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Balanites aegyptica leave extracts inhibited the growth of some human bacterial pathogens: In vitro study and GCMS analysis

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ABSTRACT

For searching for a solution to eliminate pathogens and get out of the circle of their increasing resistance to common antibiotics. This study tested different genera and species of human pathogenic bacteria for their sensitivity towards the extract of leaves of Balanites aegyptica. Water, methanol, and chloroform were used as solvents for extraction. Standard methods were used for carrying out the susceptibility tests and assessing of MIC. Varied results were shown in this study, and promising activity has been shown against three of the most worldwide problematic bacterial pathogens; Methicillin-resistant Staphylococcus aureus, Klebsiella pneumoniae, and clinical Pseudomonas aeruginosa, especially with the minimum inhibitory concentrations shown of 3.125 mg, 25 mg, and 100 mg/ml, respectively. This study introduces the Balanites aegyptica leaves as a good resource for new promising antibacterial agents.

Keywords: *Balanites aegyptica, Multi-drug resistant bacteria, GC-MS.*

INTRODUCTION

Antibiotics are the primary choice for treating bacterial infections, but misuse of them leads to the fast spread of bacterial resistance. This large spread leads to the emergence of multi-drug resistant bacteria, a problem that has been considered a major worldwide risk factor threat to public health. Researchers pay more focus to the study of phenotypic and genotypic profiles of bacterial resistance, and to the search for new antibiotic substituents of natural sources with fewer side effects and less reliability to be resisted (Pereira et al., 202; Wang & Li, 2023).

The relationship between medicinal plants and man is old and deep, where they have been used for food and healing as old as mankind. Many studies and research proved the importance of medicinal plants as a good resource for good healing agents.

This study focused to search for a wild-growing plant that has a good antibacterial activity to help other searchers for solving and manage the antibiotic bacterial resistant problem (Manoharachary & Nagaraju, 2016; Shakya, 2016; Jain et al., 2020)

Balanites aegyptiaca (L.) Del., is a plant name extracted from the Greek word Acorn, and given as a substituent name to the Arabic words "Heglig", and "Laloub", and also "Desert date".

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It is an evergreen spiny tree belonging to the family Balanitaceae, where the genus Balanites comprises nine species with eleven sub-species (Al-Thobaiti & Abu Zeid, 2018). This tree is described as an arid and semi-arid multibranched one that can grow in different soil types and sand under different moisture conditions and distributed in a wide geographic area, where it is found in Sudan, the Arabian Peninsula, South Israel, Arabia, Africa, India, Iran, and Pakistan (Singh et al., 2017; Ahmed & Alshareef, 2018).

In this study, this plant has been studied for if it has any antibacterial activity against different bacterial genera and to compare its activity with the most commonly used antibiotics.

MATERIAL AND METHODS

Plant material collection and extract preparation

Leaves of Balanites aegyptiaca plant (**Figure 1**) were collected in summer from Al-Kofra city, southeast of Libya, washed off with tap water, air dried, kept in a clean closed container, and stored at room temperature. After that, with the use of Soxhlet apparatus, twenty grams (40gm) of the dried powder of leaves of Balanites aegyptiaca was extracted successively with 250-300ml of each of the Chloroform and Methanol solvents, respectively, then the powder had been dried, and extracted with 300ml distilled water by maceration for 72 hours. The extracts liquids had been filtered, and the solvents were evaporated under reduced pressure with the use of a rotary evaporator. The extracts were weighed and kept at 4 C□ in well-labeled closed containers.

FIGURE 1: Balanites aegyptiaca plant (a), and magnified Balanites aegyptiaca plant showing the used leaves (b).

Preparation of extract stock solution, In order to prepare a stock solution of a concentration of 100mg/ml of each of the three extracts, 0.2gm of the Methanol and water extract was dissolved in 2ml of Methanol and sterile distilled water, respectively, while 0.2gm of the chloroform extract was dissolved in mixture of Petroleum ether and Methanol with a, ratio of 1:2 v/v (Figure 2).

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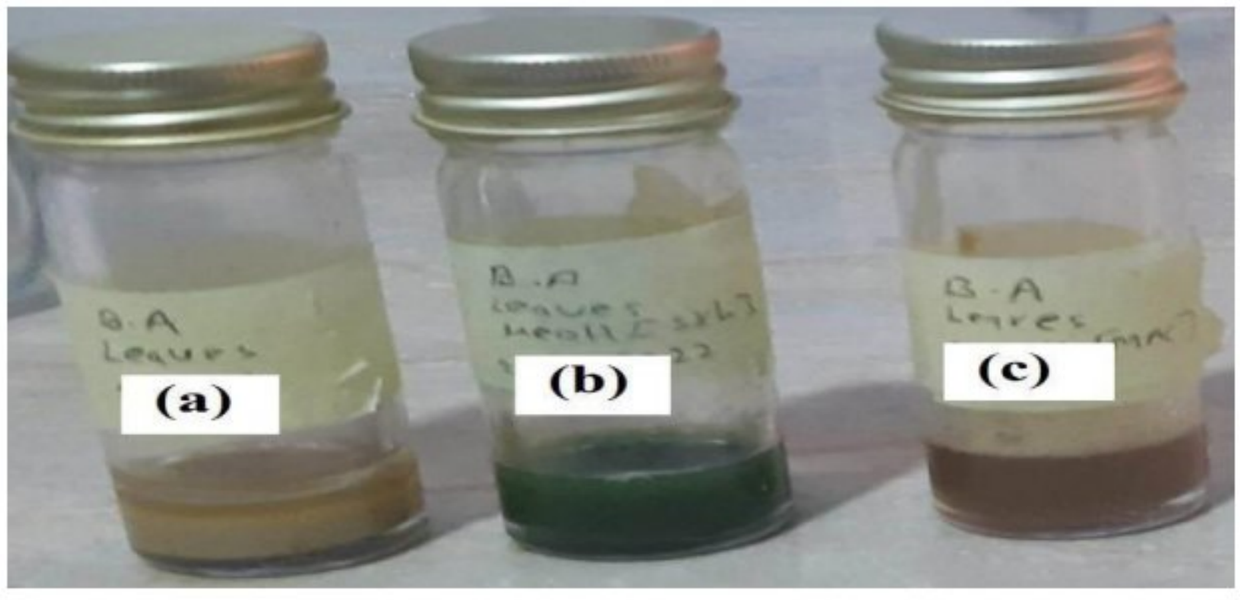

FIGURE 2: Balanites aegyptiaca leaves extraction by three different solvents where (a) chloroform extract, (b) methanol extract, and (c) water extract.

Tested bacteria

Different bacterial genera and species were included in this study; the clinical isolates Staphylococcus aureus, Methicillin-resistant Staphylococcus aureus, E.coli, Acinetobacter baumannii, Klebsiella pneumoniae, clinical P.aeruginosa, Pseudomonas aeruginosa ATCC 27853, and Bacillus cereus NCTC 8236. All bacteria tested in this study were already identified and obtained from the Laboratory of Microbiology at the Department of Biomedical Science, Pharmacy College, Omar Al-Mukhtar University, Al-Bayda City, Libya.

Preparation of bacterial suspension

In this study, a fresh bacterial suspension was concerned to be prepared for each tested microorganism. Enough colonies of overnight Nutrient agar growth culture for each tested bacteria were harvested and washed off with sterile normal saline. In order to get a suspension containing about $1*10^8$ C.F.U/ml, the suspension was adjusted to McFarland 0.5 solution. Standard antibiotics used Sixteen commercial antibiotics discs belonging to different antibiotic classes with different mechanisms of action were used as references in this study; Augmentin 30µg, Cefoxitin 40 µg, Amoxicillin 25 µg, Cefotaxime 30 µg, Meropenem 10 µg, Imipenem 10 µg, Bacitracin 10 µg, Trimethoprim 25µg, Gentamicin 10 µg, Azithromycin 15 µg, Azithromycin 15 µg, Levofloxacin 5 µg, Ciprofloxacin 5 µg, Nalidixic acid 30 µg, Doxycycline 30 µg, Tetracycline 10 µg, and Nitrofurantoin 30 µg. All antibiotics references were bought from Bioanalyse@ YSE Tibbi Malzemeler San.

Antibacterial assay

For the natural product, and according to Magaldi and his team (2004), the agar well diffusion method was used with minor modification, where an inoculum (100 µl) of tested bacterial suspension previously adjusted with 0.5 McFarland solution, was uniformly spread on the surface of a sterile Petri dish seeded with Muller Hinton agar. The plates left for 5 minutes, then 50 µL of the tested extract was added to each of the 2 duplicate wells (7 mm diameter holes cut was made in the agar gel).

The dishes were incubated aerobically for 24 hrs at 37ºC. The standard disc diffusion method was used in this study for assessment of the antibacterial activity of used antibiotic references. Inhibition zones of the bacterial growth were measured in millimeters (mm), and the median was calculated. Andrews's agar dilution method (2006), was adopted in this study to evaluate the minimum inhibitory concentration values.

TABLE 1: Activity of different extracts of Balanites aegyptica and tested standard antibiotics against tested pathogenic bacteria

Tested bacteria	Mean of Minimum Inhibition Zones (mm) of Extracts of *Balanites aegyptica* VS standard antibiotics																	
	Water 100 mg/ml	Methanol 100 mg/ml	Chloroform 100 mg/ml	Augmentin 30µg	Cefoxitin 40 µg	Amoxicillin 25 µg	Cefotaxime 30 µg	Meropenem 10 µg	Imipenem 10 µg	Bacitracin 10µg	Trimethoprim 25µg	Gentamicin 10 µg	Azithromycin 15 µg	Levofloxacin 5 µg	Ciprofloxacin 5 µg	Nalidixic acid 30 µg	Doxycycline 30 µg	Tetracycline 10 µg	Nitrofurantoin 30 µg
Staphylococcus aureus	12.5^{d}	12.5^{d}	17.5^{bcd}	16^{cd}	19^{bcd}	12^{d}	20^{abcd}	17^{bcd}	30^{a}	15^{cd}	22^{abcd}	0^{e}	19^{bcd}	24^{abc}	25^{abc}	13^{d}	27^{ab}	21^{abcd}	15^{cd}
MRSA	17^{bc}	0^{e}	7.5^{cd}	8^{cde}	16^{bcd}	10^{cd}	0^{e}	10^{cd}	20^{ab}	13^{bcd}	10^{cd}	0^{e}	0^{e}	10^{cd}	0^{e}	0^{e}	28^{a}	20^{ab}	22^{ab}
E. coli	0^{f}	19.5^{c}	0^{f}	15^{d}	22^{b}	0^{f}	22^{b}	18^{c}	25^{a}	0^{f}	0^{f}	9^{e}	0^{f}	8^{e}	0^{f}	0^{f}	0^{f}	0^{f}	15^{d}
Acinetobacter baumannii	0^{b}	9^{k}	0^{b}	0^{b}	0^{b}	0^{b}	15^{a}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	7^{ab}	0^{b}	0^{b}	16^{a}	0^{b}	0^{b}
Klebsiella pneumoniae	0^{b}	15^{a}	15^{a}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	10^{a}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	8^{a}	13^{b}	0^{b}
Pseudomonas aeruginosa ATCC 27853	16^{a}	0^{b}	22^{a}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}
Pseudomonas aeruginosa	0^{e}	33.5^{a}	23.5^{c}	0^{e}	0^{e}	0^{e}	19^{c}	33^{a}	25^{ab}	0^{e}	0^{e}	19^{c}	22^{c}	26^{ab}	33^{a}	9^{d}	10^{d}	0^{e}	0^{e}
Bacillus subtilis NCTC 8236	0^{k}	0^{k}	0^{k}	22^{gh}	25^{f}	25^{f}	16^{ij}	33^{bcd}	27^{ef}	12^{j}	30^{d}	20^{h}	23^{g}	31^{cd}	33^{bcd}	14^{ij}	36^{a}	34^{ab}	20^{h}

GC-MS Analysis

The qualitative and quantitative analysis of the sample was carried out by using GC/MS technique model. The analysis was done as the standards protocol of the National Institute of Standards Technology (NIST). Identification of components for the sample was achieved by comparing their retention times and mass fragmentation patents with those available in the library, the National Institute of Standards and Technology.

Statistics

The SPSS package version (20); One-way analysis of variance (ANOVA) followed by the LSD Post Hoc test was used for the statistical analysis, and the probability value was calculated.

RESULTS

In vitro antibacterial potential of three extracts of Balanites aegyptica

The three different extracts of Balanites aegyptica (Figure 2) were tested for their antibacterial activities against the different bacterial genera included in this study, and the results showed that the highest revealed inhibition zones were 33.5 mm, and 23.5mm shown from both methanol and chloroform extracts against the tested clinical P. aeruginosa followed by 22 mm from chloroform extract against standard P. aeruginosa, 19.5 mm revealed from methanol extract against E. coli, and 17.5 mm inhibition zones against both Methicillinresistant S. aureus and community S. aureus, from water and chloroform extract, respectively **(Table 1).**

Sixteen antibiotics of different classes have been tested in this study against the tested bacteria, and the mean of inhibition zone (IZ_m) was calculated, and compared with the antibacterial activity showed by the Balanites aegyptica extracts, and the P-value was evaluated. The results showed varied inhibition zones with significant differences ($P_{value} \leq 0.05$). The water and chloroform extract in this study revealed equal activity with IZ_m of 12.5 mm against tested community S. aureus which is less than the 17.5mm IZ_m shown from the plant chloroform extract, while only water extract

actively affect the tested Methicillin-resistant S. aureus with IZ_m of 17.5mm (Table 1).

Only Methanol extract in this study showed active and weak activity against E. coli, and Acinetobacter baumannii with 19.5 mm, and 9 mm IZ_m, respectively, while both methanol and chloroform extracts proved equal active IZ_m of 15 mm against tested Klebsiella pneumoniae (Table 1).

In addition, this study's results presented that the plant chloroform and water extracts appeared active IZ_m of 22 mm, and 16 mm against standard P. aeruginosa ATCC 27853, respectively, while the tested clinical P. aeruginosa in this study has not been affected by the water extract and actively killed by methanol and chloroform extracts with IZ_m of 33.5 mm, and 23.5mm, respectively (Table 1). Also, this study found that the tested Bacillus cereus has not been affected by the three tested extracts of leaves of Balanites aegyptica plant (Table 1).

When the antibacterial activity of the three tested extracts was compared in this study with that of sixteen tested reference antibiotics, the investigation concluded that both water and Methanol extracts proved activity nearly equal to that of Augmentin, Amoxicillin, Bacitracin, Nalidixic acid, and Nitrofurantoin with inhibition zones ranging from 12 mm – 16 mm against community S. aureus, where the chloroform extract gave higher activity closed to that given by Meropenem and Azithromycin with a range of 17 mm – 19 mm inhibition zones. The highest activity was shown from Imipenem; 30 mm, and Doxycycline; 27 mm (Table 1).

This study presented that the active IZ_m (17.5 mm) revealed against Methicillin-resistant S. aureus from the water leaves extract was near to 16 mm, 20 mm, 20 mm, and 22 mm that shown from Cefoxitin, Imipenem, Tetracycline, and Nitrofurantoin, respectively, and was higher than that shown from other tested ones except for Doxycycline which appeared as the highest one (28 mm) effective against this bacteria.

Among tested, Gram-positive bacteria in this study, non-inhibition zones were revealed from tested plant extracts against tested B. cereus, in time, almost all standard antibiotics showed good antibacterial activities (Table 1).

The tested Gram-negative bacteria investigated in this study were tested for their susceptibility toward the tested plant extracts and standard antibiotics, and the results conclude that the standard-tested P. aeruginosa ATCC 27853 appeared the only one susceptible to the plant extracts compared to tested drugs, since noninhibition zones shown from all of the tested antibiotics.

On the other hand, the tested clinical P. aeruginosa also appeared very susceptible to methanol and chloroform extracts and were inhibited with zones that either more (33.5 mm) to near close (23.5 mm) to that given by Meropenem (33 mm), Ciprofloxacin (30 mm), and 26 mm, 25mm, and 19 mm given by Levofloxacin, Imipenem, and Cefotaxime, respectively. This bacterium appeared more susceptible to methanol and chloroform extracts than other tested antibiotics (Table 1).

Minimum Inhibitory Concentrations determination

The minimum inhibitory concentration (MIC) was tested in this study and the study outputs that the lowest MICs were 3.125 mg/ml, and 25 mg/ml shown from the leaves water extract against Methicillin-resistant S. aureus, and community S. aureus, respectively. Also, the chloroform extract of the tested plant leaves showed a MIC of 25 mg/ml against tested K. pneumoniae, and Standard P. aeruginosa ATCC27853, and revealed MIC of 50 mg/ml against community S. aureus (**Table 2**).

TABLE 2: Minimum Inhibitory Concentrations of ExtractS of Balaites aegyptica

Tested bacteria	MIC of *B. aegyptica* extracts (mg/ml)		
	Water	Methanol	Chloroform
Staphylococcus aureus	25	100	50
Methicillin-resistant *Staphylococcus aureus*	3.125	-	100
Escherichia coli	-	100	-
Acinetobacter baumannii	-	100	-
Klebsiella pneumoniae	-	100	25
Standard *Pseudomonas aeruginosa* ATCC 27853	-	100	25
Clinical Pseudomonas aeruginosa	100	-	100
Bacillus subtilis NCTC 8236	-	-	-

GC-MS analysis

In this study, the analysis of the three extracts of leaves of Balanites aegyptica by GC-MS showed different constituents, where the aqueous extract (water) found contains an aldehyde, five alcohols, three aromatic compounds, three phenolic compounds, one glycoside, one alkaloid, scopolamine, and nine fatty acids as shown in Table 3 and Figure S1.

TABLE 3: GC-MS analysis of Water extract of leaves of Balanites aegyptica

Peak Report TIC

Peak#	Name	R.Time	Area	Area%
1	Urea, 1-methylcyclopropyl-	3.478	4593271	1.54
2	2-Cyclopenten-1-one, 2-hydroxy-	3.542	1847862	0.62
3	2-Furancarboxaldehyde, 5-methyl-	3.951	10181555	3.42
4	1,3-Dioxol-2-one,4,5-dimethyl-	4.795	2977201	1.00
5	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	6.232	7629400	2.56
6	Cyclohexanol, 5-methyl-2-(1-methylethyl)-	6.635	1667554	0.56
7	1-Tridecene	6.786	5362704	1.80
8	Benzofuran, 2,3-dihydro-	7.183	3746345	1.26
9	N'-(Diaminomethylidene)formohydrazide	7.549	7696132	2.58
10	4-Hydroxy-3-methylacetophenone	8.555	6375497	2.14
11	1-Pentadecene	9.462	7031819	2.36
12	Cyclohexylphenylacetic acid	10.167	4413965	1.48
13	3',5'-Dimethoxyacetophenone	11.741	7240811	2.43
14	1-Hexadecanol	11.935	7410900	2.49
15	Ethyl .alpha.-d-glucopyranoside	12.593	7829815	2.63

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16	(E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol	13.735	5157795	1.73
17	1-Nonadecene	14.172	3225467	1.08
18	n-Hexadecanoic acid	15.908	25997783	8.73
19	Hexadecanoic acid, ethyl ester	16.217	5012590	1.68
20	trans-Sinapyl alcohol	16.343	2254147	0.76
21	9,12-Octadecadienoic acid (Z,Z)-	17.588	110256091	37.02
22	trans,trans-9,12-Octadecadienoic acid, propyl ester	17.813	21286295	7.15
23	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	17.883	5361808	1.80
24	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	20.125	2265979	0.76
25	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl este	20.741	2251217	0.76
26	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl est	22.127	8158021	2.74
27	Diosgenin	27.206	11623643	3.90
28	.gamma.-Sitosterol	27.467	8951147	3.01
			297806814	100.00

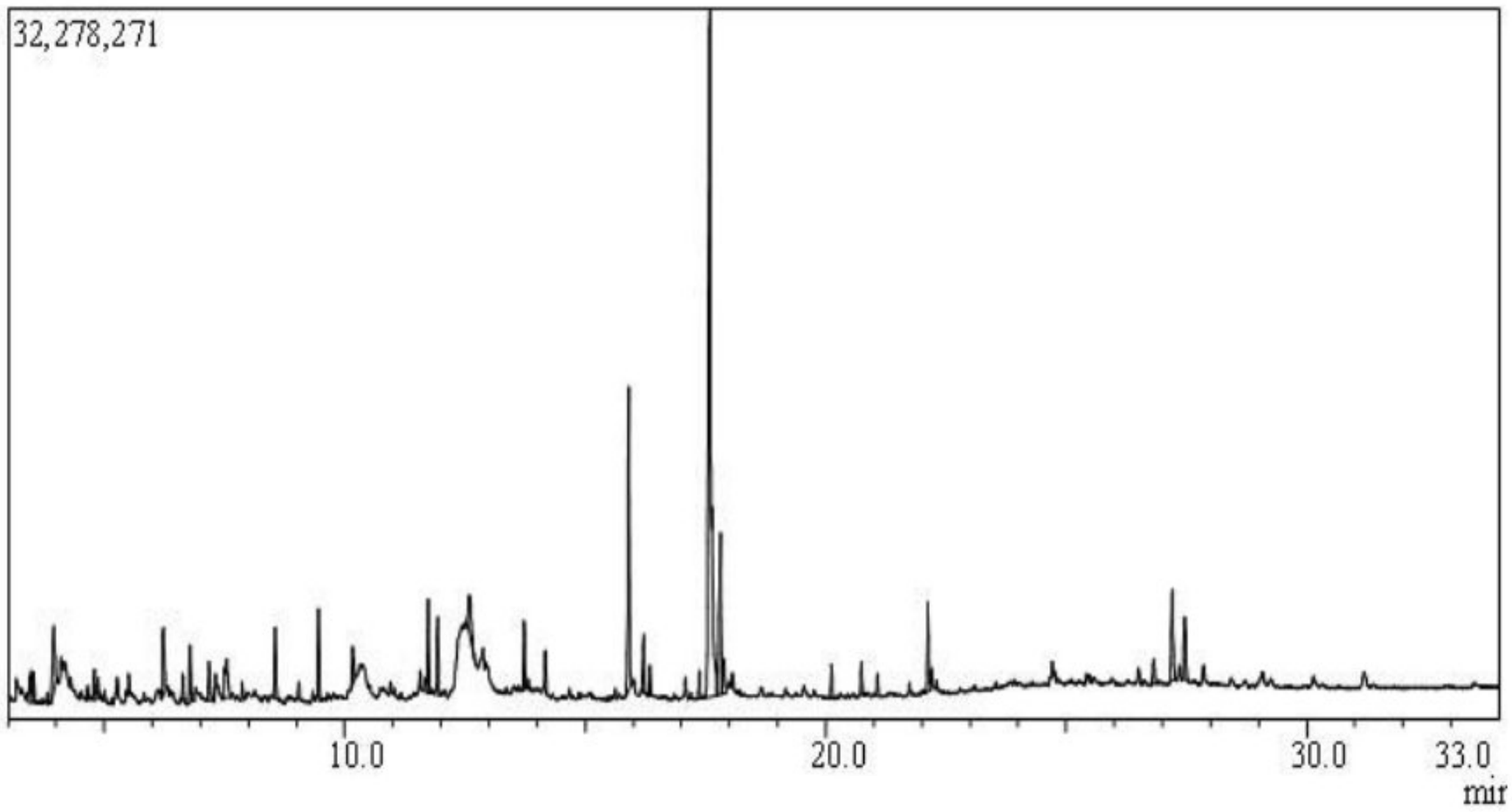

FIGURE S1: Retention time peaks of Water Extract of Balanies aegyptica

In addition, the GC-MS analysis in this study showed that the methanol leaves extract of tested plant contains two diterpene compounds, four alcohols, vitamin E, ester, Amine, and the fourteen fatty acids as shown in **Table 4** and **Figure S2.**

TABLE 4: GC-MS analysis of Methanol extract of leaves of Balanites aegyptica

Peak Report TIC

Peak#	Name	R.Time	Area	Area%

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1	1,3,5-Triazine-2,4,6-triamine	5.275	4129368	0.32
2	1-Dodecene	6.784	3433497	0.26
3	1-Tetradecene	9.461	7680148	0.59
4	Dodecanoic acid	11.714	232090040	17.84
5	1-Hexadecanol	11.934	5820423	0.45
6	Dodecanoic acid, ethyl ester	11.975	9576428	0.74
7	.beta.-D-Glucopyranose, 4-O-.beta.-D-galactopyranosyl-	12.739	13371471	1.03
8	Agaricic acid	13.739	14491858	1.11
9	Tetradecanoic acid	13.864	29552742	2.27
10	1-Nonadecene	14.171	7357923	0.57
11	Neophytadiene	14.673	5781470	0.44
12	n-Hexadecanoic acid	15.963	173356572	13.32
13	Hexadecanoic acid, ethyl ester	16.223	30507840	2.34
14	Phytol	17.375	13034451	1.00
15	9,12-Octadecadienoic acid (Z,Z)-	17.658	505736286	38.87
16	9,12-Octadecadienoic acid, ethyl ester	17.824	88872581	6.83
17	Octadecanoic acid, ethyl ester	18.074	11257171	0.87
18	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	20.748	7679432	0.59
19	4-Nonanol, 2,6,8-trimethyl-	20.983	4409258	0.34
20	Bis(2-ethylhexyl) phthalate	21.078	8243133	0.63
21	Dodecanoic acid, ethenyl ester	21.171	17428307	1.34
22	9,12-Octadecadienoic acid (Z,Z)-, 2,3-r dihydroxypropyl este	22.141	30119670	2.31
23	9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-t (hydroxyme	22.208	5978791	0.46
24	.delta.-Tocopherol	23.988	5625194	0.43
25	.delta.-Tocopherol, O-methyl-	24.650	5156616	0.40
26	Ergost-5-en-3-ol, (3.beta.)-	26.515	35230787	2.71
27	Stigmasterol	26.829	13956094	1.07
28	.gamma.-Sitosterol	27.470	11349527	0.87
			1301227078	100.00

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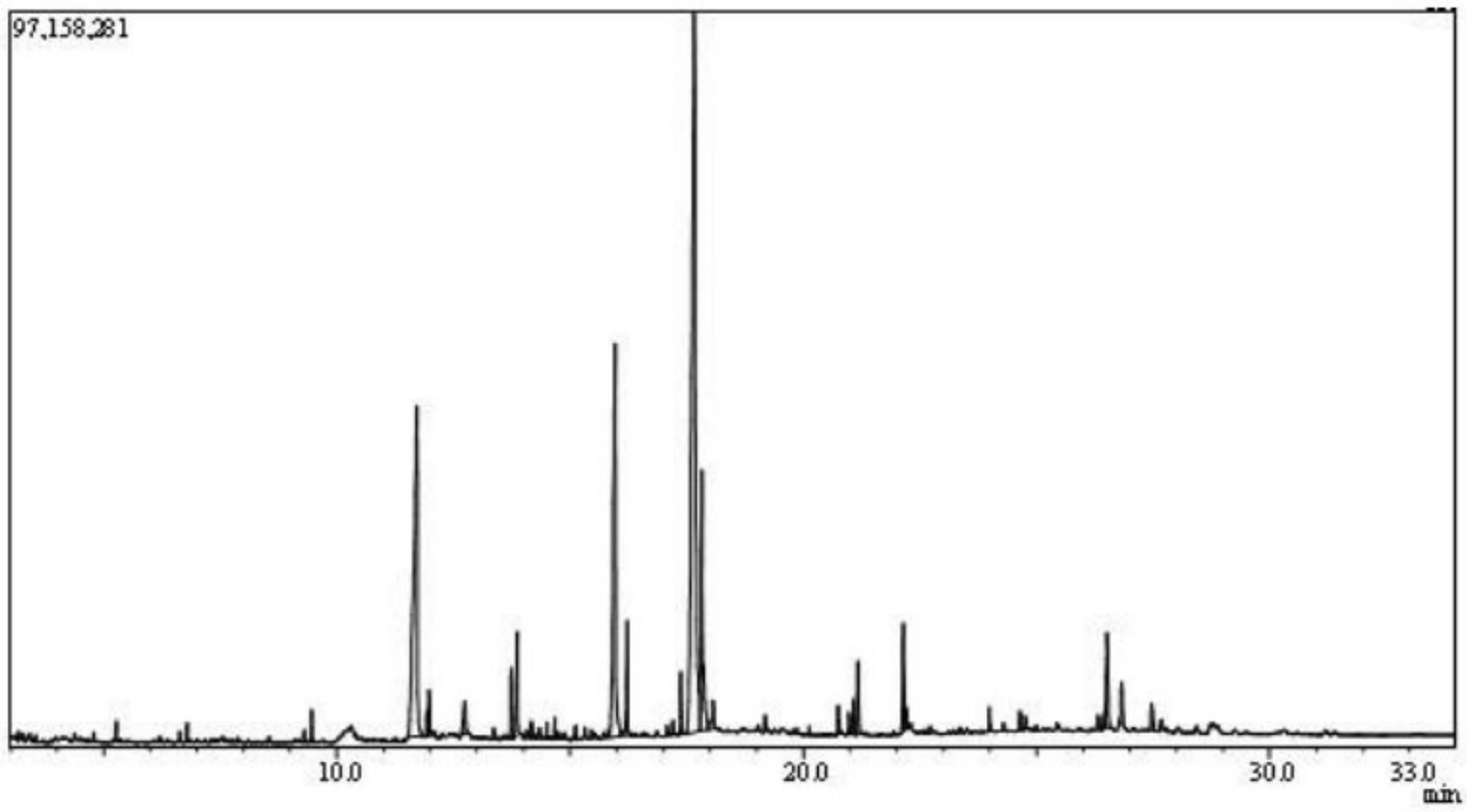

FIGURE S2: Retention time peaks of Methanol Extract of Balanies aegyptica

Furthermore, the chloroform extract analysis in this study proved that this extract contains different compounds such as aldehydes, five alcohols, three aromatic compounds, three phenolic compounds, a glucoside, an alkaloid, an Amin, and nine fatty acids as shown in **Table 5** and **Figure S3**.

TABLE 5: GC-MS analysis of Chloroform extract of leaves of B. aegyptica

Peak#	Name	R.Time	Area	Area%
1	2-Cyclopenten-1-one, 2-hydroxy-	3.542	4183647	0.84
2	2-Furancarboxaldehyde, 5-methyl-	3.950	12154267	2.45
3	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	4.101	3170219	0.64
4	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	6.227	4670327	0.94
5	1-Tridecene	6.781	3445831	0.70
6	Benzofuran, 2,3-dihydro-	7.175	3218990	0.65
7	Ethanone, 1-(2-hydroxy-5-methylphenyl)-	8.550	3037365	0.61
8	1-Tetradecene	9.459	6633615	1.34
9	1-Nonadecene	11.932	6168289	1.25
10	8-Azabicyclo[3.2.1]octan-3-ol, 6-methoxy-8-methyl-	12.381	6794779	1.37
11	Ethyl .alpha.-d-glucopyranoside	12.536	13514791	2.73
12	D-Tagatose	12.850	3920175	0.79
13	(E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol	13.732	2471640	0.50
14	1-Nonadecene	14.171	2815541	0.57
15	n-Hexadecanoic acid	15.926	49163746	9.93
16	Hexadecanoic acid, ethyl ester	16.219	11932478	2.41
17	Benzeneacetic acid, .alpha.-methylene-, 8-methyl-8-azabicyclo[3.2.1]oct-	17.294	6394452	1.29
18	9,12-Octadecadienoic acid (Z,Z)-	17.610	98219054	19.84
19	cis-9-Hexadecenal	17.641	76060563	15.36
20	9,12-Octadecadienoic acid, ethyl ester	17.816	30634485	6.19
21	Ethyl Oleate	17.858	14041162	2.84
22	Octadecanoic acid, ethyl ester	18.069	4302300	0.87
23	Atropine	18.679	82901306	16.74
24	Scopolamine	19.804	12246878	2.47
25	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	20.126	5086499	1.03
26	Bis(2-ethylhexyl) phthalate	21.075	2692027	0.54
27	9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl este	22.143	4276730	0.86
28	Ergost-5-en-3-ol, (3.beta.)-	26.507	7516267	1.52
29	Stigmasterol	26.827	4605168	0.93
30	.gamma.-Sitosterol	27.469	8861795	1.79

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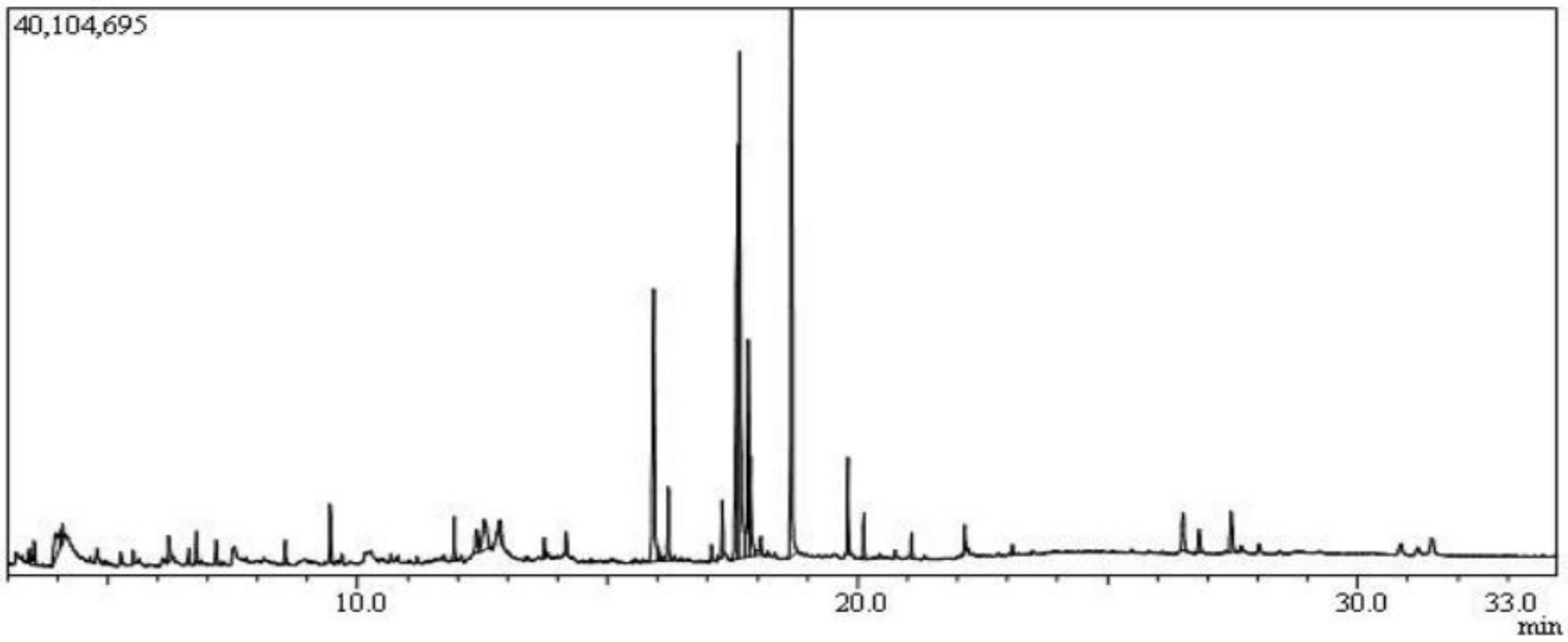

FIGURE S3: Retention time peaks of Chloroform Extract of Balanies aegyptica

DISCUSSION

It is well documented that all parts of this plant are traditionally widely used in the treatment of many ailment types, but the fruits, are the most used to manage hyperglycemia, and most plant part has been researched, while scanty research has been done on the plant leaves (Beentje et al., 1994; Yadav and Panghal, 2010; Chothani & Vaghasiya, 2011).

In Eritrea, Sudan, and Libya, it has been reported that the leaves of this plant have been traditionally used in infected wound cleaning (Oliver, 1960). No antibacterial study has been found carried out on the leaves of the Libyan Balanites aegyptica, and this is behind the focus of this study on the plant leaves, depending on its traditional use in infected wound treatment.

Little studies have been found testing the antibacterial activity of leaves of Balanites aegyptica worldwide as cleared by previous reviews, which conclude that the aqueous and ethanolic extracts have antibacterial activity against Salmonella typhi, and S. aureus bacteria. These reports cleared that the not pronounced antibacterial activity of the crude extract of leaves of Balanites aegyptica which is given at 100 mg/ml concentration is enhanced when mixed with the crude extract of leaves of Moringa oleifera plant at the same concentration. Also, these papers said that the ethanolic extract of Balanites aegyptica leaves was more active than the aqueous extract (Yadav and Panghal, 2010; Singh et al., 2017; Al-Thobaiti & Abu Zeid, 2018).

This study presented results different from that reported in the above-mentioned papers, where it is proved that the aqueous and organic leaves extract of this plant showed equal good activity against tested clinical S. aureus with an inhibition zone of 12.5 mm, while the previous studies reported a 4 mm inhibition zone from methanol and aqueous leaves extracts against Staphylococcus aureus (Yadav & Panghal, 2010).

Even though the above recent papers have not mentioned the area and season of the plant sample collection, these study results are considered promising results in comparison, especially with the 3.125 mg/ml shown as a minimum inhibitory concentration against the worldwide problematic bacterium; Methicillinresistant S. aureus.

Kulawe and his team, 2019, in their study, proved the same conclusion as this study in that the plant leaves have good antibacterial activity against S. aureus, and they reported the same MIC (25 mg/ml) as this study, but their study revealed a higher inhibition zone (20mm), with ethanol than 12.5 mm shown in this study from the methanol extract, and this could be referred to the different polarities of the two organic solvents.

In addition, when this study tested the leaves' activity against K. pneumonia, the results showed a closed near inhibition zone (15 mm) that was shown by Kulawe's team (16 mm), but this study showed higher MIC (100 mg/ml) in front of (50 mg/ml) proved in comparison.

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Another study done by Tula et al., 2014, agreed with this study's output, where both revealed near inhibition zones against S. aureus; 11.3 mm in front of 12.5 mm revealed in this study from aqueous extract of the plant leaves. The good activity shown in this study against K. pneumoniae, and both S. aureus, and Methicillinresistant S. aureus, could be referred to the leaves bio-constituents shown with GC-MS analysis in this study which showed that the leaves of the Libyan Balanites aegyptica contain numbers of fatty acids, phenols, alcohols, some amines, and these latter's are documented for having good antibacterial activity in general, and pronounce activity against Methicillin-resistant S. aureus in specific (Gopi et al., 2019; Kitahara et al., 2004).

However, this study is totally matched with that of Ibrahim, 2016, in that the aqueous extract has no activity against tested E. coli, but they disagreed about the antibacterial activity of the leaves aqueous extract against tested standard P. aeruginosa since Ibrhim's results showed a weak activity (9 mm) compared with the active inhibition zone shown in this study (16 mm). Generally, the variation in the biological activity within the same plant genera and species could be contributed to many probabilities, such as the different polarities of the used solvent, different extraction styles used, different collection seasons, plant growth stages, and different collection areas.

A study conducted by Abdallah and his team in 2012, had reported that this plant showed neargrowth inhibition zones as revealed in this study from the same solvent; methanol, but they extracted fruits instead of the leaves. Also, Jahan's study in 2013 is in agreement with this in that the plant Balanites aegyptica has antibacterial activity against most of the bacterial genera types tested in this study, but they tested the fruits too, and not investigated the leaves.

Generally, with the purpose to search more deeply, and in addition to the investigation of the antibacterial activity of the aqueous and methanol extract, this study has tested the chloroform leaves extract as well. The striking output of this study, with the situation of the scanty of studies concerned with the Balanites aegyptica leaves, is the pronounced growth inhibition zones that appeared from leaves chloroform extract against the tested Methicillinsensitive S. aureus (17.5 mm), and against the clinical P. aeruginosa (23.5 mm), the ones which are well known become worldwide a source of concern that threat health wellbeing.

What draws the attention is that when the natural product activities are compared with that of the antibiotic references against multi-drug resistant, Methicillin-resistant S. aureus, and although the varied inhibition zones revealed from the natural product and the references; either the close near IZs shown from Cefoxitin to the lower ones shown from Augmentin, Amoxicillin, Meropenem, Trimethoprim, and Levofloxacin, to the highest IZs shown from Doxycycline.

Unless, the results of LSD Post Hoc analysis showed that there is a correlation between the activity of water extract and the activity of these antibiotics despite inhibition zones revealed were significantly differed from that of the water leaves extract, the issue that draws the thought to the hypothesis of that this extract might have more than one mechanism by which fight this bacterium.

More study is needed to be carried out to stand on the fact of the mechanisms of action of this extract by which it can eliminate infections caused by Methicillin-resistant S. aureus. Another striking output in this study is that Augmentin showed the lowest inhibition zone against Methicillin-resistant S. aureus than that shown from Amoxicillin! What about the role of Clavulanic acid? The point which needs to be studied. On the other hand, the plant leaves in this study appeared as a promising solution for fighting the multi-drug resistant P. aeruginosa, where its activity was equalized with that of Meropenem, Ciprofloxacin, and was higher than the others. This study advises more studies to evaluate the safety profile of these plant leaves.

However, the comparison of results of the activities of tested antibiotics with that shown from the different extracts of the plant leaves gives an indication that these plant leaves can help with the treatment of the infection,

especially with the increasing rate of spreading of bacterial resistance.

This indication is drawn by the GC-MS results in this study which showed the presence of many bioactive constituents within the leaves components having antibacterial activity, of which, are fatty acids, alcohols, ester, amines, steroids, diterpenes, and phenols.

Also, this study result looks logical with what was mentioned in previous studies, which stated that this plant leaf contains many phenolic and polyphenolic compounds, that were well proved for their antibacterial activity against S. aureus, and other pathogenic bacteria (Murthy et al., 2020). This study advises using advanced techniques to understand the nature of this plant's actions in treating these pathogenic bacteria.

CONCLUSION AND FUTURE PERSPECTIVES

Leaves of Balanites aegyptica grow in Libya has good antibacterial activity against tested bacterial human pathogens, and this study suggests introducing this plant as a resource for the discovery of new pure compounds has a good antibacterial activity to help in the treatment of infections caused by multi-drug resistant bacterial pathogens, especially that caused by the Methicillin-resistant S. aureus, P. aeruginosa, and K. pneumonia, the types of bacteria that strained the world in searching for a final solution to eliminate them and get out of the circle of their increasing resistance to common antibiotics. Our future work will focus on the antibacterial reaction mechanism and some experiments like antibiofilm study and membrane leakage assay and also, imaging the treated microbes with SEM and HRTEM.

Finally, the introduction of nanotechnology of these three extracts must be taken into consideration through the incorporation of bimetallic nanoparticles at low concentrations to avoid toxicity and to study and increase the synergistic potential between the synthesized bimetallic NPs, and plant extracts.

Compliance with Ethical Standards

Disclosure of potential conflict of interest The authors declare that they have no conflict of interest.

Research involving Human Participation and/or Animals

Not applicable.

Informed consent

Not applicable.

Ethical approval

Not applicable.

Availability of data and materials

The datasets supporting the conclusions of this article are included within the article and its additional files.

Author's contributions

ASHT: suggested the research topic, investigated the article, planned the research methodology, wrote the original draft, and participated in data representation and article revising and editing. **SIA:** suggested the research topic, investigated the article, planned the research methodology, wrote the original draft, and participated in data representation and article revising and editing. **SSE**: suggested the research topic, investigated the article, planned the research methodology, wrote the original draft, and participated in data representation and article revising and editing. **SFB:** suggested the research topic, investigated the article, planned the research methodology, wrote the original draft, and participated in data representation and article revising and editing.

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