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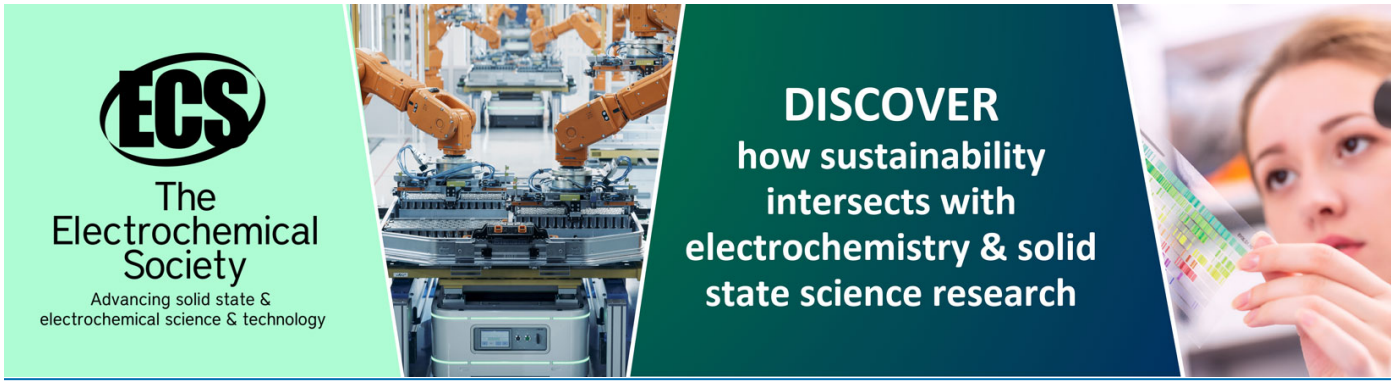

Antimicrobial activity of liquid residues of Cymbopogon citratus oil extracts

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Abstract. Cymbopogon citratus (Lemongrass) is a type of medicinal herb that widely used in Asia. This study aims to investigate the antimicrobial activity of liquid residues of ethanolic and aqueous Cymbopogon citratus oil extracts. Residues of ethanolic and aqueous Cymbopogon citratus oil extracts were used to study their antimicrobial activities for Staphylococcus aureus; Staphylococcus epidermidis; Pseudomonas aeruginosa and Candida albicans which were isolated from burned skin of patients suffering from burns at Medical City Hospital/ Specialist burns Unit/ Baghdad. Minimum inhibitory concentration and minimum bactericidal concentration were measured for all microorganisms using microtitre plates and Resazurin dye by a spectrophotometer at 517 nm. The components of liquid residues were detected by phytochemical tests; and the concentrations of Iodine were determined by Ion Chromatography. The antioxidant activity was measured by using DPPH method. The liquid residues of ethanolic and aqueous extracts of Lemongrass showed antimicrobial effect against all the test microorganisms including Pseudomonas aeruginosa. The ethanolic extraction of Cymbopogon citratus contain Alkaloids, Flavonoids, Glycosides, Phenols, Saponins, Terpenes, Tannins, Fatty acids and Couarins, but no Seroids, volatile oil and Emodins. The Minimum Inhibitory Concentrations of ethanolic extract for Staphylococcus aureus; Staphylococcus epidermidis; Pseudomonas aeruginosa and Candida albicans were 12.5, 12.5, 12.5 and 25µl/mL respectively. The extracts of lemongrass residues due to their phytochemical contents and pharmacological activity seem to be highly effective against some pathogenic microorganisms in the inflamed skin. It can be suggested that these extracts may be used for skin treatment in burned patients infected with pathogenic microorganisms.

Keywords. Cymbopogon citratus, lemongrass, phytochemicals, MIC, Resazurin dye

1. Introduction

Cymbopogon citratus or Lemongrass (LG) is one type of medicinal herbs that is widely used in Asia [1]. It belongs to the family Gramineae and is found worldwide especially in Asia and Africa. It has an odor like lemon; so the word (Lemon) comes from this property, which is mainly due to the presence of citral (a cyclic monoterpene; 3, 7- dimethyl-2, 6- octadienal or lemonal) [2]. It has been used for

respiratory tract infections, malaria and skin infections [3]. Cymbopogon citratus has been reported to have many activities against bacteria and fungi, antioxidant, antiseptic, bactericidal, fungicidal and neuronal properties [4]. Many researchers revealed that LG killed or inhibited some Gram positive bacteria and Gram negative bacteria like Salmonella spp. (Klebsiella spp.; Escherichia coli; Staphylococcus aureus, in addition to Candida albicans (fungi)) [5, 6, 7]. LG oil contains many bioactive compounds such as saponins, tannins, alkaloids, terpenoids, phenols, flavonoids and carbohydrates which are considered as natural compounds in lemongrass, and the concentration of these compounds depending on the geographical area, time and age of plant collection [8]. It was suggested that these chemical compounds produce the antimicrobial activities of Cymbopogon citratus [9]. The acidic pH of Cymbopogon citratus oil has an important role for LG biological effects against many pathogenic microorganisms [10]. Ethanolic extracts of Cymbopogon citratus leaves was shown to produce antibacterial activities against Staphylococcus aureus, presumably due to the presence of flavonoids and tannins. Whereas the Cymbopogon citratus extract from roots showed anti-fungal activity against Candida albicans because of the plant Citral content [11]. In Saudi Arabia, the medicinal herb was used because they have antimicrobial activity. Cymbopogon citrates used against Pseudomonas aeuroginosa and Staphylococcus aureus isolated from patients with urinary tract infections [6]. Previous studies have confirmed the effectiveness of LG oil against many pathogens by using minimum inhibitory concentration (MIC), but there are no studies on the use of LG liquid residues (remnants of after extracting oil from the herb). The aims of this study were to investigate the antimicrobial activity of Cymbopogon citratus liquid residues (aquous and alcoholic extractions) and Cymbopogon citratus oil on Staphylococcus aureus; Staphylococcus epidermidis, Pseudomonas aeruginosa and Candida albicans. The results will be compared with those of Streptomycin, nutrient broth and absolute alcohol as positive and negative controls respectively. In addition, the antioxidant activity of these residues will also be determined.

2. Materials and Methods

2.1. Procurement and Collection of Cymbopogon Citrates

Cymbopogon citratus leaves were collected from mountains of Sulaimania in Kurdstan, Iraq. The herb was classified at the College department of Biology, University of Baghdad.

2.2. Pathogenic Bacteria and Bacteria Isolates

Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa and Candida albicans were isolated from the skin of patients suffering from burns at Medical City Hospital, Specialist burns unit, Baghdad, Iraq.

2.3. Inoculum Preparation of Bacteria and Fungi

Staphylococcus aureus, Staphylococcus epidermidis and Pseudomonas aeruginosa were grown on Mueller Hinton agar (MHA, Difco), while Candida albicans cultured on Sabouraud Dextrose agar (SDA, Difco), aerobically for (18-24) hr at 37˚C, whereas inoculum cell suspension was prepared by using a single colony of each pathogenic microorganism in 10 mL of Mueller Hinton broth (MHB) or Sabouraud Dextrose broth (SDB) at 37˚C for 24 hr. A 1 µL of the microbial suspension was diluted into the ratio of 1:10 (Microorganism: culture medium) to yield inoculum of 107–108 CFU/mL which compared with 0.5 McFarland tube [12].

2.4. Preparation of Aqueous and Alcoholic Cymbopogon Citratus Extracts and LG Oil

1. Aqueous Cymbopogon citratus extract: 500gm of Cymbopogon citratus leaves were dried in the oven at 50 C° for 48 hr.; the dried leaves were then grounded by mortar and pestle to get the fine powder. Then 100gm of dried leaves powder were weighted and 200 mL of distilled water (1 weight: 2 volume) was added. The mixture was left in the container of the shaker of Soxhlet apparatus for 4 hr.; filter paper (Whattman no.2) was then used to separate the mixture under

pressure to collect the liquid extract. The concentration of this extract was considered to be 100% [13], and the liquid extract was stored at 4°C until use (Figure 1).

2. Alcoholic Cymbopogon citratus extract: The same method for aqueous extraction was used to prepare Alcoholic extract but instead of distilled water, 80% Ethanol was used as a solvent [13]. All extracts and residues were stored at 4□C until used.
3. The Cymbopogon citratus oil was collected from aqueous extract procedure. The plant samples were prepared for analysis within 24 hr to prevent loss of Iodin by oxidation.

2.5. Qualitative Detection of Phytochemicals in Residues from Alcoholic and Aqueous Extracts

General reagents for detection of phytochemicals groups

Alkaloids: the test was done according to Mayer's test [13], Wagner's test [13] and Dragendroff's test [14].

a) Mayer's test: 2 mL of liquid residue extract from plant extraction placed in a test tube and 2 drops of Mayer's reagent were added along the side of test tube. The appearance of white creamy precipitate indicates the presence of alkaloids [13].
b) Wagner's test: 2 mL of liquid residue from plant extraction was put in a test tube and 4 drops of Wagner's reagent were added alongside the test tube. A redish – Brown precipitate confirms the test as positive [13].
c) Dragendroff's test: 2 mL of LG liquid residue after extraction of oil from Cymbopogon citratus was added into a test tube and 0.2 mL of diluted hydrochloric acid was added. Then, 1 mL of Dragendroff's reagent [14] was added. An orange brown precipitate was formed and indicating the presence of Alkaloids.
d) Phenolic compounds detection: This was done according to Mace [15]. 2 mL of Cymbopogon citratus liquid residue was placed in a test tube, 4 drops of neutral (5%) Ferric chloride solution was added [15]. A dark blue colour formed indicates the presence of phenolic compounds.
e) Tannins detection: This was done by either 1) 5% Ferric chloride solution [15] where 2 mL of Cymbopogon citratus liquid residue was placed in a test tube and 4 drops of neutral (5%) Ferric chloride solution was added. A dark green colour formed indicating the presence of phenolic compounds. Or 2) Lead acetate test: Equal volume of 1% lead acetate and liquid residue of Cymbopogon citratus were mixed. Yellowish precipitate or creamy gelatinous precipitate indicates the presence of tannins [15].
f) Detection of Saponins: This was done by either a) Foam test: 1 mL of Cymbopogon citratus liquid residue was added to 9 mL of distilled water, and then the mixture was shacked for 15 minute to detect the formation of foam which indicates the presence of Saponins (2 cm layer of foam indicates positive result) [16]. Or b) HgCl2 test: 3 mL of 1% HgCl2 was added to 5 mL of Cymbopogon citratus liquid residue. White precipitate formation indicates the presence of Saponin [17].
g) Tests for Flavonoids detection: This was done according to Raaman [17]. Equal volume of 50% KOH and Cymbopogon citratus liquid residue was mixed. Yellow precipitate indicates the presence of flavonoids [17].
h) Test for steroids and Terpenes: This was done according to Raaman [17] where 1 mL of Cymbopogon citratus liquid residue was mixed with 2 ml of chloroform then one drop of glacial acetic acid was added. A redish brown colour should appear indicating the presence of terpenes. While the appearance of blue ring after leaving the solution for one day indicates the presence of steroids [17].
i) Test for resins: This was done according to Raaman [17]. 10 mL of Cymbopogon citratus liquid residue was mixed with 50 mL of 95% ethanol and then boiled in a water bath for 2 minute; cooled and filtered; 100 mL of 4% HCl was then added. A thickness in solution indicates the presence of resins [17].

j) Test for Coumarins: This was done according to Vimalkumar et al., [18]. 3 mL of 10% NaOH is added to 2 mL of Cymbopogon citratus liquid residue. Yellow colour formation indicates the presence of Coumarins [18].
k) Test for Volatile oils: This was done according to Raaman [17]. 10 mL of Cymbopogon citratus liquid residue was filtered on Whatman no.2 filter paper, left to dry ; and the paper offered to U.V. light. A shiny pink colour appears indicates the presence of Volatile oils [17].
l) Tests for Fatty acids: It was performed according to Raaman [17] by mixing 0.5 mL of Cymbopogon citratus liquid residue with 5 mL of Ether. Then, it was filtered and dried on a filter paper. The appearance of transparency on Whatman no.2 filter paper indicates the presence of fatty acids [17].
m) Test for Glycosides: The test was done either by Fehling test: Equal volumes of Fehling's reagent and Cymbopogon citratus liquid residue were mixed together, then boiled in a water bath for 5 minute. A brick red precipitate of cuprous oxide forms if reducing sugar exists. Positive result gives a red precipitate [17]. The other test by using Keller- Kiliani Test: 2 mL of Cymbopogon citratus liquid residue was mixed with 2 mL of glacial acetic acid. Then, one drop of 5% FeCl3 and one drop of concentrated H2SO4 were added. Reddish–brown colour forms at the junction of the two liquid layers appears confirming the presence of glycosides [17].
n) Emodins: This was done according to Raaman [17]. 2 mL of Ammonium solution was mixed with 3 mL of Benzene onto 1 mL of Cymbopogon citratus liquid residue. A red colour appears indicating the presence of Emodins [17].

2.6. Plant Sample Preparation

The concentrations of Cymbopogon citratus residue of aqueous and alcoholic extracts were (6.25, 12.5, 25, 50, 100) mg/mL, while the oil extract was used in concentrations (15, 20, 25, 30, 100) % diluted in what to study the MIC [19].

2.7. Determination of Mic and Mbc for Cymbopogon Citratus Residues

The MIC of Cymbopogon citratus liquid residue, water and Alcoholic extracts and lemongrass oil were determined by incorporation of various concentrations of extracts. 5 mL of Muller Hinton Broth (MHB) was inoculated with bacteria. All tubes were incubated at 37C° for 24 hr, and then 1.5 X 108 CFU/mL of each species of bacteria was prepared and compared with McFarland tube. A 96 wells microtiter plate was filled with bacteria cultured in Muller Hinton broth (MHB) and the Cymbopogon citratus liquid residue (1:1) volume/volum. 50 µL of each species of bacteria was added to 50 µL of each concentration of residues (ethanolic or aqueous extracts) and oil. The microtiter plates were covered and incubated at 37C° for 24 hr. After incubation, all wells were inoculated with 30 µL of Resazurin dye, the plates were left in the incubator for 2 hr. and the colour produced was read using the spectrophotometer at 517 nm [19].

Streptomycin was chosen as an antibiotic which was used against most microorganisms. It was used to compare their results with residues of water and Alcoholic extracts and lemongrass oil. Streptomycin (1gm) (Sigma) was prepared at concentrations of 200, 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78 and 0.39 mg/mL [20]. The minimum inhibitory concentration was recorded at the well in a row in which there was no change in colour.

2.8. Preparation of Resazurin Dye

Resazurin dye was prepared at concentration 0.015 % by dissolving 0.015gm of Resazurin sodium salt powder (Sigma Aldrich, China) in sterile distilled water, Sterilized by filtration, and stored at 4 C for 2 weeks after preparation [19, 21].

2.9. Separation and Determination of Iodine by Ion Chromatography

This was performed under the ideal Separation conditions previously described [22]. Briefly, the conditions used were as follows:

Column: Ion Pac AS11, AG11 (10 × 4.6 mm I.d) Eluent: 5 mmole Sodium hydroxide

Flow Rate: 2.0 mL/min Inj. Volume: 10 µL Temperature: Ambient

Sample: 10 mg/L of each analyte, standard and sample

Preparation of Sample: Samples were prepared at concentration of 10µg/mL which was dissolved in mobile phase HPLC grade methanol: water, then the mixture was passed through 2.5 µm disposable filters. A 100 µL were injected on the HPLC column. The concentration of each compound was quantitatively determined by comparison of the peak area of the standard with that of samples.

Seq	subjects	Retention time (minute)	Area (µvolt)	Concentration (µg/ml)
1	iodine	4.78	57574950	5

Calculation

$$\text{Concentration of sample } \mu g/mL = \frac{Area\ of\ Sample}{Area\ of\ Standard} \times \text{Conc. of standard} \times \text{Dilution Factor}$$

Instrument: The separation occurred on liquid chromatography Shimadzu 10 AV-LC equipped with binary delivery pump model LC-10A Shimadz, the eluted peak was monitored by UV–Vis 10A-SPD spectrophotometer.

Antioxidant determination:

The procedure was a modification of the methods previously described [23, 24]. Briefly:

1- DPPH reagent (0.1 µmol) was prepared by dissolving 50 mL of 2, 2, diphenyl 1- picryl hydrazyl (DPPH) in 50 mL of absolute ethanol and stored in a dark bottle.

2- Cymbopogon citratus liquid residue at dilutions of 1:25, 1:50, 1:100 and 1:200 each of watery and ethanolic extract were prepared, and ascorbic acid as control. A 100 µL of 0.1 µmol of DPPH reagent was added and left at 37□C for 30 minute. Three replicates were performed of each dilution.

3- The standard solutions were prepared by adding 100µL of ascorbic acid in 0.1 µmol of DPPH to 1 mL methanol for 30 minutes.

4- The O.D. was measured by UV spectrophotometer at 517 nm, and the percentage of antioxidant power was estimated by:

$$\%\ \text{antioxidant activity} = \frac{Abs\ of\ Free\ DPPH - Abs\ of\ Sample}{Abs\ of\ Free\ DPPH} \times 100$$

3. Results and Discussion

Our results represent the first results in Iraq investigating the activity of Cymbopogon citratus liquid residues of aqueous and ethanolic extracts against Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa and Candida albicans (Figure1).

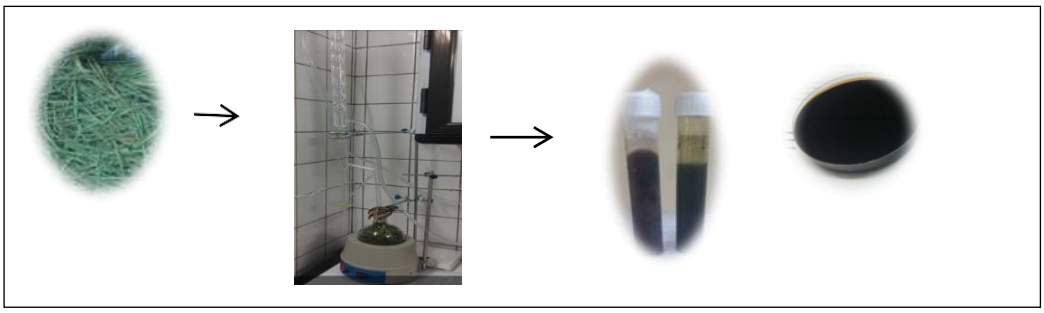


Figure 1. Extraction method by soxhlet to obtain liquid residues of aqueous and ethanolic extractions and Cymbopogon citratus oil

The results of phytochemical compositions of *Cymbopogon citratus* liquid residues of both aqueous and ethanolic extracts are shown in Table 1 and Figure 2. The ethanolic extract of *Cymbopogon citratus* contains Alkaloids, Flavonoids, Glycosides, Phenols, Saponins, Terpenes, Tannins, Fatty acids and Coumarins but no Steroids, Volatile oils and Emodins. According to our knowledge, no previous studies were reported studying the compositions of liquid residues (remainder) of *Cymbopogon citratus* after oil extraction from the plant. Most studies have only focused on the phytochemical compositions of *Cymbopogon citratus* plants and their oils. Asaolu *et al*. in 2009 [25] found that the ethanolic extract of *Cymbopogon citratus* collected from Nigeria contained alkaloids, phenols, tannins, flavonoids and saponins which possess antibacterial activity at concentration of 50mg/mL against *Escerichia coli*, *Listeria monocytogenes*, *Salmonella* typhi and *Staphylococcus aureus*. The water extract of the same plant did not show inhibitory effects on the growth of bacteria mentioned above. Other researchers found that the oil of *Cymbopogon citratus* contained tannins, alkaloids, phenols, limonene, flavonoids and caffeic acid [26].

Our results are compatible with the results of Nwachukwu *et al*. 2008 in Nigeria [13]. They found that the phytochemical studies of aqueous *Cymbopogon citratus* extracts contained tannins; saponins; alkaloids; oils; phenols; steroids and carbohydrates. The extracts showed an inhibitory effect on some microorganisms. Other researchers [27] have shown that the effect of LG oil was effective against many pathogenic microorganisms and concluded that LG oil could be used for the treatment of cases of infected burns.

Test	Aqueous extract	Ethanol extract
Alkaloids test a- Mayer's reagent	+	++
b- Dragendorff reagent	-	+++
c- Wagner's reagent	-	++
Flavonoids (KOH)	++	+++
Glycosides a- Fehling test	+	+++
b- Keller-Kiliani test	-	+++
Phenols	+	++
Saponins HgCl2	+	+++
Foam test	+	+++
Steroids	-	-
Terpenes	++	+++
Fatty acids	-	+
Tannins Lead acetate test	++	+++
Ferric chloride test	+	+++
Coumarins	+	++
Volatile oils	-	-
Emodins	-	-

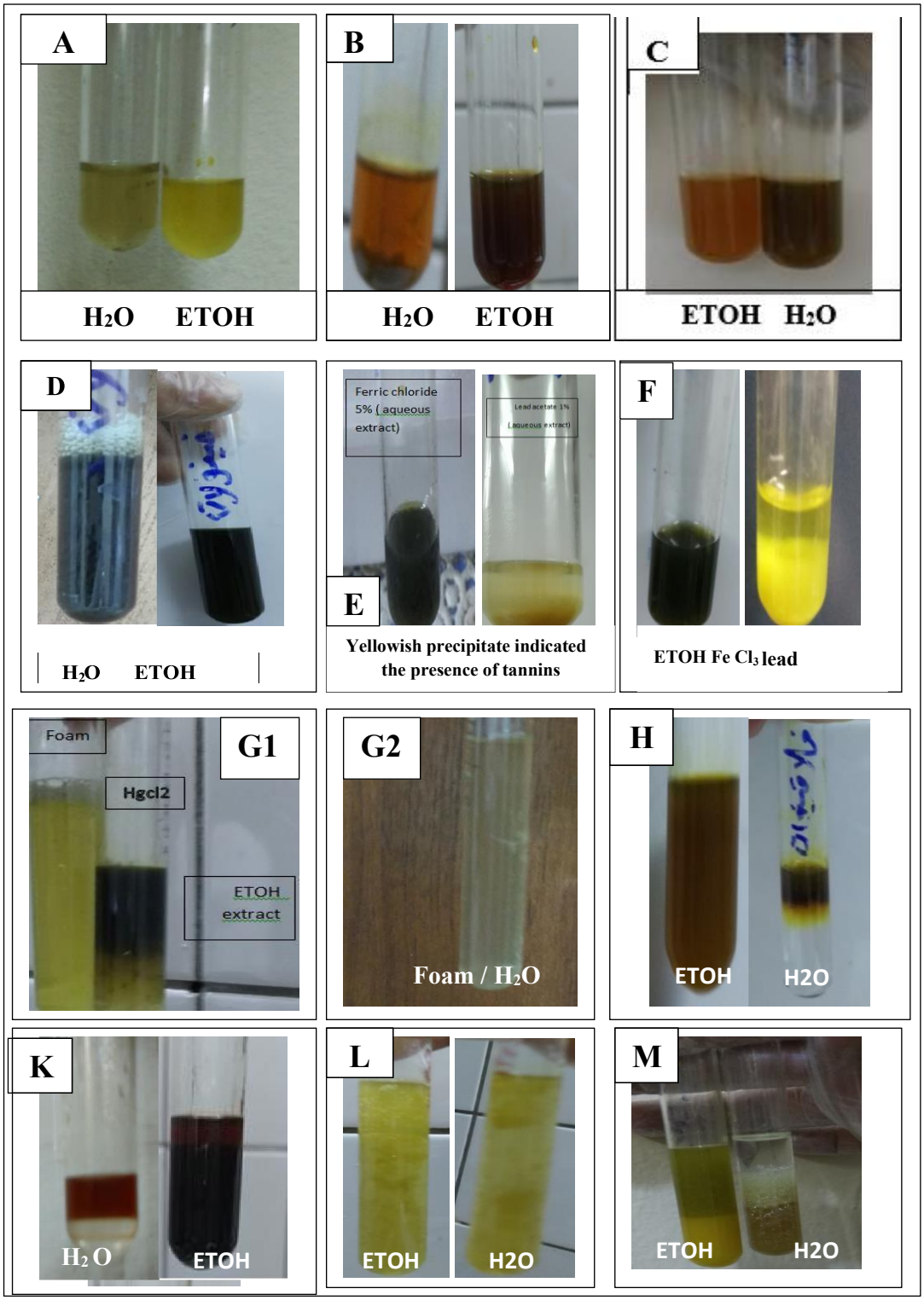
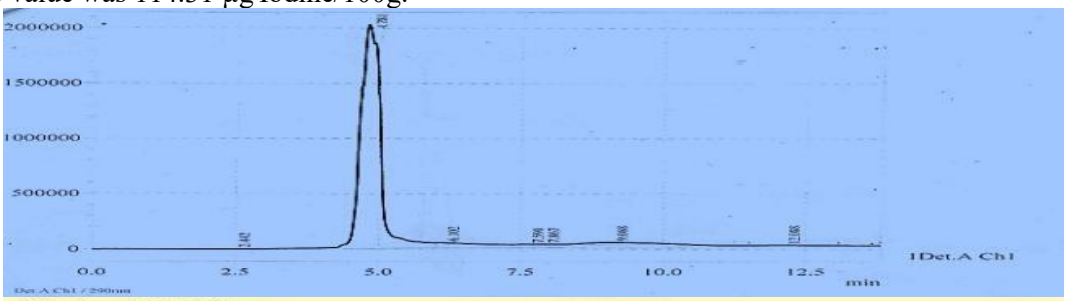


Figure 2. A. Mayer's test B. Wagner's test C. Dragendorff test D. Test for Phenolic compounds E. Lead acetate test F. Lead acetate test G1, 2. Test for Saponins H. Test for Flavonoids K. Steroids and terpenes Tests L. Resins Test M. Emodins

Figure 3 shows iodine standard chromatogram separated on Dionex IonPac™ AS11 and AG11 Columns AS11-AG11 (10 × 4.6 mm I.d). The highest peak was 97.884% at peak No. 2 and the retention time was 4.781 minute. The concentration of Iodine in the residues remaining in Soxhlet extractor after

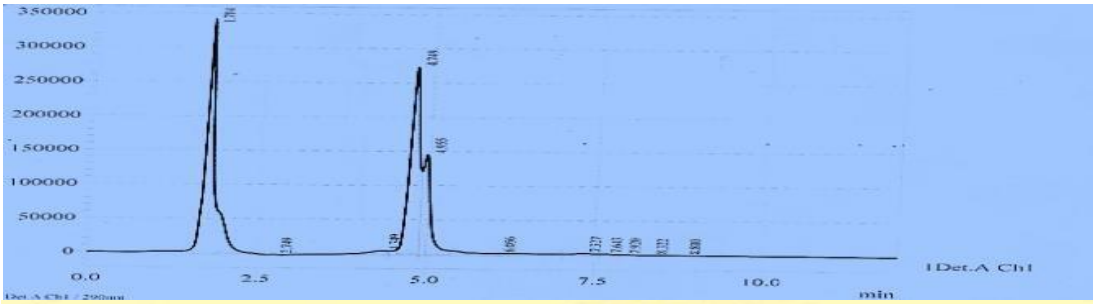
ethanolic extraction of lemongrass oil extraction was calculated from figure 5; it was 2.369µg/mL. Whereas, the concentration of Iodine in residue of aqueous extraction was much less than in the residue of ethanolic extraction; it was 0.2321µg/mL (Fig.4). All previous studies focused on measuring the concentration of Iodine in LG oil. However, there was no data reported the concentration of Iodine in residue of ethanolic extract and aqueous extract in the Soxhlet extractor after lemongrass oil extraction. Dutta *et al*., in 2014 [28] have analyzed the LG oil in India by using GC/MS and they found that the iodine value was 114.31 µg Iodine/100g.



Detector A Ch1 290nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.442	1593	106	0.003	0.005
2	4.781	57574950	2021518	94.929	97.884
3	6.102	17039	1878	0.028	0.091
4	7.590	81617	5267	0.135	0.255
5	7.867	108765	6396	0.179	0.310
6	9.068	2608092	26014	4.300	1.260
7	12.088	258787	4031	0.427	0.195
Total		60650844	2065209	100.000	100.000

Figure 3. Standard Iodine (I) separated on Dionex IonPac™ AS11 and AG11 Columns AS11-AG11 (10 × 4.6 mm I.d). Standard 2 ppm

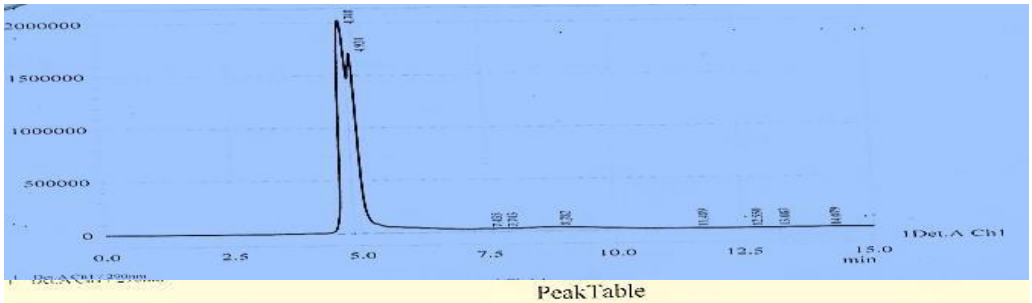


PeakTable

Detector A Ch1 290nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	1.714	3437782	342962	41.588	44.197
2	2.749	1829	257	0.022	0.033
3	4.349	208145	6357	2.518	0.819
4	4.749	2673512	275433	32.342	35.495
5	4.955	1872767	147151	22.656	18.963
6	6.056	17157	375	0.208	0.048
7	7.327	33136	1784	0.401	0.230
8	7.643	13489	1160	0.163	0.150
9	7.920	4280	304	0.052	0.039
10	8.322	1420	128	0.017	0.017
11	8.810	2747	71	0.033	0.009
Total		8266265	775982	100.000	100.000

Figure 4. Concentration of Iodine µg/mL $= \frac{area\ of\ sample}{area\ of\ standard} \times$ conc. of standard $\times$ dilution factor $= \frac{2673512}{57574950} \times 5 \times 1 = 0.2321$ µg/mL of aqueous extraction remainder



PeakTable

Detector A Ch1 290nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.748	25476850	2024145	46.528	53.771
2	4.924	27280215	1708581	49.821	45.388
3	7.435	71648	4539	0.131	0.121
4	7.743	82324	5424	0.150	0.144
5	8.782	1601562	17560	2.925	0.466
6	11.489	10038	271	0.018	0.007
7	12.550	5164	186	0.009	0.005
8	13.083	14009	689	0.026	0.018
9	14.079	214237	2983	0.391	0.079
Total		54756046	3764379	100.000	100.000

Figure 5. Concentration of 1 µg/mL $= \frac{area\ of\ sample}{area\ of\ standard} \times$ conc. of standard $\times$ dilution factor $= \frac{27280215}{57574950} \times 5 \times 1 = 2.369$ µg/ml of ethanolic remainder extraction

In the current study, the antioxidant activity was measured by using DPPH method (free radical scavenging assay). The percentages of antioxidants in the remainder residue of ethanolic extraction of LG after 30 minutes of adding DPPH solution ranged from an average of 22.06 - 94.49% (Table 2 and Figure 6), whereas the liquid residue of aqueous extraction range was from 23.08 – 86.58% (Table 3 and Figure 7). Sepahpour et al., 2018 [29] determined the antioxidant activity in oil extracted from Cymbopogon citratus by using DPPH and found that the highest DPPH values ranged from 13.78-67.83% with Turmeric solvent. Cheel et al., in 2005 [30] revealed that the values ranged from 40% to 68%.

Table 2. Ethanolic extract of lemon grass after half an hour of adding DPPH solution

	Sample concentration (µg/mL)		Absorption results	Percentage of antioxidant	No.	Antioxid. / Abs
1	1/200	A	0.022	94.83	17	98.6% / 0.013
2		B	0.029	93.19		
3		C	0.019	95.53		
4	1/150	A	0.066	84.50	18	98.2% / 0.017
5		B	0.064	84.97		
6		C	0.044	89.67		
7	1/100	A	0.107	74.88	19	83.6% /0.159
8		B	0.111	73.94		
9		C	0.131	69.24		
10	1/50	A	0.262	38.49	20	80.69% /0.187
11		B	0.253	40.61		
12		C	0.255	40.14		
13	1/25	A	0.331	22.30	21	56.60% / 0.421
14		B	0.3111	22.30		
15		C	0.334	21.59		
16	Free DPPH		0.426		22	0.426

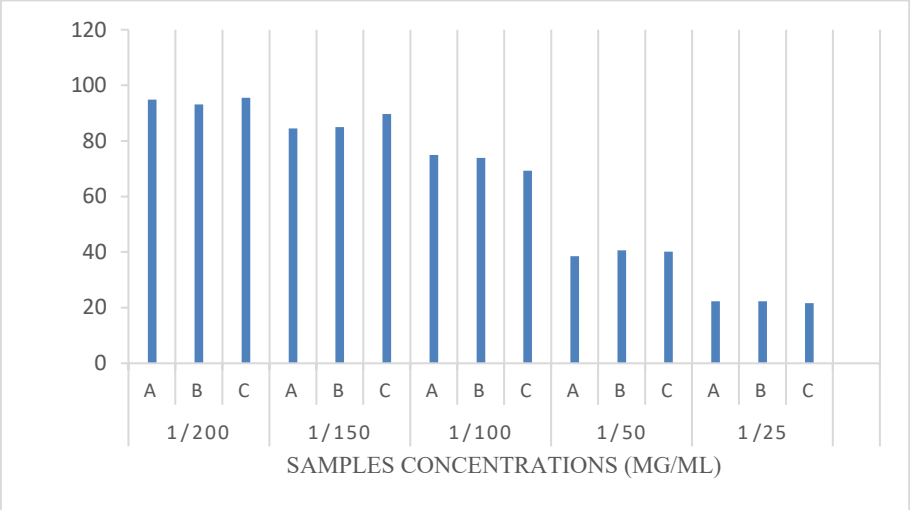


Figure 6. Ethanolic extract of lemon grass after 30 minute of adding DPPH solution

Table 3. Aqueous extract of lemon grass after half an hour of adding DPPH solution

	Sample concentration (µg/ml)		Absorption results	Percentage of anti-oxidant	Antioxid. / Abs
1	1/200	A	0.067	84.42	98.6% / 0.013
2		B	0.064	84.97	
3		C	0.041	90.37	
4	1/150	A	0.107	74.88	98.2% / 0.017
5		B	0.110	73.94	
6		C	0.107	74.88	
7	1/100	A	0.151	64.33	83.6% /0.159
8		B	0.239	66.21	
9		C	0.168	60.35	
10	1/50	A	0.262	38.49	80.69% /0.187
11		B	0.253	40.61	
12		C	0.255	40.14	
13	1/25	A	0.331	22.30	56.60% / 0.421
14		B	0.317	25.58	
15		C	0.335	21.36	
16	Free DPPH		0.426		

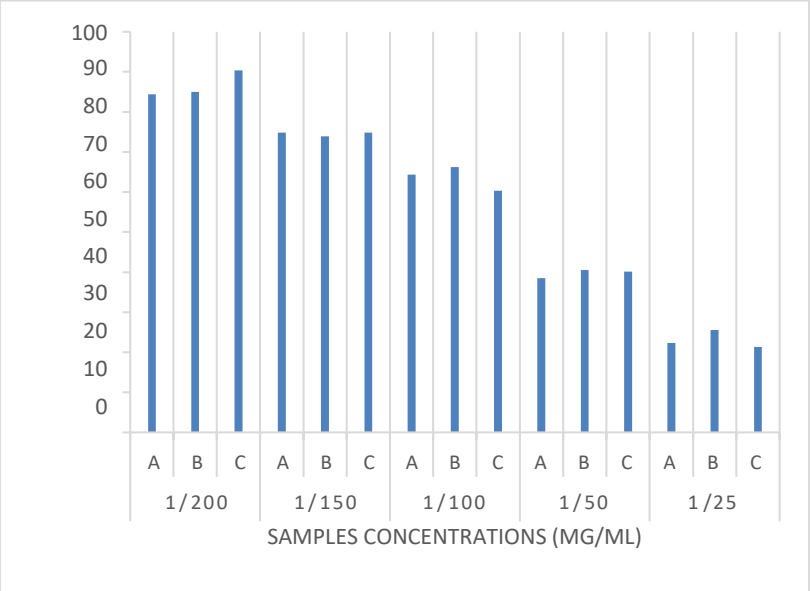


Figure 7. Aqueous extract of lemon grass after 30 minutes of adding DPPH solution

The minimum inhibitory concentration (MIC) can be defined as the lowest concentration of the residue (test compound) of ethanolic or aqueous extracts after extraction LG oil which can inhibit the growth of microorganisms under study. The minimum bacterial concentration (MBC) can be defined as the lowest concentration of the compound under test required to kill the microorganisms during a period of time. The colours in microtiter plates were measured by spectrophotometer at 517 nm. If the colour changes from purple to pink will be recorded as positive. The lowest concentration at which colour changes occurred was regarded as the MIC. From the present study, it could be seen that the residue of ethanolic and aqueous extracts of Cymbopogon citratus exhibits antimicrobial activity against Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa and Candida albicans. However, the residue of ethanolic extract was more potent than the residue of aqueous extract. The MBCs of the liquid residue of ethanolic extract for Candida albicans, Staphylococcus epidermidis, Pseudomonas aeruginosa and Staphylococcus aureus were 6.25, 6.25, 6.25 and 12.5 µl/mL respectively, while the MICs of this ethanolic extract for the same microorganisms were 12.5, 12.5, 12.5 and 25 µl/mL respectively (Fig. 6). The MBCs of residue of aqueous extract for Candida albicans; Staphylococcus epidermidis; Pseudomonas aeruginosa and Staphylococcus aureus were 25, 25, 25 and 25 µl/mL respectively, while The MIC of residue of aqueous extract for the all bacteria studied was 50 µl/mL (Fig.7).

The MBCs of Streptomycin for Candida albicans; Staphylococcus aureus; and Staphylococcus epidermidis were 1.5, 0.7 and 0.7 mg/mL respectively, but there was no effect of Streptomycin on Pseudomonas aeruginosa. The MICs of Streptomycin for the same microorganisms were 3.1, 1.5 and 1.5 mg/mL respectively (Fig.7). From these results, the liquid residue of ethanolic extract of LG oil gave the highest efficacy against microorganisms isolated from patients with burn infections. However, these residues need further analysis to investigate their cytotoxicity in order to have the potential of use, perhaps in the form of ointments to treat burns and decrease the incidence of septicemia.

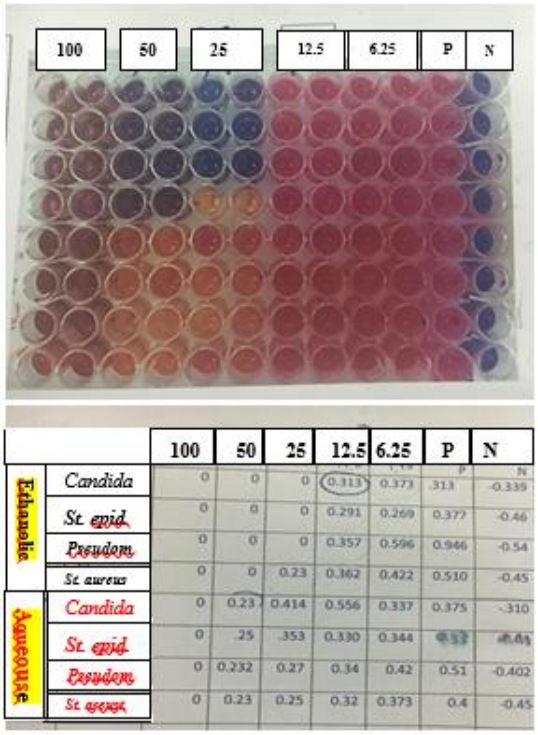


Figure 8. Plates after 2hr in modified Resazurin assay to study the effects of residues of ethanolic and aqueous of lemongrass. Pink colour indicates (growth), blue colour indicates (inhibition of bacterial or Candida growth). The test bacteria were Staphylococcus aureus; Staphylococcus epidermidis; Pseudomonas aeruginosa and Candida albicans; Positive control in well (11), Negative control in well (12); 1–10 wells represent Test extract. The numbers indicate the O.D. of each conc. of residues after ethanolic and aqueous lemongrass oil extraction (100, 50, 25, 12.5 and 6.25) %

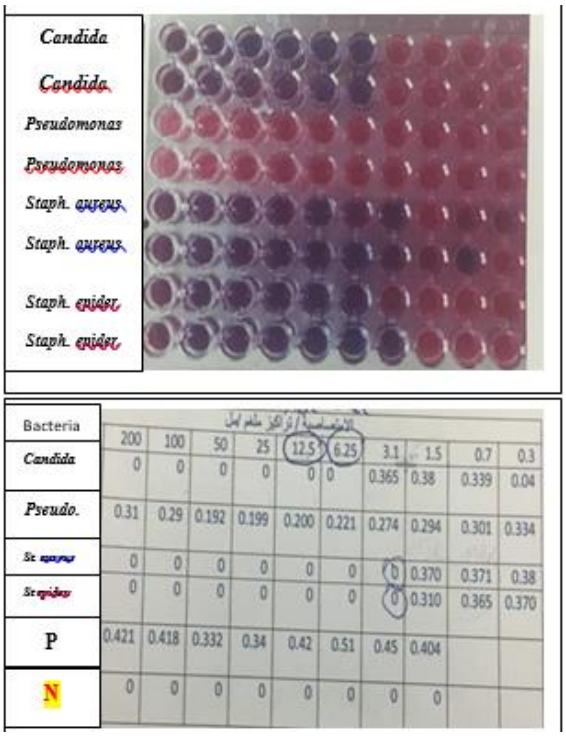


Figure 9. Plates after 2hr in modified Resazurin assay to study the effects of Streptomycin. The Pink colour indicates (growth), blue colour indicates (inhibition of bacterial or Candida growth). The test bacteria were Staphylococcus aureus; Staphylococcus epidermidis; Pseudomonas aeruginosa and Candida albicans; P: Positive control in well (11), N: Negative control in well (12); 1–10 wells represent Test extract. The numbers indicate the O.D. of each conc. Streptomycin (200, 100, 50, 25, 12.5, 6.25, 3.1, 1.5, 0.7 and 0.3) µg/mL

Streptomycin was chosen in the current study due to its role as an antibiotic against pathogenic bacteria and fungi in patients with burn infections reported in several studies in Iraq and other countries. A study in Numan hospital in Baghdad, Iraq reported that 51.75% of *Staphylococcus aureus* isolates isolated from patients with burn infection were sensitive to streptomycin [31]. Al-Hilla Education hospital in Babylon province reported that 55.6% of *Pseudomonas aeruginosa* isolates isolated from burned patients were susceptible to Streptomycin [32]. Al-Musoy [33] found that 43.75% of *Pseudomonas aeruginosa* isolates isolated from burned patients at AL-Diwaniya's teaching hospital in Iraq were sensitive to Streptomycin. Sarker *et al*. [34] have used Resazurin dye as an indicator solution to study the effect of wide spectrum antibiotics on pathogenic bacteria due to the fact that this dye can act as oxidation–reduction indicator to determine the bacterial growth. The colour of Resazurin is blue and becomes pink and fluorescent after reduction to Resorufin in the presence of oxidoreductases enzymes in viable cells and Resorufin may be further reduced to colorless compound called Hydroresorufin. Singh *et al*. [35] studied the sensitivity of many microorganisms to *Cymbopogon citratus* oil by using discs containing 50 µg oil/disc and found that 100% of yeasts, 84.3% *Bacillus spp*., 53.6% *Pseudomonas aeruginosa* and 53.1% of streptococci were sensitive. However, 69.8% of staphylococci were resistant to LG oil. The MIC of *Cymbopogon citratus* oil for sensitive isolates ranged

from 1 to 32 µg/mL and the oil had antimicrobial activity against *Staphylococcus aureus* and *Candida albicans* at 1mg/mL (LG oil killed *Candida albicans* and *Staphylococcus aureus* in 10 minutes). On the other hand, a study published by Al-Jumaily *et al*. 2008 [36] who studied the MIC and MBC of LG oil and their effect on *Staph. Aureus* and found them to be 2.5 µL/mL and 5 µL/mL respectively. The minimum fungi concentration of LG oil to kill *Candida albicans* was 0.6 µL/mL. Ewansiha *et al*. [37] used chloroform extract of LG leaf to determine MIC and MBC for *Staphylococus aureus* and *Caudida albicans* and reported to be 20.0 and 28.0 µg/mL, and 32.0 and 38µg/mL respectively.

The results of this research have shown that all extracts had antimicrobial activities in agreement with the study of AL- Jumaily *et al.* 2007[27]. The samples were collected from children with oral sores containing *Candida albicans*, *Staphylococcus aureus* and *Staphylococcus epidermidis*. Oil of lemongrass extracted by hydrodistillation was used to study their effect on microorganisms isolated from children by agar well diffusion method. At 20% concentration of LG oil, 100% of inhibition was reported for *Candida albicans* and 35.7% for *Staphylococcus aureus*. Other researchers [38] used the LG oil and microtitre plate to study the antimicrobial activities of *Cymbopogon citratus* by using 0.2% Nitrotetrazoleum (NIT). The positive control was gentamycin (Soy peptone broth). The extracts demonstrated antibacterial activity against *Streptococcus thermophillus*, *Lactobacillus plantarum* and *Escherichia coli*. The MICs were 0.0039 - 0.1250 µg/µl. While Ratananikom *et al*. [39] used Resazurin microtitre assay plate method similar to our study; pathogenic bacteria did not show visible growth by using Resazurin stain and the MIC of oil against the bacteria ranged from 62.5 to 500 ppm. Whereas another study [40] which determined the MIC of root and leaf of *Cymbopogon citratus* against many pathogenic bacteria and fungi: *Neisseria gonorrheae, Salmonella* sp., *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Staphylococcus aureus*, *Streptococcus aerugenosa, Escherichia coli*, *Salmonella typhi*, *S. aureus*, *Streptococcus pneumoniae*; *Candida albicans* and *Fusarium oxysporum* .The results showed that aqueous and alcoholic extracts were acidic in nature (pH 3 – 5). The acidity of lemongrass in addition to tannins, alkaloids and flavonoids particularly in methanolic extracts exhibited bioactivity against many microorganisms. Water extracts did not demonstrate activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus*, and the MIC of the extracts ranged from 200 to 2000 µg/mL. The antibiotic, Ofloxacin, as positive control, appeared to have the highest activity against bacteria and fungi. Gram positive microorganisms were more sensitive to *Cymbopogon citratus* oil than Gram negative microorganisms. They used the agar diffusion method and broth dilution method to determine MIC and MBC. The initial and final MIC for *Staphylococcus aureus* were 0.03% and 0.06% respectively. The zone of inhibition increased with the increase in *Cymbopogon citratus* oil concentrations.

Elshikh *et al.* [19] have used a standardized microdilution method combined with Resazurin dye for measuring the minimum inhibitory concentration of biosurfactants through tetracycline replication and gentamicin MIC determination for pathogenic bacteria. Using Resazurin dye considered to be a new method for MIC measurements that overcame any interference with bacterial growth measurements. Our results depending on the colours to determine MIC of *Cymbopogon citratus* oil and their liquid residues after watery and alcoholic extraction showed similar data. In addition, our results agreed with those of Umar *et al*., 2016 in Nigeria [41]. Their results showed that the phytochemicals: flavonoids, carbohydrates, steroids, tannins, Glycosides, phenols and alkaloids were also found in *Cymbopogon citratus* leaves after ethanolic extracts. The ethanolic leaf extract had antibacterial activity at the concentration of 100 mg/mL. The MIC of acetone extract of *Cymbopogon citratus* was 25 mg/mL for *Staphylococcus aureus*. The chemical components of *Cymbopogon citratus* were also analyzed in Nigeria, and analysis revealed that the leaves contained 5.76% of moisture, 5.10% of crude fats, 4.56% of crude proteins and the highest component was 55% Carbohydrates. The ethanolic extracts of

Cymbopogon citratus had alkaloids, saponins, tannins, anthraquinones, steroids, phenols and flavonoids. Agar diffusion technique was used to estimate the antibacterial activities against *Salmonella typhi* at 50 mg/mL of MIC but ethanolic extract was inactive on *Escerichia coli*, *Listeria monocytogenes* and *Staphylococcus aureus*. Thalaj *et al*., 2009 [42] have shown that *Cymbopogon citratus* (West Indiana Type) was investigated by pharmaceutical industries, since it contained volatile oils which presumably increased the activity against many fungal and neural diseases.

Several Medical Health Centres in Asia, Africa and America used *Cymbopogon citratus* oils against pathogenic fungi due to the fact that these pathogens exhibit resistance to antifungal drugs. Al Yousef, 2013[43] analyzed the essential oils of *Cymbopogon citratus* by gas chromatography (GC), gas chromatography/ mass spectrometry (GC-MS) and found it to have high antifungal activities (lowest MIC values with inhibition of growth in the range of 15 to 20 µl/0.4l air space). Therefore, it seems that *Cymbopogon citratus* oil has been used widely in medicine to treat mycotic infections. Other studies [44, 45 and 46] showed that the effect of ethanolic extract of lemongrass on *Staphylococcus aureus* was more than *Klebsiella pneumoniae* and *Escherichia coli* by using agar well diffusion method. In Ghana [47], they used LG oil for skin treatment, and the researchers found that *Staphylococcus aureus* and *Klebsiella pneumoniae* were more sensitive to LG oil than *Pseudomonas aureginosa* and *Candida albicans*.

4. Conclusion

The extracts of lemongrass residues due to their phytochemical contents and pharmacological activity seem to be highly effective against some pathogenic microorganisms isolated from the inflamed skin. It can be suggested that these extracts may be used for skin treatment in burned patients infected with sensitive pathogenic microorganisms.

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