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Chemical Profiling and Applications of Lemon (C*itrus limon*) and Orange (C*itrus sinensis*) fruit peels Essential oils for flavoring as a means for municipal waste Management

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Abstract

Although the peels of lemon and orange fruits are considered as a waste the essential oil extracted from them is important for food flavoring, pharmaceuticals, cosmetics and detergent production. In this work, essential oils were extracted from fresh fruit peels of lemon (1.5%) and orange (3.4%) by hydro-distillation and their physiochemical properties were recorded. The chemical composition of the essential oils was also analyzed by GC-MS. Lemon fruit peel oil was composed of 12 compounds of which limonene (49.74%) was the main component and similarly orange fruit peel oil contained four compounds with limonene (95.19%) as major ingredient. The major component of the orange fruit peel oil was isolated by silica gel column chromatography and its structure was elucidated by NMR spectroscopy. In the meanwhile, it is possible to use the lemon and orange peels as the source of limonene, an important known bioactive compound. Raw lemon peel is applicable as a flavoring agent for baking bread, whereas the fruit leftovers can be used for compost preparation, and in the course municipal dry waste management can be improved.

Keywords: Lemon/orange peels, volatile oil, GC-MS, flavoring agent, waste management

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Introduction

Citrus (family *Rutaceae*) is one of the most widespread genus comprising 140 genera and 1,300 species; of which *Citrus sinensis* (sweet orange), *Citrus limon* (lemon), *Citrus paradis* (grape fruits), *Citrus medica* (Citron), *Citrus aurantifolia* (lime), *Citrus grandis* (shaddock), *Citrus reticulate* (mandarin) and *Citrus aurantium* (sour orange) are popular and have multiple health benefits (Yerou *et al*, 2017; Sanofer *et al*, 2014; Mohanapriya *et,al*, 2013; Milind *et al*, 2012; Palazzolo *et al*, 2013). These plants grow in tropical and subtropical regions of the world. Orange, mandarin and lemon are the three most important and abundant citrus species in the world (Tongnuanchan *et al*, 2014; M'hiri *et al*, 2015; Janati *et al*,2012; Gattuso *et al*, 2007; Al-Juhaimi *et al*, 2013). The local industries and household consumers produce juices from the pulp of fruits and discard the peel as the byproduct (Jamil *et al*, 2015; Penniston *et al*, 2008).

Chemical Compositions of lemon and orange peels

Chemical compositions of the plants are affected mainly by its parts used, growing conditions, origin and maturity, sample preparation and extraction techniques. The fruits including their peels are rich sources of vitamins, minerals, sugars, carbohydrates, fats, proteins, organic acids, dietary fibers and secondary metabolites like flavonoids, terpenoids, coumarins, alkaloids and essential oils (Jamil *et al*, 2015; Penniston *et al*, 2008; Justin *et al*, 2014; Gotmare *et al*, 2018; Ezejiofor *et al*, 2011; Mamma *et al*, 2008; Javed *et al*, 2014). Citric acid is a known acidic component of citrus fruits causing them sour (Jamil *et al*, 2015; Penniston *et al*, 2008), and it is important in controlling infections and healing wounds by neutralizing harmful free radicals. Reportedly, the peels of citrus fruits contain many biologically active compounds that are extensively applied for flavoring beverages, foods, perfumes, cosmetics, etc (Ezejiofor *et al*, 2011; Mamma *et al*, 2008). Flavonoids are the major constituents of these dry fruits as well as the peels (Gattuso *et al*, 2007).

Essential oils

Essential oils are volatile and odoriferous oily liquid mixtures of polar and nonpolar compounds extracted from different plant parts (Javed *et al*, 2014). The volatile components of the fruit peels (85-99%) contain a mixture of monoterpene, sesquiterpene and their oxygenated derivatives (Javed *et al*, 2014). The common chemical components of lemon peel essential oils are d-limonene, terpinolene, γ-terpinene, geranial, heptanal/octanal, and citral, (Javed *et al*, 2014; Qiao *et al*, 2008; Hojjati *et al*, 2017; Njoku *et al*, 2014). Orange peel essential oils also contained commonly d-limonene, p-cymene, beta-myrcene, geraniol, and linalool (Javed *et al*, 2014; Qiao *et al*, 2008; Hojjati *et al*, 2017; Njoku *et al*, 2014; Wedamulla *et al*, 2022; Trytek *et al*, 2003; Burnham *et al*, 2008).

Limonene (**1**) is the major constituent of lemon and orange peel essential oils as well as other citrus essential oils. Also lemon peel essential oil has some aldehyde molecules (1.3%-1.50%) (Javed *et al*, 2014; Hojjati *et al*, 2017). Compound **1** is a colorless to colored liquid monoterpene at room temperature depending on the source; in some fruits it has strong odour. It takes its name from lemon. It exists as (*R*)-limonene in orange and (*S*)–limonene in lemon. It is insoluble in water but soluble in alcohol and ether which has boiling point of 174^{O}C (Trytek *et al*, 2003; Burnham *et al*, 2008). Peels of lemon and orange are considered as byproducts of juice processing shops and industries which are thrown away as waste material and normally discarded and dumped on the ground and can create environmental concerns (Ezejiofor *et al*, 2011; Mamma *et al*, 2008). The peels can be used as possible sources of compounds that can be applicable for the manufacture of chemicals, flavorings and fragrances, paints, cosmetics, and animal feed supplement instead of their disposal (Ezejiofor *et al*, 2011; Mamma *et al*, 2008; Wedamulla *et al*, 2022; Trytek *et al*, 2003; Burnham *et al*, 2008; Wang *et al*, 2014; Mandina *et al*, 2013; Dhanavade *et al*, 2011; Bendaoud-Boulahlib *et al*, 2017; Oboh et al,2014; Obidi *et al*, 2013; Saeb *et al*, 2016; Abdel–Salam *et al*, 2014; Aburowais *et al*, 2017; Henderson *et al*, 2018). The focus of this study was to identify the potential chemical constituents of the peel essential oils as source of commercial flavoring agents in support of municipal waste management efforts.

Materials and Methods

Collection and Extraction of Plant Materials: Lemon and orange fruits (20 Kg each) were purchased from Debre Berhan town, located 130 Km North East of Addis Ababa. The lemon and orange fruits (10 Kg each) were washed with tap water, and their peel was separated manually. The crushed fresh peel samples (225 g each) were used to isolate an essential oil by hydro-distillation using 1000 mL round bottom flask containing 720 mL of distilled water. After addition of boiling chips the flask was heated slowly for two hours. The organic phase was collected from the two layers of the distillate in the Clevenger apparatus by small bottles (vials) and dried with anhydrous sodium sulfate. The weight of essential oil was measured and the percentage yield of oil was calculated. The peel essential oil of lemon and orange were labeled as 1-5A and 1-5B respectively and stored in refrigerator until use.

Apparatus and Equipment: Grinder, chopper, Buchi rotatory evaporator, Spatula, Clevenger apparatus, refractometry, Gas chromatography-Mass spectroscopy, Electric heater, Digital weighting balance, beakers, Water bath, Measuring cylinders, Separation funnel, Round bottom flask, Dropper, cotton, Oven, density bottle, Covered glass bottles, petri dish, cotton swamp, vortex, autoclave, laminar air flow hood, Avance 400 MHz NMR were used.

Chemicals: Methanol, distilled water, chloroform, conc. hydrochloric acid, conc. sulfuric acid, 10% ferric chloride solution, diethyl ether, anhydrous sodium sulfate, iodine/potassium iodide, glacial acetic acid, carbon tetrachloride, 0.5 and 0.1 N of sodium hydroxide, sodium thiosulfate, 0.5 N of hydrochloric acid, starch solution, phenolphthalein indicator, 15% potassium iodide solution, streptomycin were used.

Determinations of physiochemical properties of essential oils

Solubility in water and ethanol: The solubility of lemon and orange essential oil was checked by adding some amount of water and ethanol in separate test tubes containing few drops of essential oils (Egbuonu *et al*, 2016).

Specific gravity: Clean and dry plastic centrifuge tube was weighed as W0 then it was filled with distilled water and again weighed as W1. Distilled water was removed and the tube was dried then it was filled with essential oil and weighed as W2 (Egbuonu *et al*, 2016). The specific gravity was determined by the following formula.

$$\text{Specific gravity} = \frac{W1-W0}{W2-W0}$$

Refractive index: Abbes refractometry was used to measure refractive index. The apparatus was standardized with distilled water (nD29.5=1.3315). It was then cleaned with acetone and dried with cotton. After cleaning the instrument essential oil was placed between the prisms of the refractometer. The telescope was rotated to bring the borderline of total refraction to the junction of cross-line in the telescope. Refractive index of essential oil was recorded at room temperature (Egbuonu *et al*, 2016).

GC-MS analysis of essential oils from lemon and orange peels

GC-MS analyses were performed at Addis Ababa University, Department of Chemistry. The instrument was fitted with a fused silica capillary column (30 m x 250 μm, i.d. 0.25 μm film thickness). The oven temperature was programmed from 60°C (2 min) to 240°C (20 min.) at 3°C/min. The injector and detector temperatures were 250°C and 280ºC respectively. Oil samples of 0.2 μL were injected into the GC with a split ratio of 1:50. Helium was the carrier gas at a flow rate of 2 mL/min. The MS was obtained with an ionization voltage of 70 eV, ion source at 230°C. MS data were acquired in the scan mode in the m/z range 40-650. Identification was made based on a comparison of their retention indices and MS data compared to those from the NIST 17 and Wiley 229 spectral libraries (National Institute of Standards and Technology) (Qiao *et al*, 2008; Getachew et al, 2019).

Isolation and characterization of limonene from orange essential oil

Use of additional characterization technique for compounds found in the essential oil is important for validation. Therefore, orange essential oil was analyzed with TLC. The retention factor (Rf) was calculated and the components of the sample were visualized using vanillin in sulfuric as spraying reagent. The column (34 mm I.D. × 50mm) was packed with 120 g of silica gel (70-230 mm mesh) slurred with 150mL n-hexane. An essential oil (2 mL) was applied on top of the column and elution was made with gradient of hexane and ethyl acetate where three fractions were collected. The purity of fractions was checked by performing TLC. Fractions of similar Rf were combined (labeled as 1-16A) and it was submitted to Addis Ababa University (Department of Chemistry) for NMR analysis.

Antibacterial activity test by disc diffusion method: Antibacterial activities of lemon and orange essential oils were determined by disc diffusion method against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella thyphi* and *Listeria monocytogene*. Gram-positive bacteria *Staphylococcus aureus* and *Listeria monocytogene* and that of gram-negative bacteria *Salmonella thyphi* and *Escherichia coli* were obtained from Microbiology Laboratory, Department of Biology in Debre-Berhan University (DBU). Antibacterial activity was evaluated in triplicate by disc diffusion method (Kirby-Bauer method) using filter paper disc (6 mm diameter). All bacterial strains were grown separately in sterile Mueller Hinton agar plate for 24 hours at 37°C in incubator. The turbidity of the inoculum was adjusted to 0.5 McFarland standards. The inoculum was spread over Mueller Hinton agar plate (90 mm) using a sterile cotton swab for obtaining a uniform microbial growth. The blank discs placed in to the plate with sterilized forceps and were loaded with 20 µL of essential oils, and streptomycin as control antibiotics and 2% dimethyl sulfoxide (DMSO) as negative control were used. The plates were incubated for 24 h at 37°C and then after the diameters of the growth inhibition zones were measured (Dhanavade *et al*, 2011; Obidi *et al*, 2013; Saeb *et al*, 2016; Abdel–Salam *et al*, 2014; Aburowais *et al*, 2017; Henderson *et al*, 2018; Egbuonu *et al*, 2016).

Demonstration of application of peel essential oils for flavoring: The application of fresh lemon peel in flavoring bread, and the lemon flesh and two peel essential oils for flavoring local *Araqe* (*Katikala*) were evaluated. Powdered lemon peel (5 g, 10 g, and 20 g) was added to the dough (3Kg) before fermentation and baked together. Full and half lemon flesh were tasted for flavoring 100 mL *Araqe*. The essential oils of lemon and orange (0.5 mL, 1mL, and 2mL) were added to clean bottles containing 100 mL of local *Araqe* purchased from the local market, Debre Berhan, separately. The flavor of the baked bread, and *Araqe* formulations were tested by a panel of people periodically starting from the beginning and then after 1 h, 2h stay, three times each (Getachew et al, 2019).

Results and Discussion

Isolation of the lemon and orange peel essential oils: Hydro-distillation of fresh lemon and orange peels afforded the corresponding essential oils. The total proportions of lemon and orange peels in the whole fruits were determined as 14.4% and 17.6%, respectively. The percentage of the essential oils were calculated relative to the mass of fresh peels of both lemon (1.5%) and orange (3.4 %) (Table 1). Orange peel has higher amount of essential oil than that of the lemon, and the percentage yield is closer with that of the literature (3%) (Hojjati *et al*, 2017; Njoku *et al*, 2014).

Table 1. Percentage yield of lemon and orange essential oils (EO).

Plant part	Lemon (g)			Orange (g)		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
Fresh peel	100	100	100	100	100	100
EO	1.6	1.5	1.4	3.4	3.5	3.4
% Yield		1.5%			3.4%	

Physiochemical properties of essential oils

Physio-chemical properties such as color, odour, refractive index, specific gravity, and solubility were determined to evaluate the quality of essential oils, and the experimental values with that of the literature were also compared. Both essential oils were liquids with characteristic pleasant smell at room temperature. Lemon essential oil was yellowish and that of orange was colorless. The solubility of each essential oil found miscible in ethanol and immiscible in water. Comparison of the specific gravity and refractive index of the extracted essential oils with that of literature revealed consistent results (Dias *et al*, 2020) and in turn confirms their equivalent quality.

Table 2. Some physiochemical properties of essential oils

Parameter	Lemon EO	Lit (Ref)	Orange EO	Lit (Ref)
Specific gravity	0.855	0.85	0.846	0.843
Refractive index	1.478	1.480	1.472	1.471

GC-MS analysis of essential oils of lemon and orange peels

The GC-MS analysis data of lemon essential oil (Table 3) showed the presence of 12 components with hydrocarbons (76.56%), monoterpene aldehydes (14.39 %), monoterpene alcohols (2.3%), and sesquiterpenes (4.97%) as major constituents. Compound **1** (49.74%), β-pinene (17.13%), γ-terpinene (7.52%), o-cymene (2.17%), β-bisabolene (2.4 %), β-caryophyllene (1.45%), trans α-bergamotene (1.12%), carbonyls citral (14.39%), heptanal (0.77%), 2-undecanone (1.02%), and α-terpineol (1.44%) and terpinen-4-ol (0.86%) were detected. Terpenes particularly monoterpenes were dominant components in the lemon essential oils. Three compounds namely 1 (95.19), beta-myrcene (1.09) and 3-carene (0.83) were detected in orange peel essential oil (99.99%) categorized as monoterpenes (97.11%) (Table 3). The common component of both essential oils found limonene as described (32%-98%) of citrus plants **(**Javed *et al*, 2014; Hojjati *et al*, 2017**)**. The differences in chemical constituents of essential oils were assumed due to their genetic characteristics **(**Javed *et al*, 2014; Qiao *et al*, 200; Hojjati *et al*, 2017; Njoku *et al*, 2014).

Isolation of limonene from orange peel essential oil

Since limonene was the dominant component of orange peel essential oil (95.19%) the peel was considered as potential source of this compound. The compound was isolated from the essential oil (2mL) using small column chromatography by hexane: ethyl acetate as eluting solvent where two of the three fractions showed a common major spot at Rf 0.77 (hexane: ethyl acetate (9:1)) visualized after spraying with vanillin in concentrated sulfuric acid and heating. This combined fraction (1-16A) was submitted, after work up, to NMR analysis (AAU, Department of Chemistry).

Tabele 3. Chemical composition of lemon and orange essential oils

No	Components	Percentage composition in	
		Lemon peel EO (%)	Orange peel EO (%)
1	β- pinene	17.13	-
2	d-limonene	49.74	95.19
3	o-cymene	2.17	-
4	γ- terpinene	7.52	-
5	terpinen-4-o1	0.86	-
6	Heptanal	0.77	-
7	α -terpineol	1.44	-
8	2-undecanone	1.02	-
9	citral	14.39	-
10	trans-α-bergamotene	1.12	-
11	Caryophyllene	1.45	-
12	β-bisaboline	2.4	-
13	β-myrcene	-	1.09
14	n-methyl-1,3-propanediamine	-	2.88
15	3-carene	-	0.83

Characterization of 1-16A (purified compound from orange peel essential oil)

The ^{1}H NMR spectrum of 1-16A (Fig 1) showed eight chemically different proton signals. It indicated two alkene proton signals at δ5.4 (q, *J* =1.6 Hz, 2 Hz) and δ4.7 (s) with an intensity ratio of 1:2 corresponding to secondary and exocyclic olefinic protons, respectively. Two singlet signals at δ1.7 and δ1.6 ppm represented methyl protons bonded to quaternary carbons. Other multiplet signals at δ2.1, 1.9, 1.8 and 1.5 were integrated for seven protons totaling 16H in the molecule. The ^{13}C and DEPT-135 NMR spectrum (Fig 2-3) demonstrated ten carbon signals corresponding to two quaternary (δ150.1, 133.6), two methyl (δ23.4, 20.7), two olefinic proton signals (δ120.6, CH 108.4, CH_2), three methylene carbon (δ30.8, 30.6, 27.9) and one tertiary methine carbon signals (δ41.1). The combination of these

NMR spectal data indicates that molecular formula of the compound was assumed to be $C_{10}H_{16}$ with three unsaturation numbers. The overall data of 1-16A was consistent with the literature data of limonene **1** (Erasto *et al*, 2008; Pétiaud *et al*, 1980) as shown in Table 4. The multiplicities of methylene protons are complex because of continuous flipping of chair conformations of cyclohexene by changing equatorial proton in to axial and axial proton in to equatorial. The axial proton resonates at up field than equatorial proton.

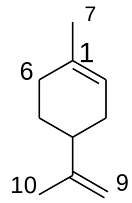

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Table 4. ^{1}H and ^{13}C NMR spectral data of 1-16A compared with reported data **1.**

C no	NMR data of 1-16A		NMR data of 1	
	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR
1	-	133.6	-	133.7
2	5.4	120.6	5.4	120.6
3	2.1/1.9	30.8	2.1/1.9	30.8
4	2.1	41.1	2.1	41.1
5	1.8/1.5	27.9	1.8/1.4	27.9
6	2.1/1.9	30.6	2.1/1.9	30.6
7	1.7	23.4	1.7	23.5
8	-	150.1	-	150.3
9	4.7	108.4	4.7	108.4
10	1.6	20.7	1.6	20.7

The two allylic methylene groups C-3 and C-6 show two very closely resonating signals and their proton signals also overlap and their splitting are not clearly separated. The axial protons appeared around 1.9 ppm and equatorial proton at 2.1 ppm. The axial C-4 appears as multiplet at 2.1 ppm. Limonene is industrially important chemical for synthesis as precursor for other monoterpenes such as perillyl alcohol (Erasto *et al*, 2008; Pétiaud *et al*, 1980; Mahajan *et al*, 2017).

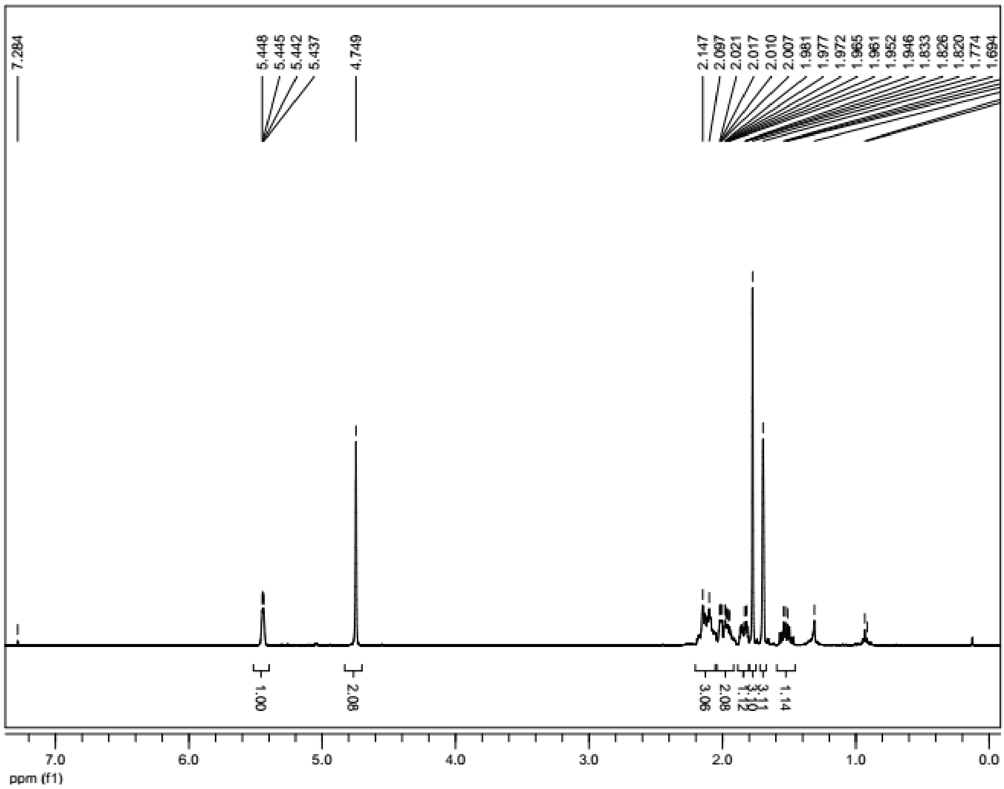


Figure 1. ^{1}H spectra of 1-16A

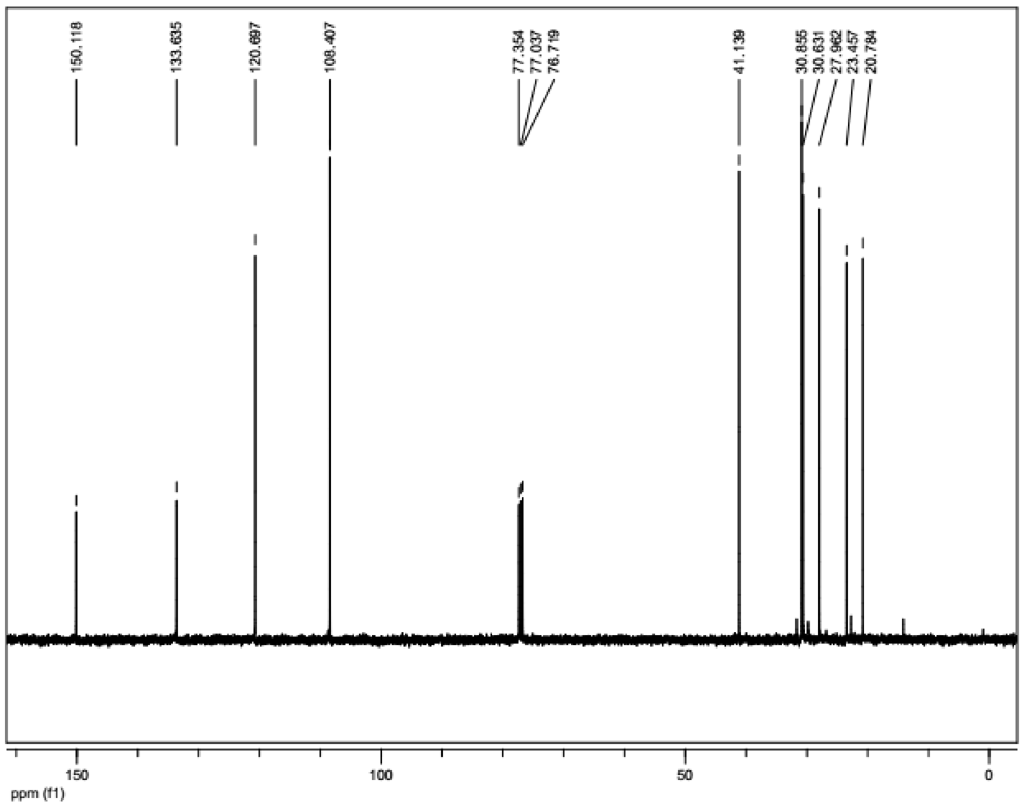


Figure 2. ^{13}C spectra of 1-16A

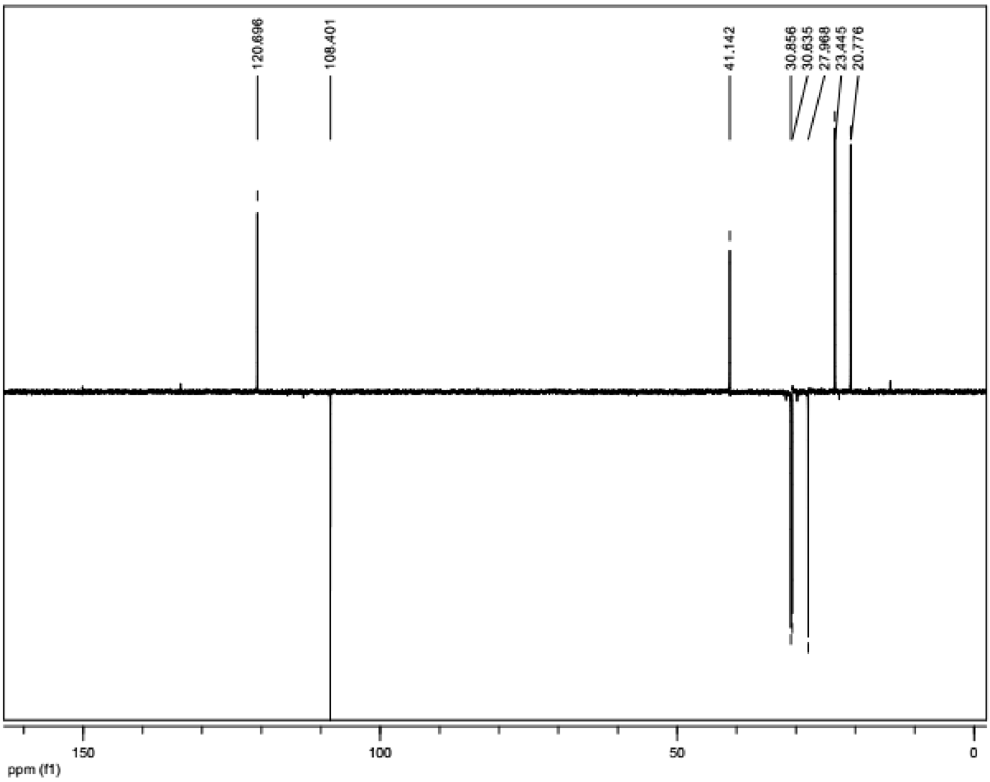


Figure 3. DEPT-135 NMR spectra of 1-16A

Antibacterial activities of lemon and orange essential oils

The experimental results of antibacterial activities of lemon and orange essential oils determined by disc diffusion method against *E. coli*, *S. aureus*, *S. thyphi* and *L. monocytogene* with streptomycin as standard antibiotic and DMSO as negative control are presented in Table 5, and Figures 4-6. The lemon peel essential oil was found better in inhibiting the growth of the test bacteria than that of orange peel where maximum and minimum inhibition zones were observed against *E. coli* (23.2 mm) and *L. monocytogene* (16.3 mm), respectively, even comparable with the standard. Orange peel essential oil showed higher antibacterial activity in the control of *L. monocytogene* (16.2mm) and *S. thypi* (13.3mm) and none against *S. aureus*.

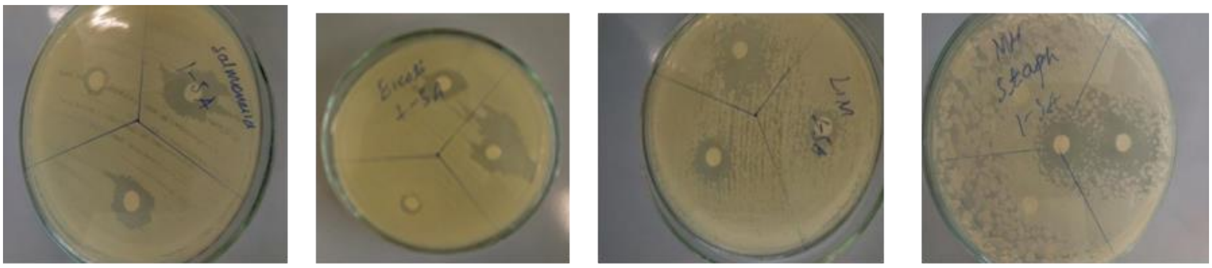
Table 5. Antibacterial activity of lemon and orange essential oils

Microorganism	Diameter of Zone of Inhibition (mm)			
	Lemon EO	Orange EO	Solvent	Streptomycin
Escherichia coli	23.2	9.5	0	25.6
Salmonella.typhi	18.3	13.3	0	26.6
Listeria monocytogene	16.3	16.2	0	23.6
Staphylococcus aureus	20.0	0	0	22.0

The results obtained in this study suggested that lemon essential oil has promising antibacterial activity compared to that of orange both in Gram positive and Gram negative bacteria. An important characteristic of such essential oil is its hydrophobicity/lipophilicity, which enables it to divide the lipids of bacterial cell membrane and mitochondrial membrane, thereby disturbing the cell structure and making it more permeable. Extensive leakage from bacterial cells or the exit of critical molecules and ions would lead to bacterial death. The antimicrobial activities of lemon and orange essential oils are due to presence of compounds that can disturb the cell membrane of the bacteria.

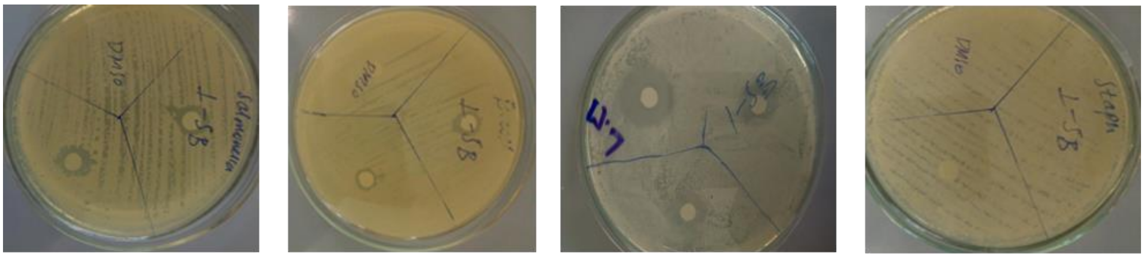
S. thypi *E.coli* *L. monocytogene* *S. aureus*

Figure 4. Antimicrobial activity of standard streptomycin



S. thypi *E.coli* *L. monocytogene* *S. aureus*

Figure 5. Antimicrobial activity of lemon essential oil



S. thypi *E.coli* *L. monocytogene* *S. aureus*

Figure 6. Antimicrobial activity of orange essential oil

Demonstration of applications of peel essential oils for flavoring: The natural products of lemon and orange peels have varieties of applications (Ezejiofor *et al*, 2011; Mamma *et al*, 2008; Giwa *et al*, 2018; Wang *et al*, 2016). The amount of the essential oil used for bioanalysis also determined their potential. The application of fresh lemon peel in flavoring bread, and the two peel essential oils for flavoring local *Araqe* (*katikala*) were evaluated by sensory panels. The bread baked with powdered lemon peel with 10 g was found tasty having eatable flavor than that of 5 g and 20 g. The essential oils were not suitable to flavor *Araqe*. But the flesh of lemon made alcohol drinks more appetizing than its peel because of the esterification reaction with the acid it contains within 1h.

Conclusion and Recommendations

This work was intended to study the chemical compositions of lemon and orange peel essential oils in Ethiopia as a source of flavoring agents and to reduce municipal waste. The percentage yield of peel essential oil from orange (3.4%) was higher than that of lemon (1.5%). The chemical compositions of the respective essential oils, isolated from their peels as identified by GC-MS, were dominated by limonene, which was isolated and characterized by NMR. Therefore, it is possible to use the lemon and orange peels as the source of limonene for different industrial applications. A better inhibitory activity was displayed by lemon peel essential oil against test bacteria. The uses of the lemon peel for flavoring bread, and its flesh for Araqe were demonstrated. These results indicated the potential applications of lemon and orange peels in flavoring foods/drinks. The fruit leftovers can be also used for compost preparation, rather than dumping outside the home in order to minimize the city municipal dry waste.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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