum gratissimum* were pulverised in 100 ml of distilled water for 1 hour. The mixture was filtered, and the filtrate was used for further investigations.

2.3.2. NaCl solution extract (NaClE)

50 g of freshly hand-picked *Ocimum gratissimum* were pulverized in 2% NaCl solution using a mortar and pestle. The solution was filtered, and the filtrate was used for further investigation. The NaCl was obtained from Molychem Laboratories, India.

2.3.3. Lime juice extract (LjE)

Lime fruits were obtained from the Lokoja International Market, Kogi State, Nigeria. The lime juice was extracted by squeezing the juice from the fruits. Then, 50 g of *Ocimum gratissimum* were pulverized in 40% v/v lime juice solution using a mortar and pestle. The solution was filtered, and the filtrate was used for further analysis.

2.4. Qualitative phytochemical analysis of the plant extracts

The ethanol, aqueous, NaCl solution, and lime juice extracts of *Ocimum gratissimum* were tested for the presence of various phytochemicals: tannins, saponins, terpenoids, glycosides, alkaloids, flavonoids, phenolic compounds, reducing sugars, steroids, and anthraquinones using the methods described by Ayoola *et al.* [11].

2.5. Quantitative phytochemical screening of EtOHE, AqE, LjE, and NaClE of Ocimum gratissimum

The total phenol content of the extracts was analysed according to the methods of Edeoga *et al.* [12]. Total flavonoids were analysed using the aluminium chloride colorimetric method as described by Chang *et al.* [13]. Total alkaloids were determined as described by Chukwuma and Chigozie [14]. Saponin content was analysed as described by Ameen *et al.* [15]. Tannin content of the extracts was determined as described by Edeogu and Ekuma [16]. The phytic acid content of the plants was measured as explained by Mallick *et al.* [17]. The permanganate titrimetric method, as outlined by Oke [18], was used to determine the amount of oxalate present in the samples.

2.6. Gas chromatography–mass spectroscopy analysis of the extracts

The ethanol, aqueous, NaCl, and lime juice extracts of *Ocimum gratissimum* were analysed using GC–MS Model QP2010 Plus (Shimadzu, Japan), as described by Magashi and Abdulmalik [19]. The GC–MS system consists of a VF-5 MS bonded silica capillary column of length 30 m, internal diameter 0.25 mm, and film thickness 0.25 μm. The column oven temperature was programmed from 80°C to 280°C at a rate of 2°C/min. The sample components were ionised in electron impact mode (EI, 70 eV). One detector was set at 200°C, and the injector temperature was maintained at 250°C. The carrier gas (99.9995% pure helium) had a flow rate of 1.5 ml/min. Mass spectra ranging from 40 to 1000 m/z were scanned at a rate of 3.0 scans per second. Using the split injection method, 1.0 μl of each extract was manually injected using a Hamilton syringe for total ion chromatographic (TIC) analysis. The total run time was 27 minutes. The relative percentage of each component was calculated using peak area normalization. Identification of compounds was achieved by comparing their mass spectra with those in the National Institute of Standards and Technology (NIST) database, which contains over 62,000 reference spectra. Compound identification was based on molecular weight, formula, and matching scores [19].

2.7. Determination of total antioxidant capacity

The total antioxidant activity was determined using the phosphomolybdenum method with slight modifications [20]. The principle is based on the reduction of Mo(VI) to Mo(V), forming a green phosphate/Mo(V) complex under acidic conditions. The reaction mixture consisted of 3 ml of reagent solution (28 mM sodium phosphate, 0.6 M sulfuric acid, and 4 mM ammonium molybdate) and 0.3 ml of extract. The tubes were incubated at 95°C for 90 minutes. After cooling to room temperature, the absorbance was measured at 695 nm against a reagent blank. The blank consisted of 0.3 ml of methanol, while ascorbic acid was used as the standard.

2.8. ABTS 2,2- azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging activity

To create the $ABTS^+$ cation radical, 5 ml of 4.9 mM potassium persulfate ($K_2S_2O_8$) solution was combined with 14 mM ABTS. The mixture was then left at room temperature for 16 hours in the dark. Before use, the ABTS/$K_2S_2O_8$ solution was diluted with ethanol in order to achieve an absorbance of 0.700 ± 0.020 at 734 nm. After homogenizing the extracts at different concentrations (25–300 μg/ml) with 1 ml of ABTS solution, the absorbance of the reaction mixture was read at 734 nm. Likewise, the reaction mixture of the standard solution of the assay was prepared by combining 950 μl of $ABTS^+$ solution with 50 μl of BHT (butylated hydroxytoluene). For every test, ethanol was used as the blank reagent; all measurements were taken after six minutes of reaction. The inhibition percentage of ABTS radical was calculated using (1) [21], where A_1 is the absorbance of the sample, and A_0 is the control's absorbance.

$$\text{ABTS Scavenging Activity (\%)} = \frac{A_0 - A_1}{A_0} \times 100. \tag{1}$$

2.9. Ferric reducing antioxidant power (FRAP) test

The reagent used for the FRAP test of the extracts was prepared as follows: sodium acetate buffer (300 mM, pH 3.6), tripyridyltriazine (TPTZ) solution (10.0 mM), and $FeCl_3 \cdot 6H_2O$ solution (20.0 mM) were mixed in a volume-by-volume ratio of 10 : 1 : 1. The extract samples at several concentrations (25–300 μg/ml) were then added to 3 ml of FRAP reagent. The reaction mixture was incubated at 37°C for 30 minutes, and the absorbance was measured at 593 nm. Calibration was performed using a fresh solution of $FeSO_4$. Using the linear calibration curve, the antioxidant capacity, based on the sample's ability to reduce ferric ions, was calculated and expressed as mmol $FeSO_4$ equivalent per gram of sample (DW) [22].

2.10. Statistical analysis of data

The SPPS (Statistical Package for Social Sciences) version 26, Chicago, IL, USA, was used for data analysis. Numerical values are represented as the mean ±SEM of duplicate assays. Analysis of variance (ANOVA) was performed on the data sets obtained following Tukey's Test. Data having values at $p < 0.05$ are considered statistically significant.

3. Results and discussion

Preparation of traditional or herbal remedies usually involves the combination of medicinal plants with other substances, with the aim of increasing their therapeutic efficacy [23]. The addition of lime juice and table salt to water for the extraction of the bioactive component of *Ocimum gratissimum* is a method commonly used for the preparation of herbal medicine. This study investigated the effects of indigenous methods of extraction (using 2% NaCl solution and 40% v/v Lime juice) on the bioactive constituents and antioxidant activities of *ocimum gratissimum* leaves.

3.1. Phytochemical profile of EtOHE, AqE, LjE and NaClE of Ocimum gratissimum

The phytochemical components of EtOHE, AqE, LjE, and NaClE of *Ocimum gratissimum* are presented in Table 1. Results of the four *Ocimum gratissimum* samples showed that all extracts contained flavonoids, phenols, tannins, steroids, alkaloids, and oxalates. Saponins were detected in the EtOHE and AqE; additionally, these extracts also contained anthraquinones. In contrast, none of the extracts contained reducing sugars. The occurrence of these phytochemicals in the various extracts of *Ocimum gratissimum* is an indication of its antioxidant potential and, hence, its possible pharmacological activity. Phenols, flavonoids, and alkaloids are essential constituents of the therapeutic profile of a plant. Flavonoids are produced by plants for defence against microbial infection; in addition, they are known to have potential anti-inflammatory and antimicrobial effects [24]. Alkaloids possess antibacterial, antiviral, anticancer, antifungal, and antimalarial properties [25], while saponins exhibit anti-inflammatory, anti-melanogenic, and antispasmodic effects, along with cancer-inhibiting potentials [26]. Scientific evidence shows that tannins are bioactive compounds that accelerate wound healing and aid in the prevention of cancer [27]. Tannins also demonstrate anti-inflammatory effects on inflamed mucous and ulcerated tissues and exhibit antibacterial activity against many human gastrointestinal pathogens [28]. Additionally, anthraquinones [29] and steroids exhibit anti-inflammatory and anticancer properties [30]. Although oxalates have been associated with some adverse health effects in humans [31], the levels of oxalates found in the analysed *Ocimum gratissimum* extracts are low.

Table 2 shows the mean percentage composition of the identified phytochemicals present in the *Ocimum gratissimum* extracts. The results revealed that the EtOHE and AqE contained significantly ($p < 0.05$) higher concentrations of the phytochemicals: phenols, tannins, steroids, saponins, anthraquinones, alkaloids, and flavonoids compared with the LjE and NaClE, with the ethanol extract having the highest concentrations of these phytochemicals. The phenol content of the EtOHE was 270.49 ± 6.35 mg/g compared with those found in the AqE (193.30 ± 2.31 mg/g), LjE (88.32 ± 0.86 mg/g), and NaClE (68.15 ± 1.10 mg/g), respectively. The flavonoid content was 110.54 ± 0.01 mg/g, 43.85 ± 1.78 mg/g, 22.45 ± 1.61 mg/g, and 22.27 ± 0.31 mg/g for EtOHE, AqE, NaClE, and LjE, respectively. The results also revealed that EtOHE (198.62 ± 1.79 mg/g) had the highest alkaloid content, followed by the AqE (55.00 ± 1.09 mg/g), NaClE (37.38 ± 1.46 mg/g), and LjE (24.80 ± 0.69 mg/g). Overall, the phytochemical content of the *Ocimum gratissimum* extract followed the order: EtOHE > AqE > NaClE > LjE for tannins and phytate. This finding suggests that ethanol is a more potent solvent for the extraction of phytochemical components of *Ocimum gratissimum*. The significantly higher concentrations of phytochemicals found in the ethanol extract may be due to the longer extraction period (72 hours) as well as the higher solubility of these bioactive compounds in ethanol compared with the other solvents used. Earlier studies [8, 32, 33] have reported the efficient use of ethanol as a potent solvent in the extraction of bioactive components of plants during phytochemical analysis. The results also revealed that the aqueous extracts of *Ocimum gratissimum* contained significantly higher concentrations of phytochemicals compared with NaClE and LjE in the following order: AqE > NaClE > LjE for phenols, tannins, steroids, alkaloids, phytate, and flavonoids ($p < 0.05$). This finding further indicates that water is also a potent extractant for *Ocimum gratissimum*. Extraction of bioactive compounds in plants using water is not uncommon. Several methods, such as decoction, infusion, and hydrodistillation, employ water as the sole extractant [34]. Water is also commonly used in combination with alcohols [35].

Our results showed that the extraction of *Ocimum gratissimum* using 2% NaCl solution as solvent yielded lower concentrations of key phytochemicals compared with the aqueous extract, suggesting that the extraction solvent and methods are less efficient for maximizing the plant's medicinal properties. Although previous studies have employed the use of 2% NaCl to increase the boiling

Table 1: Phytochemical composition of Ethanol, Aqueous, Lime juice, and NaCl extracts of *Ocimum gratissimum*.

Samples	Phenol	Tannins	Saponins	Anthraquinones	Reducing sugars	Steroids	Alkaloids	Flavonoids
EtOHE	++	+	+	+	−	+	++	++
AqE	+	+	+	−	−	+	+	+
LiE	+	+	−	−	−	+	+	+
NaCLE	+	+	−	+	−	+	+	+

+ = present and − = absent.

Table 2: Quantity of phytochemical composition of Ethanol, Aqueous, Lime juice, and NaCl extracts of *Ocimum gratissimum*.

Samples	Phenol (mg/g)	Flavonoids (mg/g)	Alkaloids (mg/g)	Tannins (mg/g)	Phytate (mg/g)	Saponins (mg/g)	Oxalates (mg/g)
EtOHE	270.49 ± 6.35*	110.54 ± 0.01*	198.62 ± 1.79*	112.86 ± 1.35*	31.71 ± 1.65*	121.99 ± 1.59	0.81 ± 0.01*
AqE	193.30 ± 2.31**	43.85 ± 1.78**	55.00 ± 1.09**	94.03 ± 0.01**	18.75 ± 0.25**	173.18 ± 1.63	0.51 ± 0.03**
LiE	88.32 ± 0.86***	22.27 ± 0.31****	24.80 ± 0.69***	64.21 ± 1.33***	12.40 ± 0.90***	ND	0.65 ± 0.04***
NaCLE	68.15 ± 1.10****	22.45 ± 1.61****	37.38 ± 1.46****	76.19 ± 0.74****	15.96 ± 0.46***	ND	0.34 ± 0.06**

Values are means ± SEM of duplicate determinations. Different numbers of asterisks (*, **, ***, ****) in the same column indicate statistical differences at $p < 0.05$.

point of a solution (to accelerate mass and heat transfer) [36], as well as to increase the polarity of the extraction solvent [37], thereby aiding the degradation of components so that the extraction process can proceed faster, our findings revealed otherwise. Specifically, the presence of 2% NaCl in the extraction solvent (water) resulted in a significant reduction in the concentrations of phytochemicals compared with the aqueous extract. This may be due to the reduced solubility of phytochemicals in the salt/solvent mixture, based on the principle that the solubility of a compound in water decreases when the ionic strength of the solution increases due to the addition of a salt such as NaCl. As shown in Table 2, the LjE contained the lowest concentration of phytochemicals compared to EtOHE and AqE. The lower concentration of phenols (88.32 mg/g) compared with EtOHE and AqE indicates a lower antioxidant potential. Flavonoids, alkaloids, tannins, and phytate concentrations (22.27 mg/g, 24.80 mg/g, 64.21 mg/g, and 12.40 mg/g, respectively) are also substantially lower, suggesting that the LjE is less effective at extracting these compounds. The efficacy of LjE as an efficient solvent for the extraction of *Ocimum gratissimum* may be influenced by its acidic properties and reactivity with other substances. Lime juice contains citric acid, water, and other nutritional components. The addition of lime juice to water produces an acidic mixture, which lowers the pH of other compounds.

3.2. Bioactive compounds present in the Ocimum gratissimum extracts

Tables 3–6 show the GC-MS profile of the EtOHE, AqE, LjE, and NaClE of *Ocimum gratissimum*. The GC-MS chromatogram of the EtOHE of *Ocimum gratissimum* as shown in Figure 1, showed twenty-one peaks, indicating the presence of twenty-one bioactive compounds. The compounds identified were Butyrolactone, 3-Hexadecyloxycarbonyl-5-(2-hydroxyethyl)-4-methylimidazolium ion, Phenol,3-ethyl-, Oleic acid, p-(2-Acetoxyethoxy) Toluene, Pentadecanoic acid 14-methyl-, methyl ester, Linoleic acid, methyl ester, 10-Octadecenoic acid methyl ester, Octadecanoic acid, methyl ester, endo-2-Methyl-2-norbornanol, 6-Methyl-2-(2-oxiranyl)-2-heptanol, 4-(4-Hydroxy-2,2,6-trimethyl-7-oxabicyclo [4.1.0] hept-1-yl)butan-2-one, 6-(3-Hydroxy-but-1-enyl)-1,5,5-trimethyl-7-oxabicyclo[4.1.0] heptan-2-ol, Benzene acetic acid, 4-hydroxy-3-methoxy-, 1-Phenyl-2-propanol, 5-Dimethylaminopyrimidine, 7-n-Hexyleicosane, Phytol, 3-Eicosyne, Glyceryl 1,3-distearate and Squalene. The result revealed that 7-n-Hexyleicosane has the highest retention time (27.148 minutes), and Butyrolactone had the least retention time (3.504 minutes). The major compound in the EtOHE, as shown in the percentage area of the peak, is p-(2-Acetoxyethoxy) toluene with a significant composition of 43.06%, indicating it is the most abundant compound in the extract. p-(2-Acetoxyethoxy) toluene is noted for its ester functionalities, which are relevant for antimicrobial or antioxidant activities. Other key compounds include Butyrolactone (9.19%) and Oleic acid (6.53%), which are both known for their broad-spectrum biological activities. The presence of Phytol (1.08%) in the EtOHE also reflects the phytochemical richness of the extract. Phytol has been noted for its antioxidant and anti-inflammatory properties [33].

As shown in Figure 2, the GC-MS chromatogram of the AqE of *Ocimum gratissimum* showed fifteen peaks, which indicated the presence of 15 bioactive compounds. The compounds identified include: 3-Undecene, (Z)-Methyl tetradecanoate, n-Hexadecanoic acid, Tetradecanoic acid, Pentadecanoic acid, 14-methyl-, methyl ester, Linolelaidic acid, methyl ester, 11-Octadecenoic acid, methyl ester, Octadecanoic acid, methyl ester, Oleic Acid, Octadecanoic acid, 1,1,1-Trifluoroheptadecen-2-one, Docosanedioic acid, dimethyl ester, E-13-Docosenoic acid, Methyl(Z)-13 docosenoate, Heneicosanoic acid, methyl ester. With 3-Undecene, (Z)- having the lowest retention time (4.056 minutes), while Heneicosanoic acid, methyl ester has the highest retention time (26.605 minutes). Oleic acid is the most dominant compound in the AqE, contributing 34.54% to its total composition. Oleic acid is a monounsaturated omega-9 fatty acid, which is commonly found in oils like olive oil. It has been linked to reduced cardiovascular risk and anti-inflammatory effects [38]. Oleic acid also inhibits cancer progression, prevents hair loss associated with immune disorders, and enhances the synthesis of red blood cells [39]. In addition to Oleic acid, the AqE contained n-Hexadecanoic acid as the second major constituent (16.75%). n-Hexadecanoic acid, commonly known as palmitic acid, is a saturated fatty acid with recognized antimicrobial, anti-inflammatory [40], hypocholesterolemic, and nematicidal properties [41]. Furthermore, the presence of 11-Octadecenoic acid, methyl ester (9.86%) is an additional value to the sample, because the unsaturated fatty acid ester is associated with lipid metabolism and plays a role in the maintenance of cellular membrane integrity.

As shown in Figure 3, the GC-MS chromatogram of LjE of *Ocimum gratissimum* displayed fifteen peaks indicating the presence of fifteen compounds. The compounds include: 1-Decene, Tridecanoic acid, methyl ester, n-Hexadecanoic acid, Methyl-11-(3-pentyl-2-oxiranyl)undecanoate, Oleic Acid, Pentadecanoic acid, 14-methyl-, methyl ester, Linolelaidic acid, methyl ester, Pentadecanoic acid, Octadecanoic acid, 11-Octadecenoic acid, methyl ester, 9-Tetradecenal, (Z)-, methyl ester, (Z)-, Octadecanoic acid, methyl ester, 16-Hexadecanoyl hydrazide, 13-Docosenoic acid, 15-Tetracosenoic acid, methyl ester. Oleic acid makes up 36.95% of the total composition of LjE of *Ocimum gratissimum*. In addition to the Oleic Acid content, another significant composition of the LjE is Pentadecanoic acid (20.57%). Recent research suggests that pentadecanoic acid could serve as a marker for dairy fat intake and may have cardiovascular and immune-protecting properties [42], as well as hepatoprotective and anti-inflammatory properties [43]. The relatively high content of 11-Octadecenoic acid, methyl ester (7.56%) aligns with the unsaturated fatty acid profile of this sample. The presence of minor bioactive compounds such as Linolelaidic acid methyl ester (2.98%) is an additional input to its lipid-rich composition. Linolelaidic acid has been shown to significantly reduce cancer cell lines and oxidative stress in the cells [44].

Figure 4 represent the GC-MS chromatogram of the NaClE of *Ocimum gratissimum*. The fifteen displayed peaks shows the extract contained 15 bioactive compounds, which include; 1-Decene, Methyl tetradecanoate, n-Hexadecanoic acid, Tetradecanoic acid, Pentadecanoic acid, 14-methyl-, methylester, Linolelaidic acid, methylester, 11-Octadecenoic acid, methylester, Stearic acid, methylester, Oleic Acid, Octadecanoic acid, 1-(4-Bromobutyl)-2-piperidinone, Cyclopropaneoctanoic acid, 2-hexyl-, methyl ester, 9-Tetradecenal, (Z)-, Methyl (Z)-13 docosenoate, Heneicosanoic acid, methylester. Oleic acid is the dominant compound found in the NaClE, contributing 29.88% of the total composition of the NaClE. The NaClE also contained n-Hexadecanoic acid (18.66%) as the second most abundant compound. Several studies have reported that n-Hexadecanoic acid possesses Antioxidant, Hypocholesterolemic, Nematicidal, Pesticidal and Antiandrogenic potentials [41, 45]. Other significant biologically active compounds found in the NaClE extract include: 11-Octadecenoic acid, methyl ester (9.95%) and Pentadecanoic acid, 14-methyl-, methyl ester (8.50%). Of significance is the occurrence of Cyclopropaneoctanoic acid, 2-hexyl-, methyl ester (4.16%). Cyclopropaneoctanoic acid has been suggested to play an important role in the human body, because of its regulatory properties on cyclooxygenase activity, actomyosin ATPase, protein kinase $C-\varepsilon$, stearoyl-CoA desaturate and inflammation [46]. The fact that only the NaCl solution extract contained Cyclopropaneoctanoic acid may be due to the salting-out effect.

The disparity in the number and components of bioactive compounds in the various extract as shown in the GC-MS profile, is consistent with previous studies [7, 25, 38], which reported variations in the bioactive components of plant extracts, using different extraction solvents and procedures.

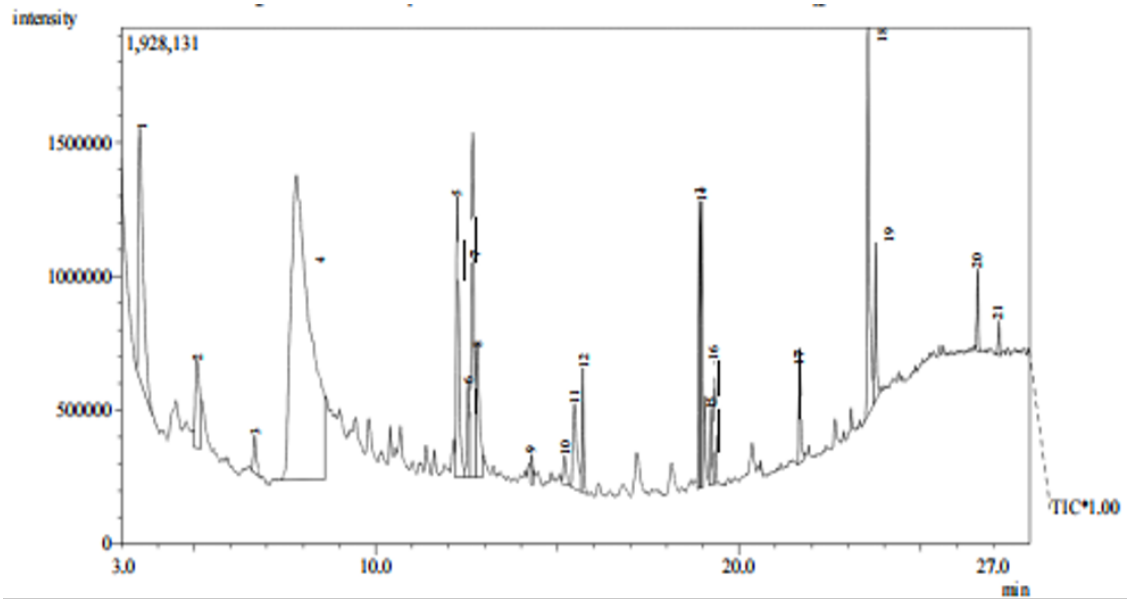


Figure 1: GC-MS chromatogram of ethanol extract of *Ocimum gratissimum*.

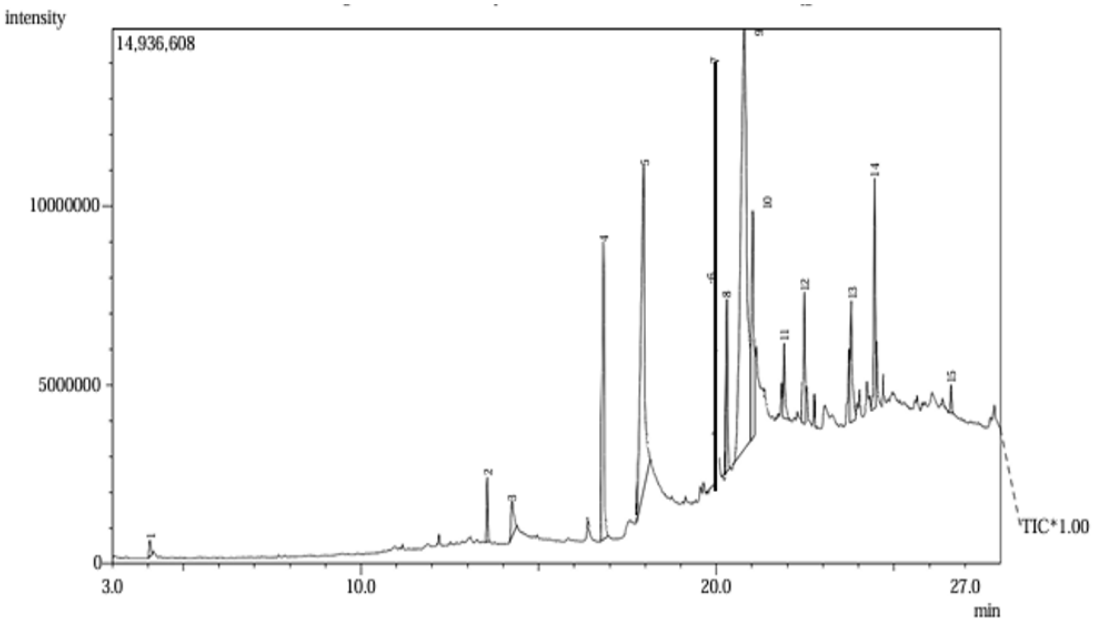
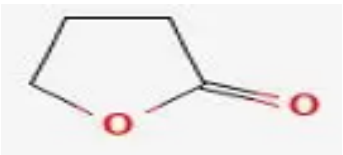
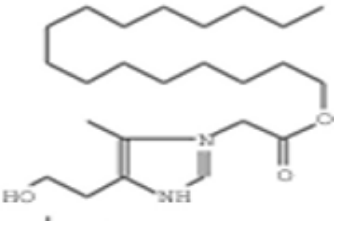
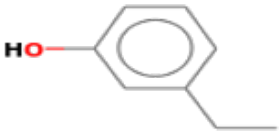
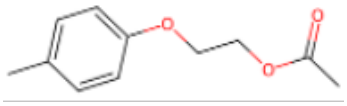
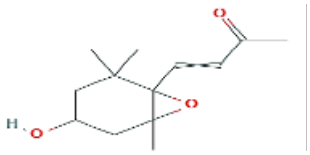
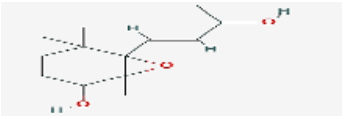
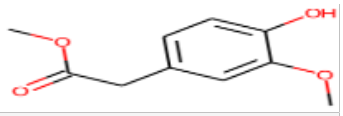
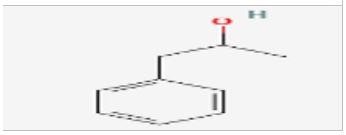
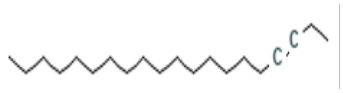
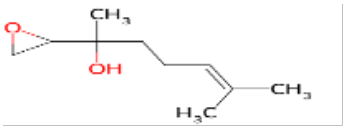
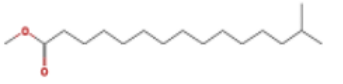


Figure 2: GC-MS chromatogram of aqueous extract of *Ocimum gratissimum*.

Table 3: Chemical composition of Ethanol extract of *Ocimum gratissimum*.

Peak	RT	Name of the compound	Area (%)	Formula	MW	Structure
1	3.504	Butyrolactone	9.19	$C_4H_6O_2$	86	

2	5.078	3-Hexadecyloxycarbonyl-5-(2-hydroxyethyl)-4-methylimidazolium ion	2.95	$C_{24}H_{45}N_2O_3$	409	
3	6.661	Phenol, 3-ethyl-	0.88	$C_8H_{10}O$	122	
4	7.818	p-(2-Acetoxyethoxy) toluene	43.06	$C_{11}H_{14}O_3$	194	
5	12.246	4-(4-Hydroxy-2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)butan-2-one	5.79	$C_{13}H_{22}O_3$	226	
6	12.544	6-(3-Hydroxy-but-1-enyl)-1,5,5-trimethyl-7-oxabicyclo [4.1.0] heptan-2-ol	1.61	$C_{13}H_{22}O_3$	226	
7	12.668	Benzene acetic acid, 4-hydroxy-3-methoxy-	7.06	$C_9H_{10}O_4$	182	
8	12.788	1-Phenyl-2-propanol	3.66	$C_9H_{12}O$	136	
9	14.287	3-Eicosyne	0.38	$C_{20}H_{38}$	278	
10	15.191	endo-2-Methyl-2-norbornanol	0.64	$C_8H_{14}O$	126	
11	15.468	6-Methyl-2-(2-oxiranyl)-2-heptanol	2.65	$C_{10}H_{20}O_2$	172	
12	15.698	Pentadecanoic acid, 14-methyl-, methyl ester	1.55	$C_{17}H_{34}O_2$	270	
13	18.907	Linoleic acid, methyl ester	3.59	$C_{19}H_{34}O_2$	294	
14	18.969	10-Octadecenoic acid, methyl ester	3.21	$C_{19}H_{36}O_2$	296	

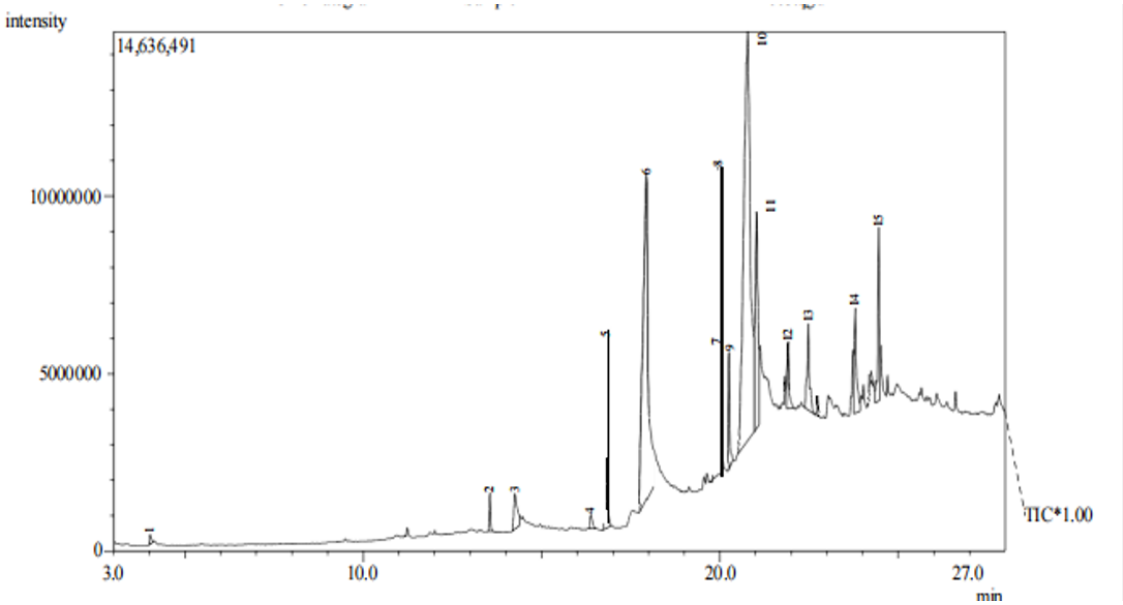
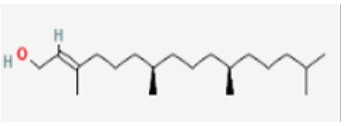
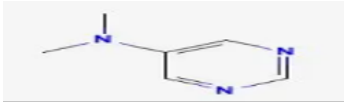
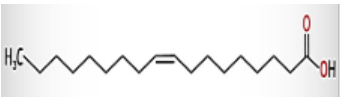
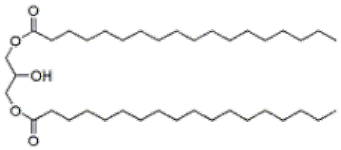
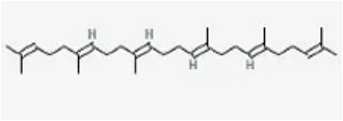
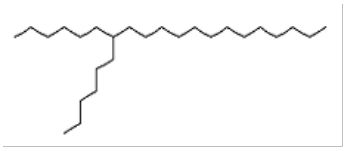


Figure 3: GC-MS chromatogram of lime juice extract of *Ocimum gratissimum*.

15	19.227	Phytol	1.08	$C_{20}H_{40}O$	296	
16	19.331	Octadecanoic acid, methyl ester	1.42	$C_{19}H_{38}O_2$	298	
17	21.669	5-Dimethylaminopyrimidine	1.54	$C_6H_9N_3$	123	
18	23.547	Oleic Acid	6.53	$C_{18}H_{34}O_2$	282	
19	23.757	Glyceryl 1,3-distearate	1.97	$C_{39}H_{76}O_5$	624	
20	26.564	Squalene	0.85	$C_{30}H_{50}$	410	
21	27.148	7-n-Hexyleicosane	0.39	$C_{26}H_{54}$	366	

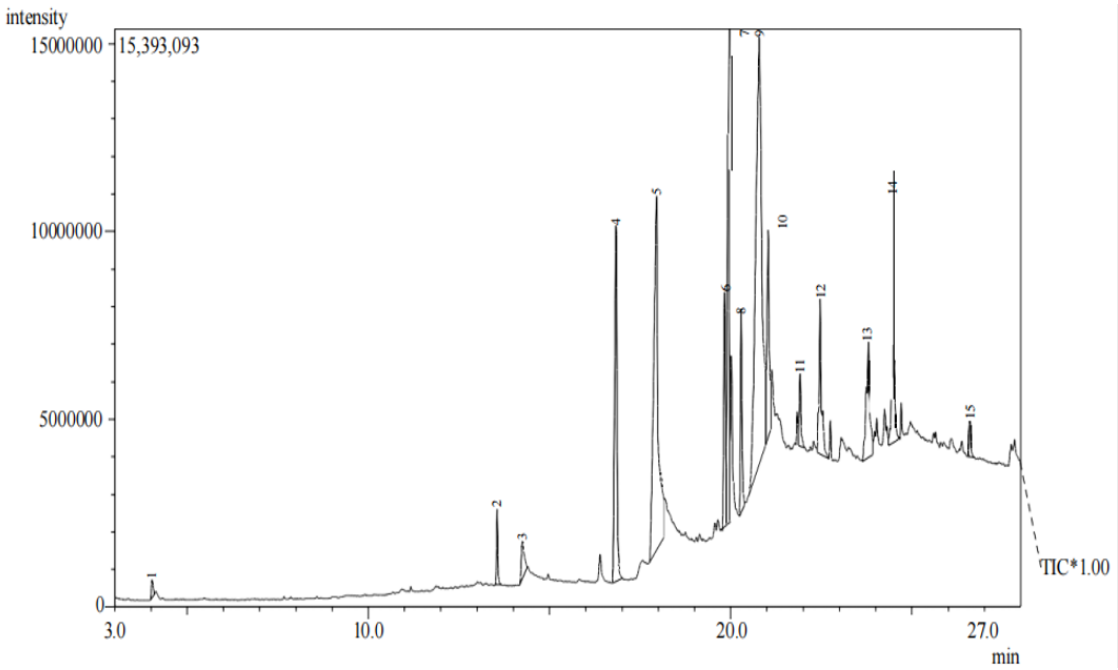


Figure 4: GC-MS chromatogram of NaCl solution extract of *Ocimum gratissimum*.

Table 4: Chemical composition of Aqueous extract of *Ocimum gratissimum*.

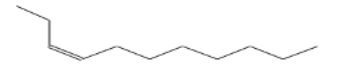
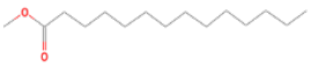
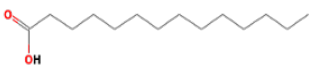
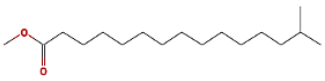
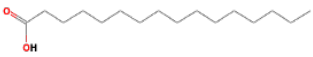
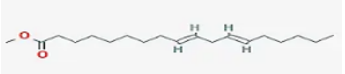
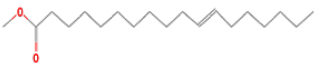
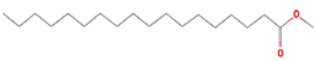
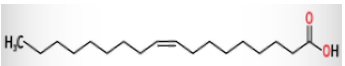
Peak	RT	Name of the compound	Area (%)	Formula	MW	Structure
1	4.056	3-Undecene, (Z)-	0.39	$C_{11}H_{22}$	156	
2	13.549	Methyl tetradecanoate	1.02	$C_{15}H_{30}O_2$	242	
3	14.252	Tetradecanoic acid	1.44	$C_{14}H_{28}O_2$	228	
4	16.822	Pentadecanoic acid, 14-methyl-, methyl ester	8.03	$C_{17}H_{34}O_2$	270	
5	17.955	n-Hexadecanoic acid	16.75	$C_{16}H_{32}O_2$	256	
6	19.853	Linolelaidic acid, methyl ester	4.45	$C_{19}H_{34}O_2$	294	
7	19.954	11-Octadecenoic acid, methyl ester	9.86	$C_{19}H_{36}O_2$	296	
8	20.300	Octadecanoic acid, methyl ester	2.90	$C_{19}H_{38}O_2$	298	
9	20.788	Oleic Acid	34.54	$C_{18}H_{34}O_2$	282	

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Table 4 – continued from previous page

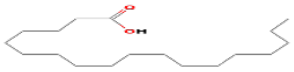
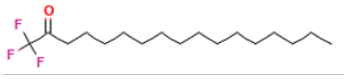
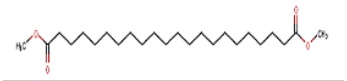
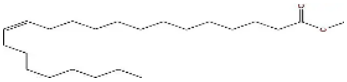
Peak	RT	Name of the compound	Area (%)	Formula	MW	Structure
10	21.027	Octadecanoic acid	7.54	$C_{18}H_{36}O_2$	284	
11	21.907	1,1,1-Trifluoroheptadecen-2-one	1.72	$C_{17}H_{31}F_3O$	308	
12	22.478	Docosanedioic acid, dimethyl ester	3.17	$C_{24}H_{46}O_4$	398	
13	23.92	E-13-Docosenoic acid	3.15	$C_{22}H_{42}O_2$	338	
14	24.454	Methyl(Z)-13 docosenoate	4.49	$C_{23}H_{44}O_2$	352	
15	25.612	Squalene	0.58	$C_{30}H_{50}$	410	

Table 5: Chemical composition of lime juice extract of *Ocimum gratissimum*.

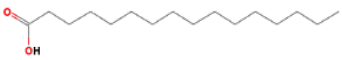
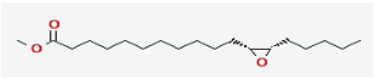
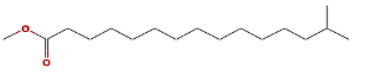
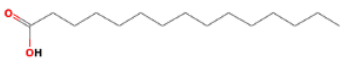
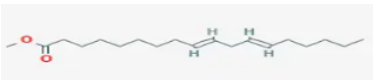
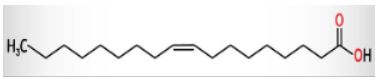
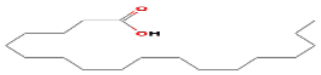
Peak	RT	Name of the compound	Area (%)	Formula	MW	Structure
1	4.025	1-Decene	0.24	$C_{10}H_{20}$	140	
2	13.549	Tridecanoic acid, methyl ester	0.96	$C_{14}H_{28}O_2$	228	
3	14.250	n-Hexadecanoic acid	1.87	$C_{16}H_{32}O_2$	256	
4	16.386	Methyl-11-(3-pentyl-2-oxiranyl) undecanoate	0.51	$C_{19}H_{36}O_3$	312	
5	16.615	Pentadecanoic acid, 14-methyl-, methyl ester	5.81	$C_{17}H_{34}O_2$	270	
6	17.943	Pentadecanoic acid	20.57	$C_{15}H_{30}O_2$	242	
7	19.849	Linolelaidic acid, methyl ester	2.98	$C_{19}H_{34}O_2$	294	
8	19.945	11-Octadecenoic acid, methyl ester	7.56	$C_{19}H_{36}O_2$	296	
9	20.299	Octadecanoic acid, methyl ester	2.26	$C_{19}H_{38}O_2$	298	
10	20.780	Oleic Acid	36.95	$C_{18}H_{34}O_2$	282	
11	21.020	Octadecanoic acid	8.49	$C_{18}H_{36}O_2$	284	

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Table 5 – continued from previous page

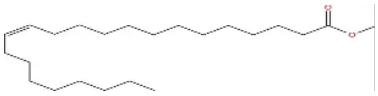
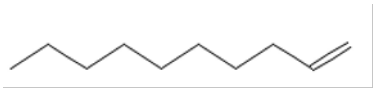
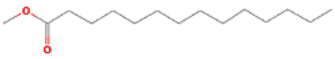
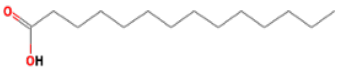
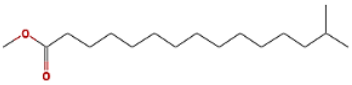
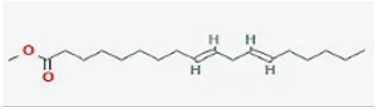
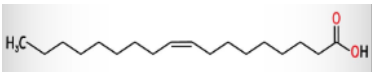
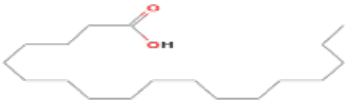
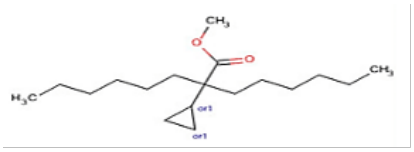
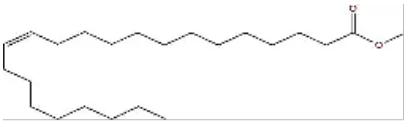
Peak	RT	Name of the compound	Area (%)	Formula	MW	Structure
12	21.906	16-Hexadecanoyl hydrazide	1.79	$C_{16}H_{34}N_2O$	270	
13	22.478	15-Tetracosenoic acid, methyl ester	2.99	$C_{25}H_{48}O_2$	380	
14	23.793	9-Tetradecenal, (Z)-	3.20	$C_{14}H_{26}O$	210	
15	24.475	13-Docosenoic acid, methyl ester, (Z)-	4.10	$C_{23}H_{44}O_2$	352	

Table 6: Chemical composition of NaCl solution extract of *Ocimum gratissimum*.

Peak	RT	Name of the compound	Area (%)	Formula	MW	Structure
1	4.034	1-Decene	0.39	$C_{10}H_{20}$	140	
2	13.552	Methyl tetradecanoate	1.14	$C_{15}H_{30}O_2$	242	
3	14.249	Tetradecanoic acid	1.26	$C_{14}H_{28}O_2$	228	
4	16.827	Pentadecanoic acid, 14-methyl-, methyl ester	8.50	$C_{17}H_{34}O_2$	270	
5	17.953	n-Hexadecanoic acid	18.66	$C_{16}H_{32}O_2$	256	
6	19.585	Linolelaidic acid, methyl ester	4.43	$C_{19}H_{34}O_2$	294	
7	19.859	11-Octadecenoic acid, methyl ester	9.95	$C_{19}H_{36}O_2$	296	
8	20.305	Stearic acid, methyl ester	3.43	$C_{19}H_{38}O_2$	298	
9	20.787	Oleic Acid	29.88	$C_{18}H_{34}O_2$	282	
10	21.026	Octadecanoic acid	5.47	$C_{18}H_{36}O_2$	284	
11	21.910	1-(4-Bromobutyl)-2-piperidinone	1.32	$C_9H_{16}BrNO$	233	

Continued on next page

Table 6 – continued from previous page

Peak	RT	Name of the compound	Area (%)	Formula	MW	Structure
12	22.482	Cyclopropaneoctanoic acid, 2-hexyl-, methyl ester	4.16	$C_{18}H_{34}O_2$	282	
13	3.795	9-Tetradecenal, (Z)-	4.71	$C_{14}H_{26}O$	210	
14	24.459	Methyl (Z)-13 docosenoate	6.07	$C_{23}H_{44}O_2$	352	
15	26.608	Heneicosanoic acid, methyl ester	0.62	$C_{22}H_{44}O_2$	340	

3.3. Antioxidant properties of the extracts

The antioxidant activities of the extracts were analysed using three different methods: Total antioxidant capacity (TAC) method, ABTS (2,2- Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging activity assay and Ferric reducing antioxidant power (FRAP) assay. As shown in Figure 5, the TAC of the EtOHE, AqE, NaClE, and LjE were 26.08 ±0.76 µg/mL, 7.79 ± 0.61 µg/mL, 7.82 ± 0.81 µg/mL and 3.49 ± 0.48 µg/mL, respectively expressed as mg ascorbic acid equivalent/g extract. These results show that the EtOHE exhibited a significantly higher total antioxidant activity compared with other extracts $p < 0.05$. While the LjE has the lowest TAC compared with AqE and the NaClE. The study shows that the total antioxidant capacity of the *Ocimum gratissimum* extracts correlates with the concentrations of phytochemicals found in them. The higher concentrations of phenols, flavonoids, Alkaloids, saponins, and tannins found in the EtOHE, AqE, NaClE, and LjE, respectively, correlate with their antioxidant capacity in the same order. It has been reported that having a higher TAC value positively correlates with greater antioxidant capacity [47, 48].

Figure 6 shows the IC_{50} values obtained from antioxidant analysis using the ABTS method. The EtOHE, AqE, LjE, and NaClE IC_{50} values were 60.39 µg/mL, 73.25 µg/mL, 66.75 µg/mL and 78.52 µg/mL, respectively. Although all the extracts (EtOHE, AqE, LjE and NaClE) demonstrated strong scavenging activities against ABTS radicals, as indicated by the IC_{50} values, EtOHE, having the lowest IC_{50} value, indicates that it has the highest free radical scavenging activity against ABTS radicals compared with AqE, LjE, and NaClE. This also confirms its higher antioxidant capability, which directly correlates with its high flavonoid and phenolic content.

The Ferric reducing antioxidant power (FRAP) assay is used for the determination of the antioxidant activity of a sample based on the ability of antioxidants in the plant extracts to reduce ferric (III) iron to ferrous (II) iron in FRAP reagent, i.e., by inactivating free radicals by electron transfer [49]. The FRAP of the extracts is shown in Figure 6, with EtOHE, AqE, LjE and NaClE having IC_{50} values of 126.77 µg/mL, 117.37 µg/mL, 228.94 µg/mL and 166.83 µg/mL, respectively. The aqueous fraction exhibited the highest antioxidant activity at an IC_{50} of 117.37 µg/mL. Based on the results of the antioxidant activity, it implies that the constituents of the various *Ocimum gratissimum* extracts (EtOHE, AqE, NaClE, and LjE) have the potential to function as antioxidants based on their electron-donating abilities in the ABTS and FRAP assays.

This study revealed that the concentration of phytochemicals and bioactive compounds in *Ocimum gratissimum* was highest in the EtOHE, higher in AqE and NaClE, and high in LjE, respectively. This may be due to the longer period used for the ethanol extraction (72 hours), while the aqueous, NaCl solution, and lime juice extracts were prepared within one (1) hour of steeping. The short period used for the extraction may have resulted in the release of lesser composition of phytochemical/bioactive compounds in the different indigenous extracts (especially the flavonoids and phenolic compounds). The antioxidative potential of an extract is dependent on its level of phytochemical compounds; for instance, higher concentrations of phenolic compounds have been shown to correlate with higher antioxidant properties [49].

This corroborates our findings on the contents of phytochemicals found in the various extracts, which correlate significantly with their antioxidant activities. Furthermore, the higher concentrations of phenols, tannins, steroids, alkaloids, flavonoids, and oxalate found in the EtOHE have contributed to the higher antioxidant activity exhibited by the extract.

Values are means ± SEM of duplicate determinations. Bars with different numbers of asterisks * indicate a statistical difference at $p < 0.05$.

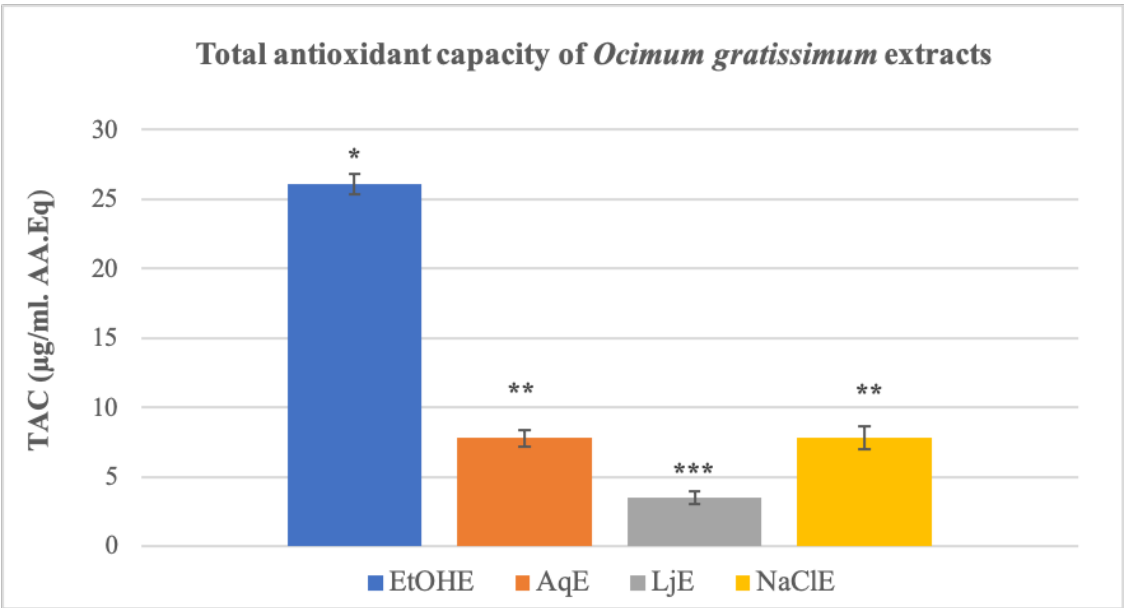


Figure 5: Total antioxidant capacity of the ethanol extract (EtOHE), aqueous extract (AqE), NaCl solution extract (NaClE), and lime juice extract (LjE) of *Ocimum gratissimum*.

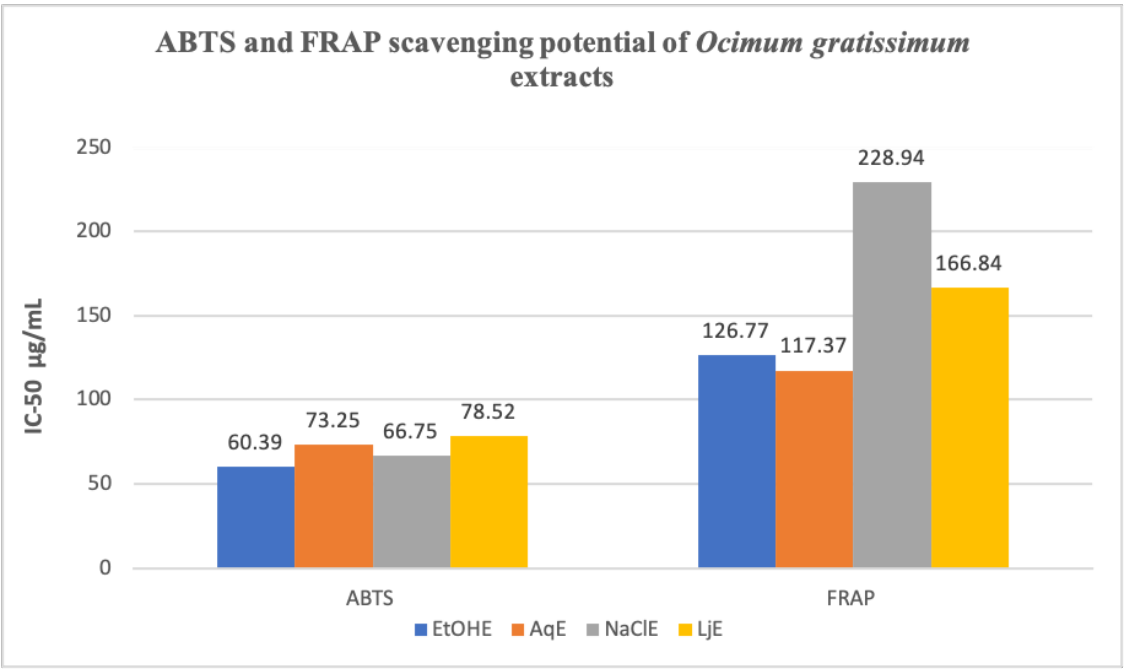


Figure 6: ABTS and FRAP scavenging potential of ethanol extract (EtOHE), aqueous extract (AqE), NaCl solution extract (NaClE) and lime juice extract (LjE) of *Ocimum gratissimum*.

The higher concentrations of phytochemicals found in the ethanol extract and its corresponding greater antioxidant activities is an indication of its efficiency for extraction compared with the indigenous solvents and methods. The reduced antioxidant potentials of the NaClE and LjE, correlate with the lower concentrations of phytochemicals and bioactive compounds in the extracts. The reduction in the phytochemical composition of the NaClE may be a result of the reduced solubility of the organic compounds in solution, which occurs due to the salting-out effect of 2% NaCl on the phytochemicals. The use of 40% Lime juice as an extractant results in a reduction in the biologically active compounds and antioxidant potentials of the LjE of *Ocimum gratissimum*, because of its effect on the solubility of the phytochemical. Lime juice alters the solubility of ions present in a solution. Additionally, the herb-water (50%) ratio of the Aqueous, Lime juice and NaCl solution extract used by many herbal practitioners is too high for optimum extraction of the plant's components, suggesting the need for a modified procedure of extraction.

4. Conclusions

This study investigated the effect of indigenous methods of extraction and extractants on the phytochemical and biological activities of *Ocimum gratissimum* leaves. Our findings showed that the antioxidant potentials of the extracts correlate significantly with the quantity of phytochemicals and bioactive compounds found in them. Extraction using indigenous extractants (Aqueous, 2% NaCl, and 40% Lime juice) for a short period of steeping (1 hour) resulted in a significant decrease in the quantity of phytochemical/bioactive compounds found in the different extracts compared with the ethanol extract. Additionally, the herb-water (50%) ratio of the Aqueous, Lime juice and NaCl extract used by many herbal practitioners is too high for optimum extraction of the plant's bioactive components, suggesting the need for a modified procedure of extraction. In conclusion, our findings revealed that extraction by steeping *Ocimum gratissimum* leaves in Aqueous, 2% NaCl and 40% Lime juice for 1 hour reduced the quantity of phytochemicals, biologically active compounds and antioxidant properties of the extracts when compared with the common ethanol extract. Hence, there is a need to ascertain which extraction methods or extraction solvents are best for the preparation of potent herbal remedies that meet the consumers' safety requirements.

Data availability

In this study, no primary datasets were generated or collected.

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