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Evaluation of Antibacterial Potency of *Citrus Limon* (Lemon) Juice Against Some Pathogenic Organisms as Alternative Source of Chemotherapy

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ABSTRACT

The development of antibiotic resistance by pathogenic microorganisms have necessitated the quest for alternative drug therapy. The efficacy and safety of extract from *Citrus limon* for the development of alternative antibacterial drug using the cold-pressing extraction methods and column chromatography to obtain crude juice extracts and fractions respectively while the agar well diffusion and tube dilution methods were used to screen the juice extract and fractions for antibacterial activity against *Salmonella enterica*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae* and *Streptococcus pyogenes.* A total of 277.5mL (12.313%) of juice was obtained from 2253.8g of fruit while the phytochemical analysis revealed the presence of 10 phytocomponents namely tannins, flavonoids, anthraquinones, alkaloids, steroids, phenols, cardiac glycoside, terpenes, resins and saponins. One hundred percent (100%) of the juice crude extract exhibited the highest activity with mean inhibition zones (MIZ) ranged from 25.00±0.57mm to 32.33±0.33mm while 25% (6.67±1.15mm to 10.00±1.00mm) exhibited the least activity. The minimum inhibitory concentration and minimum bactericidal concentration (MIC and MBC) for the juice crude extract ranged from 25% to 12.5% and 100% respectively while *S. paratyphi C, S. typhi*, *K. pneumoniae* and *S. pneumoniae* were still viable at 100%. Out of the three fractions eluted, only one (JEtOAc) was active against all the test organisms with MIZ ranged from 14.00±0.33mm to 22.33±1.20mm while 27 compounds were identified in the fractions by GCMS. Notable group of compounds identified include fatty acids, terpenes, aliphatic and aromatic hydrocarbons. The MIC and MBC for the fractions ranged from 30mg/mL to 15mg/mL and >120mg/mL to 30mg/mL. Based on these findings, it can therefore be concluded that the juice extract and ethyl acetate fraction of *Citrus limon* possess antibacterial activity due to the abundant presence of secondary metabolites and it is recommended that toxicity test be carried out on the crude extract and fraction for the development of alternative drugs for the control and treatment of infections caused by resistant organisms.

***Key Words*: *Citrus limon*, Juice, Antibacterial, Cold pressing, Fractions, Chemotherapy.**

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I. Introduction

Medicinal plants are increasingly gaining acceptance and well acknowledged even among the rural and urban settlements, due to the increasing development of drug resistance, inefficacy of many modern chemotherapeutic drugs used for the control of microbial infections as well as side effects by several antibiotics and the increasing cost of drugs prescription, for the maintenance of personal health [1]. Increases also in human population has made it impossible for modern health facilities to meet health demands all over the world, thus causing more demands on the use of natural herbal health remedies. Medicinal plants are used in traditional health care systems since prehistoric times and are still the most important health care source for the vast majority of the population around the world [2]. [3] reported that an estimate of 70-80% of the world population rely on traditional herbal medicine to meet their primary health care needs. It has been reported that a substantial percentage of modern chemotherapeutic drugs contained one or more of the natural products which are of plant origin. [4] Suck (1989) reported that more than 75 pure compounds derived from higher plants are used in herbal medicine but most of those applied in modern medicine are now produced synthetically. Citrus limon is an evergreen plant native to Asia. It is popularly utilized for its juice, pulp & also the peels. It is used worldwide in cooking dishes because its juice provides a unique sour taste. It is rich in citric acid which gives a pH of 2-3. Lemon is used to make lemonades, cocktails, beverages, etc. It is also a plant of interest because of its medicinal value [5]. It exhibits antimicrobial properties thus, its potentials to inhibit microbial growth should be explored. Since it is easily available and common in use, its extracts

can serve as medicines. Lemon is an important medicinal plant of the family Rutaceae. It is cultivated mainly for its alkaloids, which are having anticancer activities and the antibacterial potentials in crude extracts of different parts (viz., leaves, stem, root, juice and flower) of Lemon against clinically significant bacterial strains has been reported [6]. Citrus flavonoids have a large spectrum of biological activity including antibacterial, antifungal, antidiabetic, anticancer and antiviral activities

[7] [8]. The aim of this study was to evaluate the antibacterial potential of lemon fruit juice crude extracts against some pathogenic bacteria and its toxicological properties using standard routine antibacterial assay techniques.

II. MATERIALS AND METHODS

A Collection, identification and processing of Plant sample

Fruits of *Citrus limon* (Plate I) were collected from Bosso estate Minna, Nigeria and identified/authenticated at the Herbarium Department of the National Institute of Pharmaceutical Research, and Development, Idu, Abuja where voucher specimens were deposited with voucher number: NIPRD/H/6780.

Fig. 1. *Citrus limon*

B Test Organisms.

The test organisms, *Salmonella enteric subs. enterica serotype typhi, Salmonella enteric serotype paratyphi* A, B & C, *Klebsiella pneumoniae, Streptococcus pneumoniae* and *Streptococcus pyogenes* were obtained from stock cultures in the Microbiology laboratory Federal University of Technology, Minna. The microorganisms were reconstituted by sub-culturing onto freshly prepared nutrient agar and then incubated at 37^0C for 24hours, after which their identities were confirmed using gram staining and molecular identification.

III. *GRAM STAINING*

An overnight culture of the test organisms were Gram stained according to the method described by [9], Gram positive and Gram negative organisms were recorded. A control smear of known Gram positive organism (*Staphylococcus aureus*) and a known gram negative organism (*Escherichia coli*) was stained simultaneously to confirm the accuracy of the procedure.

VI. MOLECULAR IDENTIFICATION.

The organism's identity were molecularly authenticated according to Promega Protocol (Technical Manual #TM050) (www.promega.com) and their identities and accession numbers were determined by BLAST (comparison of the extracted GENE sequence with the known sequence from the GENE bank) (*www.ncbi.nlm.nih.gov*).

Extraction of Fruit Juice.

Lemon fruit were washed with distilled water and weighed on a digital weighing balance and the weight was recorded. The peels were removed with newly purchased razor blade, to prevent contamination of the juice with the contents of the peel during extraction. The fruits were massaged gently to release the juice within the pulp and again they were washed with distilled water and then wiped with cotton soaked in 70% alcohol. The juice were extracted by cold pressing method, which was achieved by dividing the fruits into two halves with a sterile laboratory knife after which the juice were squeezed out gently and aseptically. The juice was then filtered through a whatman filter paper with pore size of 20µm. The volume of the juice was measured in a graduated cylinder while the percentage yield was determined by dividing the total volume of the extracted juice by the total original weight of the fruit juice before extraction.

$$Percentage\ Yield\ (v/w) = \frac{Volume\ of\ fruit\ juice\ (ml)}{Weight\ of\ whole\ fruit\ juice\ (g)}\ x100/1 \qquad (1)$$

V DETERMINATION OF THE PH OF *CITRUS LIMON* FRUIT JUICE

The pH (power of hydrogen) of the fruit juice extract of *Citrus limon* was determined using a digital pH meter. The pH meter was first calibrated using buffer 4, 7 and 9 pH, this was achieved by insertion of the pH electrode in the buffer solutions while the calibration control nub was adjusted to the desired pH value. After calibration, the pH of the fruit juice extract was then taking by the insertion of the PH electrode in the sample and the pH values recorded.

VI. PHYTOCHEMICAL SCREENING

Phytochemical analyses was performed using the method as described by [10] to screen the extracts for the presence of the following active principles: alkaloids, tannins, saponins, flavonoids, anthraquinones, cardiac glycosides, volatile oils, terpenoids, resins, steroids and phenol.

Standardization of Inoculums

Zero point two millilitre (0.2ml) of overnight cultures of the test organism was transferred into 20ml of sterile nutrient broth and the culture was incubated for 3 – 5h at 37^0C to standardize the culture to 10^6 CFU/mL McFarland. A loopful of the standardized inoculum was used for the antibacterial assay [11].

VII. PREPARATION OF EXTRACT CONCENTRATION

One hundred percent of the lemon juice (100%) was directly used against the test organisms, while 75%, 50% and 25% were prepared by transferring 7.5, 5, and 2.5mL of the juice crude extract into tubs containing 2.5, 5 and

7.5mL distilled water respectively for the antibacterial susceptibility test.

VIII. ANTIBACTERIAL SUSCEPTIBILITY TEST

The antibacterial activity of the fruit juice extract was carried out using the agar-well diffusion method as described by [12]. Muller-Hinton agar was prepared according to manufacturer instructions and seeded with the standardized test organisms by the spread plate method using a sterile rod spreader to obtain uniform microbial growth. Wells were made in the inoculated media using sterile cork-borer (6 mm diameter) after which a little molten media was used to seal the base of the wells to prevent unwanted spread of the extracts. 100µl each of the prepared juice extract equivalent to the desired concentrations per millilitre was transferred into the wells with a sterile micropipette and it was well labelled, while 100µl of water (free of juice extract) was transferred into wells to serve as the negative control. Ciprofloxacin (1mg/ml) was used as the positive control. This was done by transferring 100µl of the prepared standard antibiotics into the well and the cultures were allowed to stand for 30min after which the seeded plates were incubated at 37°C for 24 hours. The experiment was carried out in triplicate and the mean values with the corresponding standard deviation of the inhibition zone diameters (IZD) were calculated. The performed agar well diffusion susceptibility test was based on the modified methods of the Science Laboratory Standards Institute [13].

IX. THIN LAYER CHROMATOGRAPHY OF CRUDE EXTRACTS

The Analytical thin layer chromatographic technique was done according to the method described by [14] to spot, separate and determine the Rf (Retension factors) values and a suitable solvent systems for fractionation of the phytochemical coponents by column chromatography on the crude extracts. This was achieved by using the TLC silica gel 60 F_{254} Aluminium sheet made by Merck KGaA, Millipore Corporation Germany was used as the stationary phase for the most active *limon* juice. The solevent system that gave the best separation based on the Rf values were used to fractionate the crude extracts by column chromatography, while the number of spots seen was recorded.

X. COLUMN CHROMATOGRAPHY (PARTIAL PURIFICATION) OF CRUDE EXTRACT

The micro scale column chromatographic method according to [15] was used to separate the fractions of the fruit juice extract. The column (40mm diameter width and 150mm length) was prepared by packing it with 150g silica gel (0.015-0.04mm mesh size) desolved in 500ml n-hexane to make a slorry using the wet method. The fractions were collected in test tubes according to their colour development, bulked using thin layer chromatography and the eluting solvents were allowed to vapourise until a constant weight was obtained.

XI. DETERMINATION OF THE MINIMUM INHIBITORY AND BACTERICIDAL CONCENTRATION (MIC AND MBC) OF THE JUICE EXTRACTS

A Serial Dilution of Juice Extracts

The tube dilution method as described by [16] [17] with slight modification using spectrophotometer was used to determine the minimum inhibitory concentration. 100% and 75% as described above was used directly while two fold serial dilutions of the lemon juice were prepared to give a decrease in concentration ranging from 50, 25, 12.5, 6.25, 3.125, 1.563 & 0.78%/ml of lactose broth.

B Determination of MIC And MBC

All the prepared dilutions were properly shaken to obtain a homogenous mixture and they were next inoculated with 100µl of the test organisms appropriately. Positive and negative control tubes were also maintained for each test batch of extract concentrations and test organisms respectively [16] [17]. For the positive control, sterile nutrient broth was inoculated with 100µl of the test organisms without the addition of the extract while for the negative control, the prepared serially diluted juice were incubated without the test organisms. The test tubes were all incubated at 37^0C for 24 h. At the end of the incubation period, the optical density of the cultures in the test tubes were read using spectrophotometer at a wavelength of 600nm while the spectrophotometer was adjusted to zero using sterile lactose broth void of extracts and test organism. The MIC was determined by subtracting the absorbance of the negative control from the absorbance of the test and comparing the result with the absorbance of the positive control (see below formulae). The concentration/test tube where significant reduction in absorbance was observed, was recorded as the MIC.

Absorbance of Test (T) minus absorbance of negative control (C_0) equal to the absorbance of positive control (C_1):

$$T\text{-}C_0 = C_1 \qquad (2)$$

The minimum bactericidal concentration (MBC) was determined by subculturing the cultures with the lowest optical density beginning with the test tube containing the minimum inhibitory concentration and above onto a freshly prepared nutrient agar medium. The cultures were incubated for 24 hours at 37^0C, after incubation, the culture concentration without visible growth was regarded as the minimum bactericidal concentration [16] [17].

XII. QUANTITATIVE ANALYSIS AND IDENTIFICATION OF COMPOUNDS

The determination of the identity of components in the most active fraction (JEtOAc) were done by GC-MS analysis using GC-MS-QP 2010 Plus, Shimadzu system (SHIMADZU, JAPAN) as described by [18]. The gas chromatograph interface to a mass spectrometer (GC-MS) instrument was used while the Column elite-1 was fused with silica capillary column (30m x0.25mm 1D x µL df, composed of 100% dimethyl polysiloxane). An electronic ionization system with ionization energy of 60eV was used for the GC-MS detection while Helium gas (99.99%) was used as the carrier gas at a flow rate of 1ml/min and

injection size of the fraction was 2µl (0.002ml with split ratio of 1:40 and film thickness of 0.20µm). Total GC running time was 28.00minutes. Relative percentages and amount of each components were deduced by comparing individual average peaks area to the total areas. Turbomass was used for the mass spectra and chromatogram while the detection of compounds was done using the database from the library of National Institute of Standard and Technology (NIST) NIST Ver. 2.0 year 2009.

XIII. RESULTS AND DISCUSSION

A. Results

TABLE I: IDENTITY AND ACCESSION NUMBER OF TEST ORGANISMS

Test Organisms	**Gram s reacti on**	**Total Score**	**Identity**	**Accession** number
S.enterica subsp. enterica serovar paratyphi A strain SPA2. (1460bp)	-	2697	100%	*KM977902.1*
S.enterica subsp. enterica serovar paratyphi B strain 374.(1467bp)	-	2676	100%	*JQ694526.1*
S.enterica subsp. enterica serovar paratyphi C strain DT4. (1503bp)	-	2776	100%	*JF951185.1*
S.enterica subsp. enterica serovar typhi strain T4. (1546)	-	2856	100%	*EU118111.1*
Klebsiella pneumoniae strain BYK-9 (1504bp)	-	2778	100%	*KP255917.1*
Sreptococcus pneumoniae strain ATCC 33400. (1515bp)	+	2795	100%	*NR028665.1*
Sreptococcus pyogenes strain JCM 5674. (1480bp).	+	2734	100%	*LC071824.1*

TABLE II: PERCENTAGE YIELD OF JUICE EXTRACTS

weight of fruit **(g)**	**TY mL (%)**
2253.8	277.5(10.08)

Key: WtF = weight of fruit; TY = total yeild

TABLE III: PHYTOCHEMICAL CONSTITUENTS OF CITRUS LIMON JUICE EXTRACTS

	Phytochemical constituent										
Plant Extracts	Flavonoid	Phenols	Alkaloids	Tannins	Steroids	Cardiac glycosides	Saponins	Terpenes	Volatile oil	Anthraquinon Resins
CL J	+	+	+	+	+	+	+	+	-	+

Key: CL J = *Citrus limon* Juice

TABLE IV: MEAN INHIBITION ZONES (MM) AND PH OF CITRUS LIMON JUICE EXTRACT

	Juice extracts **(%)/pH**				Control	
Organisms	25/2.61	50/2.11	75/1.80	100/1.42	Cpx (5µg/ml)	**D** (100µl)
S. paratyphi A	8.67± 1.15^{a}	9.00± 1.00^{a}	16.00 ± 2.00^{b}	31.33 ± 0.33^{d}	23.50 ± 1.50^{c}	NA
S. paratyphi B	1.15^{a}	± 1.00^{b}	± 0.57^{c}	± 0.33^{de}	± 0.88^{d}	
S. paratyphi C	9.33± 0.57^{a}	12.33 ± 0.57^{c}	14.00 ± 1.00^{a}	29.00 ± 0.00^{b}	24.66 ± 1.45^{a}	NA
S. typhi	10.00 ± 1.0^{b}	13.33 ± 0.57^{c}	15.33 ± 0.57^{a}	30.00 ± 0.00^{b}	25.33 ± 0.33^{a}	NA
K. pneumoniae	8.67± 0.57^{ab}	13.67 ± 1.53^{c}	14.33 ± 0.57^{a}	25.00 ± 0.57^{a}	25.66 ± 0.88^{a}	NA
S. pneumoniae	6.67± 1.15^{ab}	11.00 ± 1.00^{b}	14.33 ± 1.53^{a}	30.00 ± 0.00^{b}	25.33 ± 0.88^{a}	NA
S. pyogenes	8.67± 1.53^{b}	13.33 ± 2.08^{c}	14.67 ± 1.15^{a}	29.66 ± 0.33^{b}	30.00 ± 1.00^{b}	NA

Key: Cpx: ciprofloxacin, D: distilled water, NA: no activity, *Specification for Cpx and E are: ≤15 (resistance), 16-20 (intermediate), ≥21 (susceptible) [13]. Values on the same column with different superscript are significantly different ($p<0.05$), n = 3.

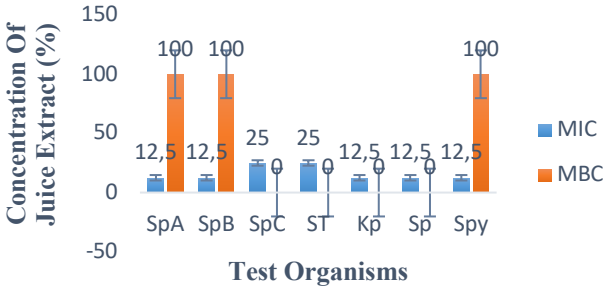


Fig. 2: Minimum inhibitory concentration (MIC) and minimum bactericidal concentrations (MBC) of *Citrus limon* juice extract.

Key: SpA: *Salmonella paratyphi* A, SpB: *Salmonella paratyphi* B, SpC: *Salmonella paratyphi* C, ST: *Salmonella typhi,* Kp: *Klebsiella pneumoniae*, Sp: *Streptococcus pneumoniae*, Spy: *Streptococcus pyogenes.*

TABLE V: SOLVENT SYSTEM, NUMBER OF SPOTS AND RETENTION FACTORS OF *CITRUS LIMON* FRUIT JUICE CRUDE EXTRACT.

Solvent System	**NS**	**DMS (cm)**	**DMF (cm)**	**Rf (cm)**
100% n-hexane	-	5.0	-	-
9:1 hexane : chloroform	-	5.0	-	-
4:1 hexane : chloroform	1	5.0	0.8	0.16
1:1 hexane : chloroform	1	5.0	1.6	0.32
1:4 hexane : chloroform	3	5.0	2.0, 4.4, 4.9	0.40-0.98
1:9 hexane : chloroform	4	5.0	1.2, 1.5, 2.1, 4.9	0.24-0.98
100% chloroform	4	5.0	1.4, 2.0, 4.6, 4.9	0.28-0.98
9:1 chloroform : ethyl acetate	3	5.0	2.9, 3.6, 4.8	0.58-0.96
4:1 chloroform : ethyl acetate	2	5.0	4.2, 4.8	0.84, 0.96
1:1 chloroform : ethyl acetate	1	5.0	4.4	0.88
1:4 chloroform : ethyl acetate	1	5.0	4.6	0.92
1:9 chloroform : ethyl acetate	1	5.0	4.8	0.96
100% ethyl acetate	1	5.0	4.8	0.96
9:1 ethyl acetate : methanol	1	5.0	4.9	0.98
4:1 ethyl acetate : methanol	1	5.0	4.9	0.98
1:1 ethyl acetate : methanol	-	5.0	-	-
1:4 ethyl acetate : methanol	-	5.0	-	-
1:9 ethyl acetate : methanol	-	5.0	-	-

100% methanol	-	5.0	-	-

Key: DMS= distance moved by the solvent (mobile phase), DMF= distance moved by fraction, Rf = Retention factor, NS = Number of spot.

TABLE VI: PERCENTAGE YIELD AND RETENTION FACTOR OF *CITRUS LIMON* FRUIT JUICE FRACTIONS (50ML)

Fraction	**Solvent system &Volume (ml)**	**Description**	**Percentage Yield g (%w/v)**	**NS**	**Rf (cm)**
$JCHCl_3$	100% $CHCl_3$ (500)	Yellow semi solid	1g (2)	1	0.86
JEtOAc	100% EtOAc (700)	Brown semi solid	5g (10)	1	0.76
JH_2O	100% water (400)	White pellet	9g (18)	NA	NA

TABLE VII: MEAN ZONES OF INHIBITION OF *CITRUS LIMON* JUICE FRACTIONS (MM)

Organism	Juice fractions			Control	
	$JCHCL_3$ (40mg /mL)	JEtOAc (40mg /mL)	JH_2O (40mg /mL)	Cpx (1mg /mL)	D (100µL)
S. paratyphi A	0.00[a]	15.00±1.00[a]	0.00[a]	23.50±1.50[a]	
S. paratyphi B	0.00[a]	22.33±1.20[b]	0.00[a]	26.66±0.88[a]	0.00[a]
S. paratyphi C	0.00[a]	17.00±1.00[a]	0.00[a]	24.66±1.45[a]	0.00[a]
S. typhi	0.00[a]	17.00±0.57[a]	0.00[a]	25.33±0.33[a]	0.00[a]
K. pneumoniae	0.00[a]	15.00±0.00[a]	0.00[a]	25.33±0.33[a]	0.00[a]
S. pneumoniae	0.00[a]	14.00±0.33[a]	0.00[a]	25.33±0.88[a]	0.00[a]
S. pyogenes	0.00[a]	16.65±0.63[a]	0.00[a]	30.00±1.00[b]	0.00[a]

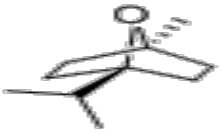
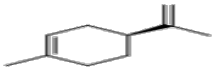
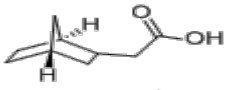
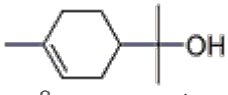
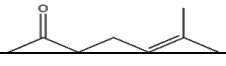
Key: JEtOAc = Juice ethyl acetate fraction, $JCHCL_3$ = Juice chloroform fraction, JH_2OC = Juice aqueous fraction, Cpx = ciprofloxacin, D = dimethyl sulfoxide, Values on the same column with different superscript are significantly different ($p<0.05$), n = 3.

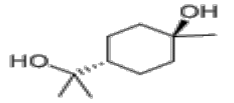
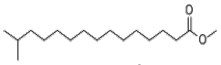
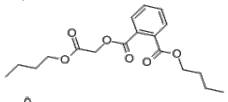
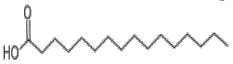
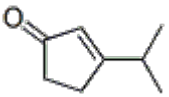
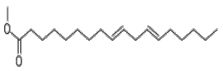
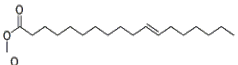
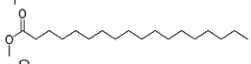
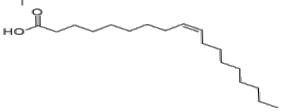
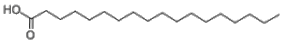
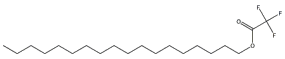
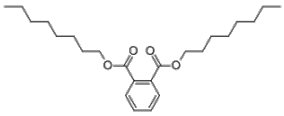
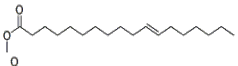
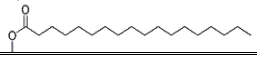
TABLE VIII: MINIMUM INHIBITORY CONCENTRATION (MIC) AND MINIMUM BACTERICIDAL CONCENTRATION (MBC) OF *CITRUS LIMON* JUICE FRACTIONS

Organisms	JEtOAc (mg/mL)	
	MIC	MBC
S. paratyphi A	30	120
S. paratyphi B	15	60
S. paratyphi C	30	>120
S. typhi	30	>120
K. pneumoniae	30	>120
S. pneumoniae	15	120
S. pyogenes	15	60

Key: JEtOAc = Ethyl acetate juice fraction,

TABLE IX: PERCENTAGE COMPOSITION AND STRUCTURE OF PROBABLE COMPOUNDS IDENTIFIED IN *CITRUS LIMON* FRUIT JUICE FRACTION (JETOAC)

Peak No.	RT	PA (%)	MW (g/mol)	M F	Compound Name	Structure
1	4.364	20.45	112.08	$C_5H_4O_3$	2,5-Furandione, dihydro-3-methylene-	
2	4,812	2.41	140.27	$C_{10}H_{20}$	1-Decene	
3	5.234	0.57	154.25	$C_{10}H_{18}O$	7-Oxabicyclo[2.2.1]heptane, 1-methyl-4-(1-methylethyl)-	
4	5.457	1.29	136.23	$C_{10}H_{16}$	Cyclohexene, 1-methyl-4-(1-methylethenyl)-, (S)-	
5	5.659	4.53	112.08	$C_5H_4O_3$	2,5-Furandione, dihydro-3-methylene-	
6	6.319	0.57	94.15	C_7H_{10}	1,3,6-Heptatriene, 2,5,5-trimethyl-	
7	7.267	2.31	154.21	$C_9H_{14}O_2$	2-norbornaneaccetic acid	
8	7.941	0.54	154.25	$C_{10}H_{18}O$	p-menth-1-en-8-ol	
9	8.889	36.61	126.19	$C_8H_{14}O$	2-Hepten-6-one, 2-methyl-	

10	9.690	2.93	172.26	$C_{10}H_{20}O_2$	p-Menthane-1,8-diol	
11	13.036	0.73	196.37	$C_{14}H_{28}$	1-Tetradecene	
12	15.527	0.76	210.4	$C_{15}H_{30}$	1-Pentadecene	
13	17.889	0.57	270.45	$C_{17}H_{34}O_2$	Methyl 14-methylpentadecanoate	
14	18.773	1.23	336.38	$C_{18}H_{24}O_6$	Phthalic acid, butyl ester, ester with butyl glycolate	
15	19.010	5.98	256.42	$C_{16}H_{32}O_2$	n-Hexadecanoic acid	
16	19.248	0.92	224.43	$C_{16}H_{32}$	1-Hexadecene	
17	19.655	1.01	124	$C_8H_{12}O$	3-Isopropyl-2-cyclopenten-1-one	
18	20.911	0.46	294.47	$C_{19}H_{34}O_2$	Linolelaidic acid, methyl ester	
19	21.000	0.91	296	$C_{19}H_{36}O_2$	11-Octadecenoic acid, methyl ester	
20	21.360	0.29	298.5	$C_{19}H_{38}O_2$	Octadecanoic acid, methyl ester	
21	21.818	10.74	282.46	$C_{18}H_{34}O_2$	Oleic Acid	
22	22.081	2.38	284.48	$C_{18}H_{36}O_2$	Octadecanoic acid	
23	22.255	0.64	266.50	$C_{20}H_{37}F_3O_2$	Octadecyl trifluoroacetate	
24	23.485	0.20	310	$C_{20}H_{38}O_2$	Methyl 5-(2-undecylcyclopropyl)pentanoate	
25	24.487	0.32	308.59	$C_{22}H_{44}$	1-Docosene	
26	25.961	0.48	390.56	$C_{24}H_{38}O_4$	Di-n-octyl phthalate	
27	26.331	0.16	396.73	$C_{27}H_{56}O$	1-Heptacosanol	
Total	-	100.00	-	-	-	-
19	21.000	0.91	296	$C_{19}H_{36}O_2$	11-Octadecenoic acid, methyl ester	
20	21.360	0.29	298.5	$C_{19}H_{38}O_2$	Octadecanoic acid, methyl ester	

Key: RT = Retention time; PA = Peak area; MW = Molecular weight; MF = Molecular formula

B. Discussion

The extraction result reveals a considerable percentage of the juice (277.5mL (10.08) from 2257.8g of whole lemon juice. It is reported that extraction yield of medicinal plant has a direct relationship with the extracting solvent and methods employed [19], but in this case the cold pressing method which is a direct method was used and therefore was not affected by any external factor. Of the 11 phytochemical constituents analysed for, 10 namely tannins, flavonoids, anthraquinones, alkaloids, steroids, phenols, cardiac glycoside, terpenes, resins and saponins were present while volatile oil was absent. The qualitative phytochemical analysis method was used which limits the research work to determine only their presence and not their quantity. The presence of most of these constituents has been reported in several research findings and have been linked to antimicrobial potency of several medicinal plants. Tannins are known to be astringent, plants polyphenols that bind to protein, precipitate and shrink them [20]. Flavonoids is a potent antioxidant which increases with increase in hydroxyl groups and reduces in glycosylation [21], this could be responsible for the antibacterial activity of the juice. The antioxidant activity has been reported to be concomitant with the development of reducing power [22]. Tannins also possess the ability to chelate metal ions such as Fe (II) and interact with one of the process in the Fenton reaction and retard oxidation [23]. The presence of these constituents are responsible for the potent antibacterial activity of the extract.

Notable compounds where identified in the fractions of the active extract by GCMS, compounds such as Oleic Acid, Octadecanoic acid, Linolelaidic acid etc. these are groups of lipids which are reported to possessing antimicrobial potency in different capacity. Free fatty acids are reported to having the ability to kill or inhibit the growth of bacteria, owing to the fact that they are used by many microorganisms to defend themselves against parasitic or pathogenic bacteria [24]. The antibacterial properties of fatty acids are well recognized and they are reported to act through different mechanisms to most conventional antibiotics they offer potential for commercial exploitation. They can also be unstable and have the tendency to bind non-specifically to proteins [25]. [26] reported that most free fatty acids possess an exciting potential applications as topical antibacterial medicine used for the prevention and treatment of bacterial diseases. The crude extract was found to be more active than the fractions, therefore it could be standardized for direct use provided it is safe.

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2. **J. U**. **Ewansiha**, S. A. Garba, M. Galadima, S.Y. Daniyan, M.B. Busari, J.H. Doughari, Bioactivity of Chromatographic Fractions from *Eucalyptus citriodora* Leaf against Some Bacterial Pathogens. *International Journal of Clinical Medicine Research*; 4(1): 1-14, 2017.
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