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Antibacterial activity of the bioactive compounds identified in three woody plants against some pathogenic bacteria

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ABSTRACT

Three bacterial isolates were identified from infected potato tubers showing soft and blackleg like symptoms as well as one isolate from infected pear tree showing crown gall symptom. Conventional and molecular identification proved that bacterial isolates belonging to *Pectobacterium carotovorum* subsp. *carotovorum*, *Pectobacterium atrosepticum*, *Dickeya solani* and *Agrobacterium tumefaciens*. The above plant bacterial isolates and human pathogenic bacteria *Escherichia coli*, *Sarcina lutea*, and *Staphylococcus aureus* were used for the bioassay. The chloroform leaf extracts from *Duranta plumieri variegata, Lantana camara*, and *Citharexylum spinosum* were assayed for their antibacterial activity by measuring the inhibition zones and minimum inhibitory concentrations (MICs). The suggested chemical compositions of extracts were analyzed using GC/MS apparatus. The main compounds in leaf extract of *L. camara* were 5,8-diethyl-dodecane, pyrimidin-2-one, 4-[*N*-methylureido]-1-[4-methylaminocarbonyloxymethyl, oleic acid,3-(octadecyloxy)propyl ester; in *D. plumieri* were 4,7-dimethoxy-2-methylindan-1-one and 5-(hexadecyloxy)-2-pentadecyl-,trans-1,3-dioxane; and in *C. spinosum* were *N*-[5-(3-hydroxy-2-methylpropenyl)-1,3,4,5-tetrahydrobenzo[cd]indol-3-yl]-*N*-methylacetamide. Promising activity was found against *A. tumefaciens*, *E. coli*, *P. carotovorum*, *Sar. lutea,* and *Staph. aureus* with MIC values of 8, 128, 64, 500 and 500 μg/mL, respectively, as *L. camara* leaf extract was applied. *D. plumieri* leaf extract showed good activity against *D. solani* and *P. atrosepticum* with MIC values of 16 μg/mL and 128 μg/mL, respectively. On the other hand, weak bioactivity was found with leaf extract from *C. spinosum*. It could be concluded that leaf extracts from *D. plumieri* and *L. camara* have a promising antibacterial agents.

1. Introduction

Potato (*Solanum tuberosum*, L.) is one of the most important vegetable crops in Egypt. Potato production of approximately 4,800,000 tons, produced from approximately 178,000 ha, making Egypt Africa's biggest potato producer [1]. The soft rot *Enterobacteriaceae Pectobacterium* and *Dickeya* species cause soft rot diseases on potato and other horticultural crops [2]. They affect the growing potato causing blackleg and they are responsible for tuber soft rot in storages thereby reducing yield and quality. The causal agent of crown gall bacterium, *Agrobacterium tumefaciens,* is well-known in soil and infects a wide host range of susceptible hosts belong to more than 90 families of gymnosperms and dicotyledons [3].

Natural products such as essential oils, phenolic compounds, saponins, flavonoid compounds, lignans, steroids, fatty acids, alkaloids, and others, isolated from medicinal and aromatic plants as well as the higher plants have a long history for their bioactivity against certain pathogenic microbes [4–12].

Family Verbenaceae is consider to be a large one among the plant families, which contains 100 genera and around 3000 species [13,14], and have a potential source of natural products especially flavonoids, essential oils [15,16].

Lantana camara L. originated in subtropical America is used widely as ornamental plants [17,18]. The essential oils have promising

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antibacterial activity [15,19]. The extracts have antimycobacterial, antifungal, nematicidal and insecticidal activities [20–23]. Also, Silver nanoparticles synthesized by *L. camara* leaf extract have been shown good activity against *Escherichia coli*, *Pseudomonas* spp., *Bacillus* spp. and *Staphylococcus* spp [24].

Duranta plumieri L. (*D. repens* Linn. or *D. erecta* Linn.) leaves and berries is toxic, and are confirmed to have killed children, dogs and cats [25]. Alkaloids, flavonoids, steroids, triterpenoids, tannins and phenols were found in methanolic leaf extracts [26,27]. *β*-sitoserol, naringenin, acteoside, sucrose, raffinose were identified in from *D. repens* Linn. var. variegate [28]. Aqueous and methanolic leaf extracts of *D. repens* showed promising antifungal activity [26]. It also showed antifungal activity against *Candida albicans* and *Aspergillus niger* and *A. flavus* [29].

Citharexylum spinosum (*C. quadrangulare* or *C. fruticosum*), is distributed through the Caribbean, United States (Southern Florida), Guyana, Suriname, and Venezuela [30–32]. Different chemical component have been identified from the aerial parts or leaves such as 7-*ß*-O-acetate of lamiide (iridoid glucoside) 8-epiloganin, duranterectoside C, lamiidoside, and lignan glucoside (+)-lyonirenisol-3a-*O*-*ß*-*D*-glucopyranoside [16], Biologically, it was reported that flowers essential oil and extracts exhibited antibacterial and antioxidant efficacy [32]. The methanol extract from *C. spinosum* wood showed remarkable inhibition against the growth of *Paecilomyces variotii* at 8 μg/mL [33].

The objective of the present study was to evaluate the antibacterial activity of chloroform leaf extracts of *L. camara, D. plumieri*, and *C. spinosum* grown in Egypt on the growth of different plant and human pathogenic bacteria, and find an alternative, safer control agent for the control of some bacterial diseases. In addition, identify the bioactive molecules of these extracts by Gas chromatography–mass spectroscopy (GC-MS).

2. Materials and methods

2.1. Bacterial isolates

2.1.1. Isolation and identification of phytopathogenic bacteria

Naturally infected potato tuber showing typical symptoms of soft rot or blackleg disease were collected from fields and stores, as well as, soft galls formed on Pear (*Pyrus communis*) trees (Fig. 1). Diseased samples were first washed with tap water then surface sterilized with 10% sodium hypochlorite solution (NaOCl) for 3 min then washed thoroughly 3 times with sterilized distilled water; the rotted/galled tissues were put into sterilized mortar and homogenized then left to stand for 20 min then a loopful of the resulting suspension was streaked into plates containing growth medium selective for each pathogen [34–36]. After 24–48 h at 30 °C, bacterial colonies will be appeared depending on the medium used [37]. The morphological and biochemical characteristics of the isolated plant bacteria were performed using standard methods recommended [38–40].

2.2. Molecular identification

DNA extraction protocol and molecular identification was described in our previously studies [3,[6],38,41]. The following bacterial plant isolates were identified and used in the present work, *Agrobacterium tumefaciens*, *Dickeya solani*, *Pectobacterium atrosepticum*, and *Pectobacterium cartovorum* subsp. *cartovorum*.

2.3. Human bacterial cultures

Bacterial cultures of *Escherichia coli* ATCC 8739, *Sarcina lutea* ATCC 9341, and *Staphylococcus aureus* ATCC 6538 were provided by Bacteriology Lab. Faculty of Agriculture, Alexandria, Egypt.

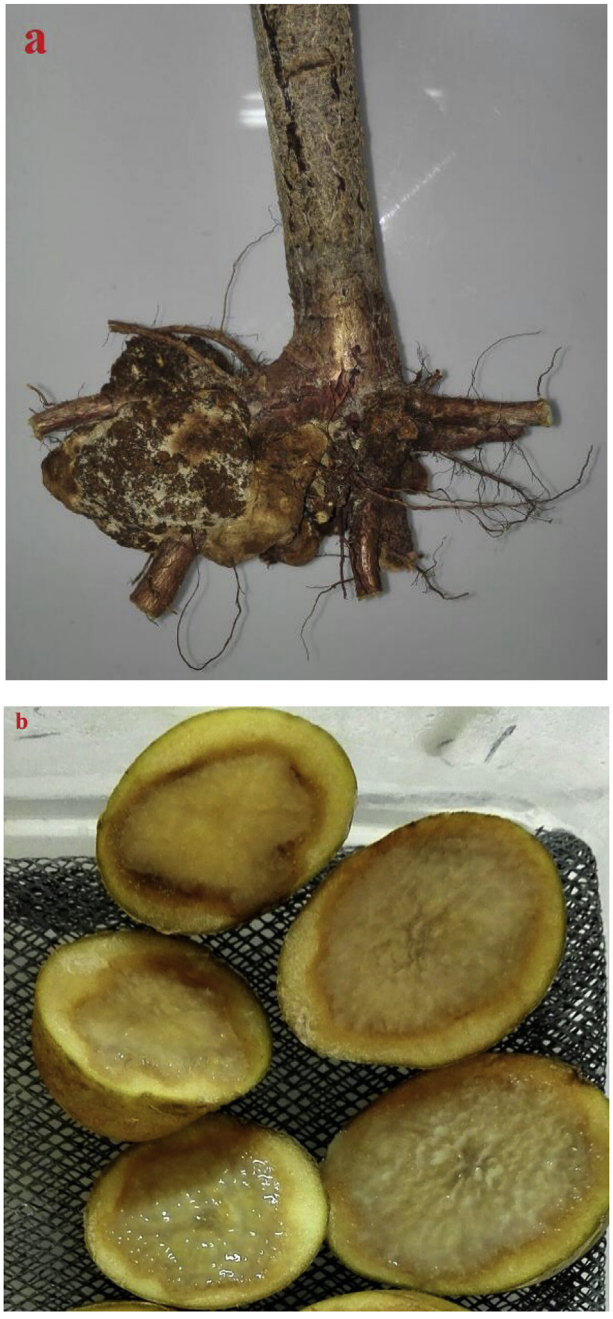

Fig. 1. Symptoms of naturally infected plant samples, A; Crown gall caused by *Agrobacterium tumefaciens*, B; soft rot cased by *Pectobacterium* and *Dickeya* species.

2.4. Plant material and extraction

Leaves of L. *camara*, *D. plumieri variegata* and *C. spinosum* were collected from Antoniadis gardens, Alexandria, Egypt. The leaves were air-dried at laboratory temperature for 7 days then ground to small pieces using small laboratory mill. The air-dried powdered leaves (50 g) from each plant were subjected to the extraction by immersing method in chloroform solvent (150 mL) for three times to insure completing the extraction [42]. The extract quantities were 15.45%, 17.15% and 14.20%, from *L. camara, D. plumieri variegata* and *C. spinosum*, respectively.

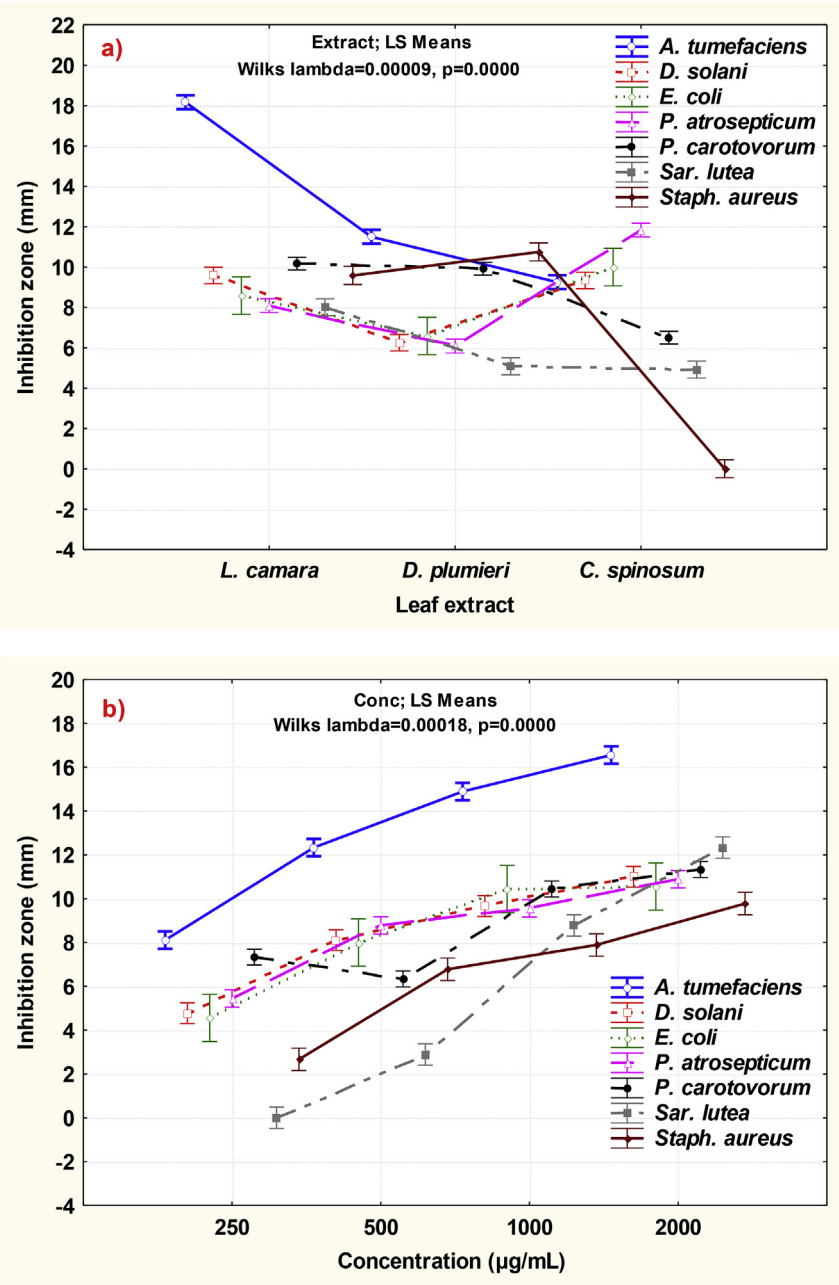

Fig. 2. Antibacterial activity of leaf extracts from *L. camara*, *D. plumieri*, and *C. spinosum* (a) and the their concnetrations (b) against the growth of pathogenic bacteria.

2.5. GC–MS analysis of the extracts

The extracts samples were analyzed for their chemical composition using Focus GC-DSQ Mass Spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (length 30 m × 0.25 mm ID x 0.25 µm film thickness) apparatus. The GC/MS program and temperatures used can be found in previous published work [43].

2.6. Antibacterial activity assay

The chloroform extracts isolated from leaves of *D. plumieri*, *L. camara*, and *C. spinosum* were assayed for their antibacterial activity using disk diffusion method [44] against the growth of selected plant pathogenic bacteria; *A. tumefaciens*, *D. solani*, *P. atrosepticum*, and *P. cartovorum* subsp. *cartovorum*. Human pathogenic bacteria *E. coli* ATCC 8739, *Sar. lutea* ATCC 9341, and *Staph. aureus* ATCC 6538 were also used to show the activity of extracts. A suspension of freshly 24-h's old bacterial strains with 1.0×10^5 CFU/mL were used. The bacterial suspensions were spread over the Mueller Hinton Agar media in sterilized Petri dishes. Discs with diameter of 5 mm were located over the inoculated media, then impregnated with 20 µL of the respective extract concentrations (250, 500, 1000 and 2000 µg/mL, prepared by dissolving in 10% dimethyl sulfoxide). After 24 of incubation at 37 °C for human bacterial pathogens and at 30 °C for plant pathogens, the inhibition zones (IZs) around the discs were measured in millimeters using a ruler. Controls discs with negative dimethylsulfoxide (DMSO) and positive (Tetracycline (20 µg/disc) were performed, and all tests were measured in triplicate [45,46]. Minimum inhibitory concentrations (MICs) was performed in 96-well micro-plates [47] using serial dilutions of the extract ranged between 4 and 4000 µg/mL.

Table 1
Antibacterial activity of the interaction between chloroform leaf extracts and their concentrations from *D. plumieri*, *L. camara*, and *C. spinosum* against the growth of some pathogenic bacteria.

Plant extract	Conc µg/mL	*A. tumefaciens*			*D. solani*			*E. coli*			*P. atrosepticum*
		IZ (mm)	−95%	+95%	IZ (mm)	−95%	+95%	IZ (mm)	−95%	+95%	IZ (mm)
L. camara	250	8.3 ± 0.58	6.9	9.7	6.6 ± 0.58	5.23	8.1	6.3 ± 0.58	4.9	7.7	6.6 ± 0.58
	500	17.3 ± 0.58	15.9	18.7	8.3 ± 0.58	6.90	9.7	8	8	8	7.6 ± 0.58
	1000	22.67 ± 0.58	21.2	24.1	11 ± 1.73	6.70	15.3	9.6 ± 0.58	8.2	11.1	8.3 ± 0.58
	2000	24.3 ± 0.58	22.9	25.7	12.3 ± 1.15	9.46	15.2	10.3 ± 0.58	8.9	11.7	9.6 ± 0.58
D. plumieri	250	8	8	8	0	0	0	0	0	0	0
	500	11.6 ± 0.58	10.2	13.1	7	7	7	7	7	7	7
	1000	12.6 ± 0.58	11.2	14.1	8	8	8	8.6 ± 0.58	7.2	10.1	8
	2000	13.6 ± 0.58	12.2	15.1	10	10	10	10.6 ± 0.58	9.2	12.1	9.3 ± 1.15
C. spinosum	250	8	8	8	7.6 ± 0.58	6.23	9.1	7.3 ± 0.58	5.9	8.7	9.6 ± 0.58
	500	8	8	8	9	9	9	9	9	9	11.6 ± 0.58
	1000	9.3 ± 1.15	6.4	12.2	10	10	10	10	10	10	12.3 ± 0.58
	2000	11.6 ± 0.58	10.2	13.1	10.6 ± 0.58	9.23	12.1	10.6 ± 0.58	9.2	12.1	13.6 ± 0.58
Negative control[a]		0			0			0			0
Positive control[b]		19.6 ± 1.15			14.6 ± 0.58			18 ± 1.00			12.6 ± 1.15

Plant extract	*P. atrosepticum*		*P. carotovorum*			*Sar. lutea*			*Staph. aureus*		
	−95%	+95%	IZ (mm)	−95%	+95%	IZ (mm)	−95%	+95%	IZ (mm)	−95%	+95%
L. camara	5.23	8.10	8	8	8	0	0	0	0	0	0
	6.23	9.10	9.6 ± 0.58	8.23	11.1	8.6 ± 0.58	7.23	10.1	10.6 ± 1.15	7.8	13.54
	6.90	9.77	10.6 ± 0.58	9.23	12.1	10.6 ± 1.15	7.80	13.54	12.3 ± 0.58	10.90	13.77
	8.23	11.1	12.3 ± 0.58	10.9	13.77	12.6 ± 1.15	9.80	15.54	15.3 ± 0.58	13.90	16.77
D. plumieri	0	0	7.6 ± 0.58	6.23	9.10	0	0	0	8	8	8
	7	7	9.3 ± 0.58	7.9	10.77	0	0	0	9.6 ± 1.15	6.80	12.54
	8	8	11.3 ± 0.58	9.9	12.77	7.3 ± 0.58	5.90	8.77	11.3 ± 0.58	9.90	12.77
	6.46	12.20	11.3 ± 0.58	9.9	12.77	13 ± 1	10.52	15.48	14 ± 1.73	9.70	18.30

(*continued on next page*)

Table 1 (*continued*)

Plant extract	*P. atrosepticum*		*P. carotovorum*			*Sar. lutea*			*Staph. aureus*		
	−95%	+95%	IZ (mm)	−95%	+95%	IZ (mm)	−95%	+95%	IZ (mm)	−95%	+95%
C. spinosum	8.23	11.10	6.3 ± 0.58	4.90	7.77	0	0	0	0	0	0
	10.23	13.10	0	0	0	0	0	0	0	0	0
	10.9	13.77	9.3 ± 0.58	7.9	10.77	8.3 ± 0.58	6.9	9.77	0	0	0
	12.23	15.10	10.3 ± 0.58	8.90	11.77	11.3 ± 1.15	8.46	14.20	0	0	0
Negative control[a]	0		0			0			0		
Positive control[b]	12.6 ± 1.15		20.3 ± 0.58			17.6 ± 0.58			16.6 ± 0.58		

[a] Dimethyl sulfoxide (DMSO 10%).
[b] Tetracycline (20 μg/disc).

2.7. Statistical analysis

Inhibition zones values were analyzed using two way analysis of variance in completely randomized design using SAS software version 8.2. The data reported as mean ± standard division. LSD at 0.05 level of probability was used to test the significant difference among the treatments.

3. Results and discussion

3.1. Isolation and identification of bacterial isolates

Three bacterial isolates were isolated from infected potato tubers and one isolate from infected pear tree. Morphological, physiological, biochemical and molecular characteristics of the isolated bacteria indicated that isolates were belonging to *Pectobacterium carotovrum* subsp. *carotovorum*, *Pectobacterium atrosepticum*, *Dickeya solani*. and *Agrobacterium tumefaciens*. These results in agreement with previous studies [6,38].

3.2. Antibacterial activity

Fig. 2a shows the overall effects of extracts against the studied bacterial strains; where the extract from *L. camara* observed the highest activity against *A. tumefaciens*, *P. carotovorum*, *D. solani*, and *Sar. lutea*. *D. plumieri* extract showed the highest activity against *Staph. aureus,* while *C. spinosum* observed the highest activity against *E. coli* and *P. atrosepticum*. Fig. 2b presents that, with increasing the extract concentration, the activity as measured by inhibition zone (IZ) was increased.

L. camara extract at 2000 and 1000 μg/mL showed the highest activity against *A. tumefaciens* with IZ values of 24.3 mm and 22.6 mm; *D. solani* with IZ values of 12.3 mm and 11 mm, respectively, compared to 19.6 mm (Tetracycline 20 μg/disc). Extracts at the concentration of 2000 μg/mL showed good activity against the growth of *E. coli* with IZ values of 10.3 mm (*L. camara*), 10.67 mm (*D. plumieri*) and 10.6 mm (*C. spinosum*), which lower than those reported from the antibiotic used (18 mm). *C. spinosum* extract observed the highest activity against *P. atrosepticum* at 2000 and 1000 μg/mL with IZ values of 13.6 mm and 12.3 mm, respectively, which nearly equal to reported from the positive control (12.6 mm). For *P. carotovorum*, extracts from *L. camara* at 2000 μg/mL (12.3 mm), *D. plumieri* at 2000 μg/mL and 1000 μg/mL (11.3 mm) and *C. spinosum* at 2000 μg/mL (10.3 mm), which were lower than those reported from antibiotic (20.3 mm). At 2000 μg/mL extracts from *L. camara*, *D. plumieri* and *C. spinosum* showed the highest activity against the growth of *Sar. lutea* with IZ values of 12.6 mm, 13 mm, and 11.3 mm, respectively, which were lower than the value from positive control (17.6 mm). The highest activity against the growth of *Staph. aureus* was observed by the extracts of *L. camara* at 1000 μg/mL (12.3 mm) and 2000 μg/mL (15.3 mm); and *D. plumieri* at 2000 μg/mL (14 mm), which lower than the value from antibiotic used (16.6 mm) (see Table 1).

According to MIC values (Table 2), *L. camara* leaf extract showed good activity against *A. tumefaciens*, *E. coli*, *P. carotovorum*, *Sar. lutea,* and *Staph. aureus* with MIC values of 8, 128, 64, 500 and 500 μg/mL, respectively. Leaf extract of *D. plumieri* showed good antibacterial activity against *D. solani* (16 μg/mL), and *P. atrosepticum* (MIC value of 128 μg/mL). While, leaf extract from *C. spinosum* observed weak activity against the studied bacterial pathogens and only had an activity against *E. coli* with MIC value of 128 μg/mL.

3.3. Chemical composition of extracts

Gas chromatography–mass spectroscopy (GC-MS) is a valuable tool for reliable identification of phytocompounds [48]. In the present study, 8 compounds for *L. camara* extract, 10 compounds from the *D.*

Table 2
Minimum inhibitory concentration (MIC) of chloroform leaf extracts from *D. plumieri*, *L. camara*, and *C. spinosum* against some pathogenic bacteria.

Extract	MIC (μg/mL)						
	A. tumefaciens	*D. solani*	*E. coli*	*P. atrosepticum*	*P. carotovorum*	*Sar. lutea*	*Staph. aureus*
L. camara	8	64	128	500	64	500	500
D. plumieri	32	16	500	128	128	1000	2000
C. spinosum	32	32	128	500	1000	1000	> 4000

MIC: Minimum inhibitory concentration (μg/mL).

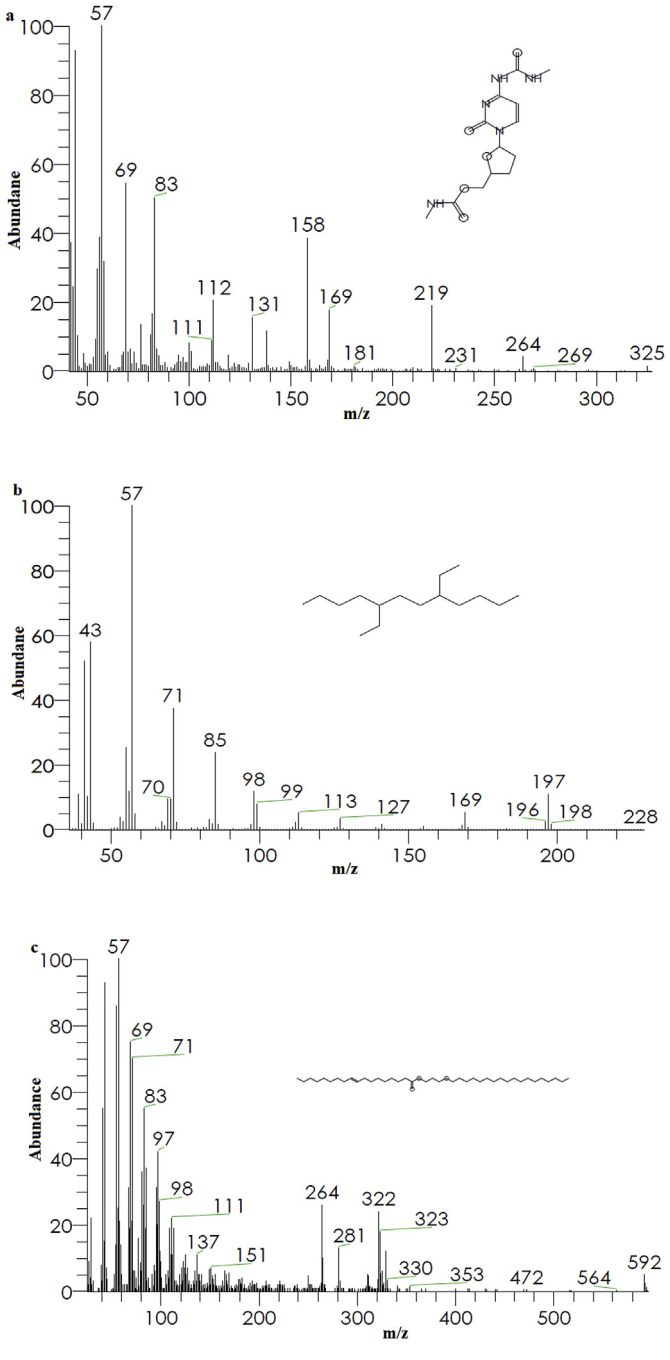

Fig. 3. The 70 eV mass spectrum of pyrimidin-2-one, 4-[*N*-methylureido]-1-[4 methylaminocarbonyloxymethyl (a), 5,8-diethyl-dodecane (b) and Oleic acid, 3-(octadecyloxy)propyl ester (c) component of *L. camara* leaf extract.

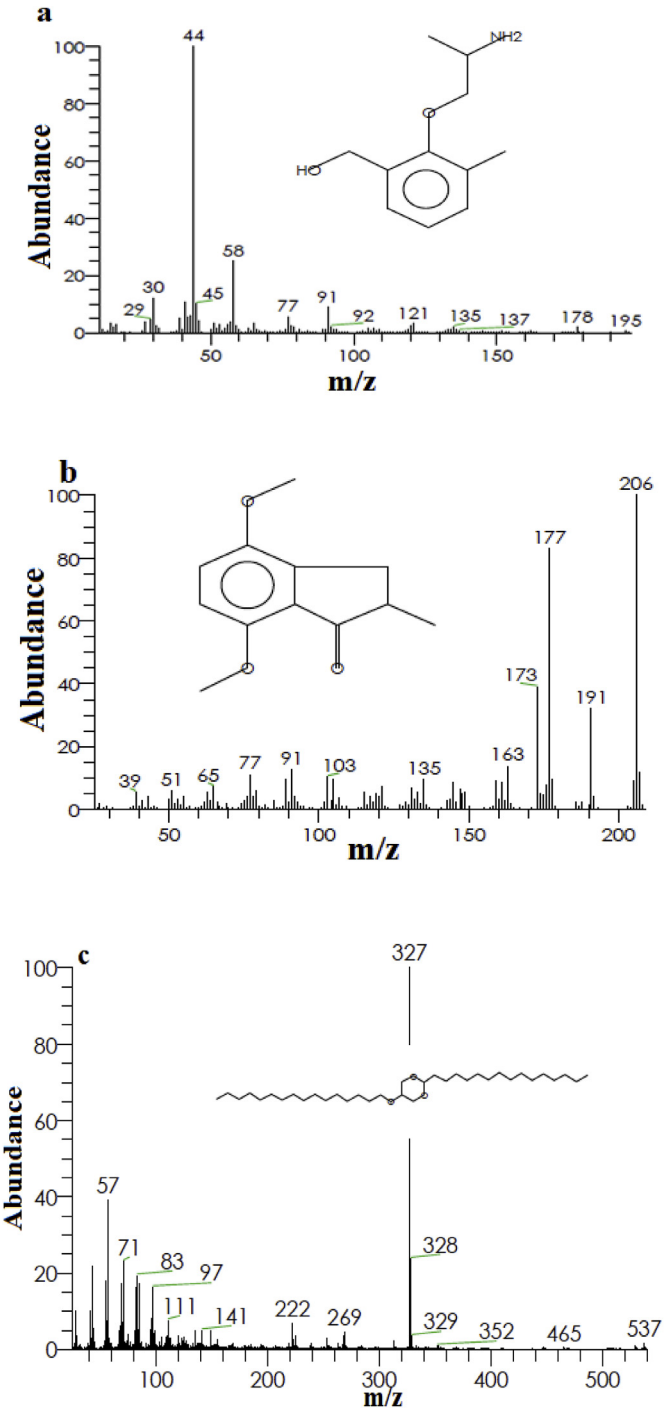

Fig. 4. The 70 eV mass spectrum of 2-(2-aminopropoxy)-3-methyl-benzene-methanol (a), 4,7-dimethoxy-2-methylindan-1-one (b), and 5-(hexadecyloxy)-2-pentadecyl-trans-1,3-dioxane, (c) as main compounds isolated from component of *D. plumieri* leaf extract.

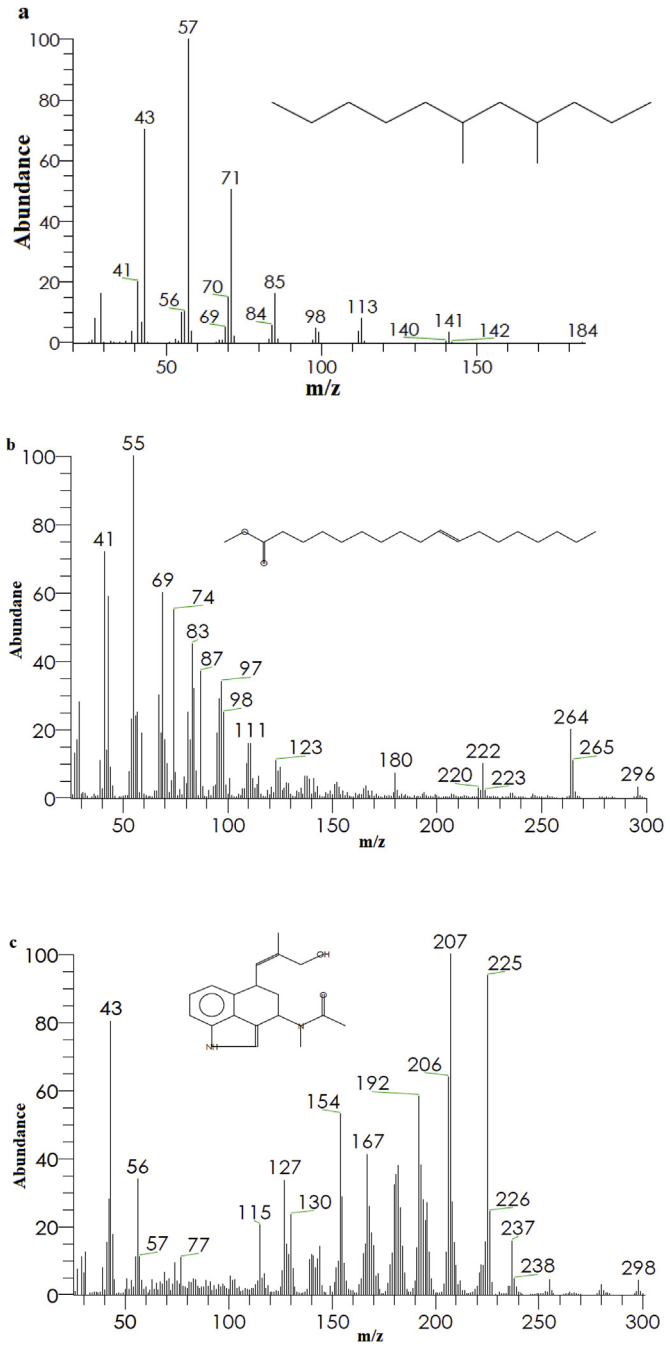

Fig. 5. The 70 eV mass spectrum of 4,6-dimethyl-undecane(a), 10-octadecenoic acid, methyl ester (b), and *N*-[5-(3-hydroxy-2-methylpropenyl)-1,3,4,5-tetrahydrobenzo[cd]indol-3-yl]-*N*-methylacetamide component of *C. spinosum* leaf extract.

plumieri extract, and 13 compounds for *C. spinosum* extract have been identified by GC-MS analysis. The mass spectrometric analysis of organic compounds gives information on the molecular mass, chemical formula, chemical structure, or quantity of the analyte. Based on the measured *m/z* and their peak intensities, the formula and chemical structure can be determined manually and/or by comparison with a reference database of spectra [49]. The pharmacological, biological activity of any molecule is dependent upon its structure and that a change in the structure can lead to a change in activity. Therefore, the study the mass spectra of the obtained components of the studied three extract samples will share in understanding the biological activities of these components.

The mass spectra of the major components of the studied three plant extracts are shown in Figs. 3–5, and the most fragment ions of the four studied samples were reported at Table 3.

1 The MS of pyrimidin-2-one, 4-[*N*-methylureido]-1-[4 methylaminocarbonyloxymethyl component of *L. camara* extract 70 eV (Fig. 3a) show that the molecular ion peak at m/z 325 have RI = 5% and the other fragment ions are 219 (25%), 158(45%), 112(25%) and 83(50%). While the fragment ion at m/z 57 represent the base peak with RI = 100%.
2 The MS of 5,8-diethyl-dodecanecomponent of *L. camara* extract at 70 eV (Fig. 3b) show that the molecular ion peak at *m/z* 226 have RI = 2% the most intense peak at m/z 57 with RI = 100% and the other fragment ions are 197 (20%) 85(30%), 71(40%) and 71(30%).
3 The MS of oleic acid,3-(octadecyloxy)propyl ester component of *L. camara* extract at 70 eV (Fig. 3c) show that the molecular ion peak at m/z 592 have RI = 10% the most intense peak at m/z 57 with RI = 100% and the other fragment ions are 322 (25%), 264(30%), 97(45%), 83(55%) and 69(80%).
4 The MS of benzenemethanol,2-(2-aminopropoxy)-3-methyl- component of *D. plumieri* extract at 70 eV (Fig. 4a) show that the molecular ion peak at m/z 195 have RI = 3% and the other fragment ions at m/z 91(15) and 58(25)
5 The MS of 4,7-dimethoxy-2-methylindan-1-one component of *D. plumieri* extract at 70 eV are recoded as shown in (Fig. 4b). Using this MS one can note that the molecular ion peak at m/z 206 have RI = 100% represent the most intense peak the other fragment ions are 191(35%), 177(85%) and 173(40%).
6 The MS of 5-(hexadecyloxy)-2-pentadecyl-trans-1,3-dioxane component of *D. plumieri* extract at 70 eV (Fig. 4c) show that the molecular ion peak at m/z 538 have RI = 2% the most intense peak at m/z 327 with RI = 100% and the other fragment ions are 222(15%), 83 (22%) and 71(30%).
7 The MS of 4,6-dimethyl-undecane component of *C. spinosum* extract at 70 eV (Fig. 5a) show that the molecular ion peak at m/z 184 have RI = 3% the most intense peak at m/z 57 with RI = 100% and the other fragment ions are 141 (7%), 113(10%), 85(20%) and 71(55%).
8 The mass spectrum of 10-octadecenoic acid, methyl ester component of *C. spinosum* extract at 70 eV (Fig. 5b) show that the molecular ion peak at m/z 296 have RI = 8% the most intense peak at m/z 55 with RI = 100% and the other fragment ions are 264 (20%), 222(15%), 97(35%) 83(50%), 74(55%) and 69(60%).
9 The MS of *N*-[5-(3-hydroxy-2-methylpropenyl)-1,3,4,5 tetrahydrobenzo[cd]indol-3-yl]-*N*-methylacetamide component of *C. spinosum* extract at 70 eV (Fig. 5c) show that the molecular ion peak at *m/z* 298 has RI = 5% the most intense peak at *m/z* 207 with RI = 100% and the other fragment ions are 237 (20%), 225(95%), 192(60%), 167(45%), 154(55%), 127(40%) and 115(25%).

From the present work, chloroform extracts from leaves of *D. plumieri variegata*, and *L. camara*, were showed promising antibacterial activity, while the extract from *C. spinosum* observed weak activity.

Generally, from the following studies of the literature there some variations in their results and our results which could related to geographical regions and the season of collecting leaves [50,51]. Antimicrobial activities of several plant extracts against bacterial soft rot of potatoes were evaluated and a quite satisfactory result was obtained [6,15,41]. It was reported that, methanolic leaf extract of *D. erecta* has a good antifungal and antibacterial activities, which revealed the presence of excellent amount of alkaloids, flavonoids, terpenoids, saponins and phenolic compounds which has good antibacterial activity [52,53]. Flavonoids, *C*-alkylated, and some alkaloids were isolated from *D. plumieri* [54–56]. It was reported previously that, *D. erecta*. Ethyl acetate and aqueous extracts of leaves showed significant antimalarial activity when administered to mice [57]. The total ethanol extract of

Table 3
Phytochemicals screening of chloroform extracts from the studied plant samples by GC-MS.

No.	RT[1]	Name of the compound	Molecular Formula	MW[2]	Peak Area %	SI[3]	RSI[4]	Most Fragment ions with RI[5] (%)
L. camara leaf extract								
1	3.27	14-Formol-9-hydroxyarteether	$C_{17}H_{26}O_7$	342	1.33	349	342	310(10),264(20),193(15),181(20),86(45) and 55(32)
2	3.42	Pyrimidin-2-one, 4-[*N*-methylureido]-1-[4-methylaminocarbonyloxymethyl	$C_{13}H_{19}N_5O_5$	325	36.26	500	614	325(5),219(25),158(45),112(25),83(50) and 57(100)
3	3.65	5,8-Diethyl-dodecane	$C_{16}H_{34}$	226	49.50	539	578	226(2),197(20), 85(30),71(40) and 57(100)
4	8.24	Alanine	$C_3H_7NO_2$	89	0.37	640	822	89(3),74(5)
5	28.71	3-Acetoxy-7,8-epoxylanostan-11-ol	$C_{32}H_{54}O_4$	502	1.06	400	407	502(5),304(25),244(43),109(45), 95(65) and 57(85)
6	36.51	2-(3-Acetoxy-4,4,14-trimethylandrost-8-en-17-yl)-propanoic acid	$C_{27}H_{42}O_4$	430	1.61	411	430	430(25), 415(40), 355(100),281(20),121(30) and 83(45)
7	37.33	Oleic acid,3-(octadecyloxy)propyl ester	$C_{39}H_{76}O_3$	592	9.17	417	443	592(10), 322(25), 264(30), 97(45),83(55), 69(80) and 57(100)
8	40.50	3,4-didehydro-1,1′,2,2′-tetrahydro-1′-hydroxy-1-methoxy-psi.,.psi.-Carotene	$C_{41}H_{60}O_2$	584	0.70	395	413	584(3), 478(10), 106(30), 91(50) and 73(100)
D. plumieri leaf extract								
1	3.64	Decane	$C_{10}H_{22}$	142	3.79	615	807	142(15),85(35),71(45) and 57(100)
2	13.03	2-(2-Aminopropoxy)-3-methyl-benzenemethanol	$C_{11}H_{17}NO_2$	195	5.46	373	512	195(3),91(15) and 58(25)
3	13.13	7,11,18-triacetoxy-3-methoxy-3,9-Epoxypregn-16-ene-14,20-diol	$C_{28}H_{40}O_{10}$	536	1.46	390	410	458(25),398(20),180(25) and 124(65)
4	13.19	9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol,(3á,5Z,7E)-	$C_{27}H_{44}O_3$	416	2.09	424	492	416(20),207(25),136(100,118(95) and 59(85)
5	19.12	,(2-Phenyl-1,3-dioxolan-4-yl)methyl ester, cis-9-octadecenoic acid	$C_{28}H_{44}O_4$	444	3.79	376	410	367(5),264(10),105(45),73(100) and 55(35)
6	19.42	3-amino-2-ethyl-4(3H)-Quinazolinone	$C_{10}H_{11}N_3O$	189	2.95	422	552	189(80),173(55),130(45),119(100),90(35)and 76(6)
7	22.49	4,7-Dimethoxy-2-methylindan-1-one	$C_{12}H_{14}O_3$	206	66.67	589	640	206(100),191(35),177(85) and 173(40)
8	33.75	5-Hydroxy-6,7,8-trimethoxy-2,3-dimethyl-chromone	$C_{14}H_{16}O_6$	280	1.09	345	421	280(80),265(100),151(35),91(25) and 57(40)
9	33.88	(5,6,7-Triacetoxy-4b,8-dimethyl-2-oxo-tetradecahydro-phenanthren-1-yl)-acetic acid, methyl ester	$C_{25}H_{36}O_9$	480	4.05	401	432	480(10),407(35),318(25),227(23),135(42) and 121(22)
10	37.35	5-(Hexadecyloxy)-2-pentadecyl-, trans-1,3-dioxane	$C_{35}H_{70}O_3$	538	8.64	402	420	538(2),327(100),222(15),83(22) and 71(30)
C. spinosum leaf extract								
1	3.63	4,6-Dimethyl-undecane	$C_{13}H_{28}$	184	13.35	668	854	184(3),141(7),113(10),85(20),71(55) and 57(100)
2	27.22	10-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	24.66	471	489	296(8),264(20),222(15),97(35),83(50), 74(55),69(60) and 55(100)
3	31.55	Carbamic acid,(methylenedi-4,1-phenylene)bis-, diethyl ester	$C_{19}H_{22}N_2O_4$	342	5.09	339	402	342(80),313(45),296(75),250(45),223(40),132(42),106(35) and 77(25)
4	32.03	(5á)Pregnane-3,20á-diol, 14à,18à-[4-methyl-3-oxo-(1-oxa-4-azabutane-1,4-diyl)]-, diacetate	$C_{28}H_{43}NO_6$	489	4.60	381	399	489(2),459(10),399(25),328(65),161(30),73(45) and 57(85)
5	32.14	3-acetoxy-7,8-Epoxylanostan-11-ol	$C_{32}H_{54}O_4$	502	3.93	384	389	502(5),304(30),244(42),109(50),95(65),69(70) and 57(80)
6	32.47	4-Piperidineacetic acid,1-acetyl-5-ethyl-2-[3-(2-hydroxyethyl)-1H-indol-2-y-l]-à-methyl-, methyl ester	$C_{23}H_{32}N_2O_4$	400	6.05	367	406	400(30),385(35),327(37),240(30),152(25),110(75)
7	33.50	Stearic acid,3-(octadecyloxy)propyl ester	$C_{39}H_{78}O_3$	594	2.08	414	439	594(5),325(60),281(67),85(50) and 71(100)
8	34.83	(2-Phenyl-1,3-dioxolan-4-yl)methyl ester, cis-octadecanoic acid	$C_{28}H_{46}O_4$	446	4.24	393	438	446(5),327(7),267(15),179(10),105(100),77(25) and 57(30)
9	37.33	N-[5-(3-Hydroxy-2-methylpropenyl)-1,3,4,5-tetrahydrobenzo[cd]indol-3-yl]-N -methylacetamide	$C_{18}H_{22}N_2O_2$	298	17.02	395	471	298(5),237(20),225(95),207(100),192(60),167(45),154(55),127(40) and 115(25)
10	38.34	2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)-Propanoic acid	$C_{27}H_{42}O_4$	430	3.71	407	430	430(20),415(45),355(100) and 83(45)
11	38.43	9-Desoxo-9-x-acetoxy-3,8,12-tri-O-acetylingol	$C_{28}H_{40}O_{10}$	536	4.55	352	389	536(3),477(5),357(20),165(20) and 122(25)
12	38.83	Astaxanthin	$C_{40}H_{52}O_4$	596	6.67	365	371	596(10),580(15),203(55),147(70),105(75) and 91(100)
13	39.67	Folic Acid	$C_{19}H_{19}N_7O_6$	441	1.94	365	416	263(10),177(45),120(40),93(70) and 84(100)

1. RT: Retention Time (min.).
2. MW: Molecular Wight (g/mol).
3. SI: Standard Index.
4. RSI: Reverse Standard Index.
5. RI: Relative Intensities.

Arial parts from *D. repens* showed 76% inhibition, while the ethylacetate/methanol fraction showed 88% inhibition of Hepatitis A virus by the plaque reduction assay [24]. The MICs of extracts from *D. repens* (stem and fruits), and their fractions (chloroform and diethyl ether) and compound (mixture of *β*-Amyrin and 12-Oleanene 3*β*, 21*β*-diol) were found to be in the range of 32–128 μg/mL [58] against *E. coli* and not active against *P. aeruginosa*. Methanolic seed extract of *D. erecta* didn't show inhibition against *B. subtilis*. However petroleum ether, *n*-hexane, ethyl acetate, chloroform, ethanol, acetone and water extracts showed no antibacterial activity towards *P. aeruginosa, S. aureus* and *E. coli* [59,60]. The activity of extracts from *D. erecta* may be attributed to the aceteoside or lamiide content which showed high antioxidant activity [31]. The triterpene saponins from the leaves methanol extract and durantanin IV and V and *E/Z* acteoside showed significant cytotoxic activity against a HepG2 cell line as well as showed radical scavenging activity [23]. Different extracts from different plants including *D. repens* demonstrated the most potent cytotoxic activity against tumor cell lines. The chloroform soluble fractions of *D. repens* flowers led to the isolation of quercetin which showed moderate cytotoxic activity [61].

Ethanolic extracts of L. *camara* leaves and roots were shown promising activity against gram-positive and gram-negative strains standard and multi-resistant bacteria isolated from clinical material [(*P. aeruginosa* and *E. coli* (Ec 27)] [62].

From the above results and discussed literature data and in order to establish the safety of leaf extracts from those plants, possible clinical application in therapy of infectious diseases for human pathogens as well as filed trials for plant pathogens should be evaluated further.

4. Conclusions

Antibacterial activity from the present results are clearly varies within the studied species of the plants. Thus, the study ascertains the moderate activity of leaf extracts against *E coli*, *Sar. lutea*, and *Staph. aureus*. Moreover, good activity was found against *A. tumefaciens*, *D. solani*, *P. atrosepticum*, and *P. cartovorum* subsp. *cartovorum*. The value of extracts from these plants could be considered interesting in development of new drugs and safe bactericides used to control plant pathogenic bacteria.

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