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Evaluation of the biological activity of Capparis spinosa var. aegyptiaca essential oils and fatty constituents as Anticipated Antioxidant and Antimicrobial Agents

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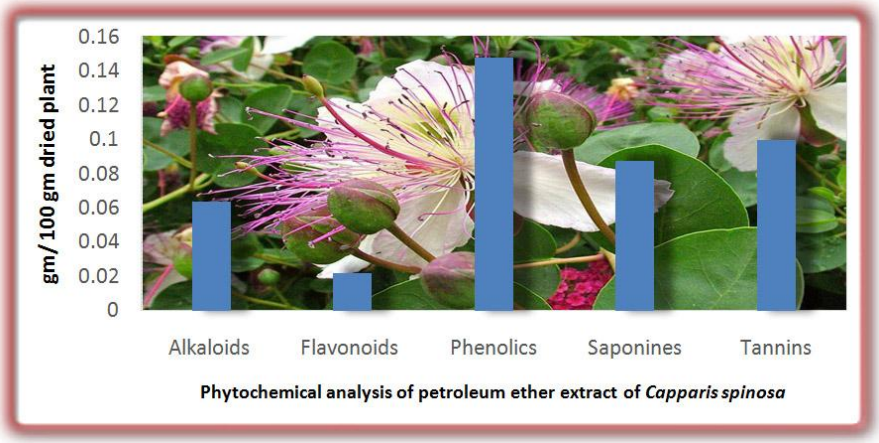
GRAPHICAL ABSTRACT



KEYWORDS

Capparis spinosa
GC/MS
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ABSTRACT

The essential oils and fatty constituents of *Capparis spinosa* as an important medicinal plant were extracted in petroleum ether and tested as antioxidant and antimicrobial agents. The components of the petroleum ether extract were identified by spectroscopic analysis using GC/MS. The spectral analysis showed that this extract contains mainly tetracosane (14.368%), methyl-cyclopentane (11.979%), heptadecane (11.794%), heptacosane (10.098%), eicosane (3.481%), vitamin E (4.280%), α-Cadinol (3.079%), Spathulenol (0.868%), α-Curcumene (1.188%), Muurolol (0.917%), phytol (1.727%), trans geranyl geraniol (0.862%), Palmitic acid (1.132%) and stigmasterol (2.172%). The functional chemical groups were estimated using Fourier transform infrared" FTIR" spectroscopy. The main phytochemical constituents present in this extract were quantitatively estimated. The antioxidant scavenging activity of petroleum ether extract was also examined using DPPH·, FIC, ABTS+· and FRAP assays. The petroleum ether extract of *C. spinosa* expressed a good antioxidant activity. The antimicrobial activity of *C. spinosa* extract was evaluated against several pathogenic bacterial strains such as *Bacillus subtilis, Klebsiella pneunomonia, Erwinia carotovora and Escherichia coli* in addition to *Candida albicans* as pathogenic fungal strain. The extract exhibited a broad antimicrobial spectrum against the tested microorganisms. MIC and MBC of the extract were determined. The results showed inhibitory activity against all the tested organisms while showed cidal activity against only *B. subtilis*, *K. pneunomonia* and *C. albicans*.

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1. Introduction

The popularity of essential oils and extracts from plants is of importance for the characterization of bioactive components that could be used in food processing and several pharmaceutical applications [1, 2]. Formerly, in folk medicine, the essential oils were used for the treatment of many infectious illnesses [2]. The development of antibiotic-resistant bacteria has necessitated the search of novel antimicrobial drugs from the natural origin such as plants [3, 4]. Consequently, diverse applications of essentials oils or plants against pathogenic microbial strains in a large scale including antibiotic-resistant bacterial, fungal and yeast species have been developed [5-8]. *Capparis spinosa* var. *aegyptiaca* (Lam.) Boiss (Capparaceae) comprises about 250 different species. It is characterized by its economic importance [9, 10]. There are six species of genus *Capparis* in Egypt [11]. It is called the caper and grows in the deserts as a perennial winter-deciduous plant that have leaves in a round shape, fleshy, and alternate and the completed flowers are thick, shiny, large white to pinkish-white. It is an edible plant. It is globally founded in dry rocky and desert regions of the Mediterranean, Iran, India, Armenia, and Pakistan [9, 12]. *Capparis* species are well known for their nutritional value and active chemical constitutes present like phenolics, flavonoids, alkaloids, phytosterols, vitamins, and organic acids. It possesses wide antimicrobial spectrum including antibacterial and antifungal activity in addition to their antioxidant, hepatoprotective, anticancer, antiallergic, anthelmintic, antidiabetic, anti-inflammatory, anticancer, and antihyperlipidemic along with their uses in the traditional medicine for controlling of many diseases. The leaves, roots and buds are used for treating gastric, earache, dermatological, liver and kidney disorders, while the fruits used for treating fever, diabetes, rheumatism and headache. [9, 12-16].

2. MATERIALS AND METHODS

2.1. Plant material

C. spinosa was collected in its vegetative state from its natural habitat during the period from mid-August to mid-September 2016 from different localities in the South Sinai region, Egypt. Aerial parts of plant materials were air-dried in shade for fifteen days, later grinded into a fine powder using grinding mill and kept in well stoppered bottles for analysis. The samples' botanical identities were identified and authenticated according to Boulos [17].

2.2. Extraction of the essential oil

The collected aerial parts of *C. spinosa* plant were air-dried and finely ground. Ten grams were extracted by petroleum ether (200 ml) for 5 hours using a Soxhlet apparatus (extraction yield 2.568%). Anhydrous sodium sulfate was used for the drying process of the extracted essential oils, which were stored at 4°C for further analysis.

2.3. Phytochemical analysis of secondary metabolites

2.3.1. Total phenolic contents

The total phenolic contents of the petroleum ether extract expressed as gm gallic acid equivalent/100 gm dried plant material was measured using the modified Folin Ciocalteu colorimetric assay developed by Wolfe *et al* [18].

2.3.2. Total flavonoids content

Flavonoids content of the petroleum ether extract expressed as g catechin equivalent/100 g dried plant material was measured using $AlCl_3$ colorimetric method adopted by Zhishen *et al* [19].

2.3.3. Total Alkaloids content

The alkaloids content of the petroleum ether extract was calculated as gm alkaloids per 100-gm of the dried plant according to Harborne [20].

2.3.4. Total Saponins content

Saponin content of the petroleum ether extract calculated as gm alkaloids per 100-gm dried plant was measured using the assay adopted by Obadoni & Ochuko [21].

2.3.5. Total Tannins content:

The tannin content of the petroleum ether extract expressed as gram gallic acid equivalent/ 100 gram of the dried plant was estimated using Vanillin hydrochloride assay [22].

2.4. Screening of the antioxidant scavenging activity

2.4.1. 2, 2- Diphenyl picryl hydrazyl method (DPPH•)

The antioxidant scavenging activity of *C. spinosa* petroleum ether extract was determined using DPPH radical following the assay described by **Kitts *et al*** [23] with some modifications [24] and the calculations followed by **Parejo *et al.*** [25] were used. An aliquot of one ml of the prepared concentrations of light petroleum extract were tested against one ml of DPPH• (0.135 mM). The absorbance was recorded at 517 nm after 30 minutes at the exclusion of light.

The remaining DPPH˙% for each tested concentration at steady-state was determined using the equation:

$$\% \text{ DPPH}^{\cdot} \text{ remaining} = [\text{DPPH}^{\cdot}]_T / [\text{DPPH}^{\cdot}]_{T=0} \times 100$$

This % DPPH˙ remaining was plotted against mg of plant extract/ mg DPPH˙ using the exponential curve to calculate EC_{50} (the concentration of the extract that has the ability to diminish 50% of the initial DPPH˙ concentration [26].

Antiradical efficiency **$AE = 1 / EC_{50}$**

2.4.2. Antioxidant capacity of plant extract using ($ABTS^+$) assess

ABTS method was estimated according to the technique described by Re *et al* [26]. 2 ml of ABTS solution (phosphate buffer 1 mg/ml, 0.1 M, pH 7.0) were added to 3 ml of MnO_2 (25 mg/ ml). Then centrifugation of the mixture was accomplished and the clear supernatant was obtained. 20 µL of the extract was added to the ABTS solution. Ascorbic acid was used as a control sample.

The absorbance of the sample was determined at 734 nm, and the percentage of inhibition was calculated using the following equation:

$$\% \text{ Inhibition} = \frac{\text{Abs}_{\text{Blank}} - \text{Abs}_{\text{Test}}}{\text{Abs}_{\text{Blank}}} \times 100$$

2.4.3. The ferrous ion chelating (FIC) assay

The FIC assay was measured following the method described by Singh & Rajini [27]. 1 ml of 2 mM $FeSO_4$ was mixed with 1 ml sample then 1 ml of 5 mM ferrozine was added and the mixture left for 10 minutes then the absorbance was estimated at 562 nm. Different dilutions of each sample were assayed. Catechin, Ascorbic acid and gallic acid were used as references.

2.4.4. Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay was adapted by Benzie & Strain [28]. 300 mM acetate buffer pH 3.6 were added to 10 mM TPTZ (2,4,6-tripyridyl-*s*-triazine) and 20 mM $FeCl_3$. 6 H_2O in a ratio of 10:1:1 for preparation of FRAP solution. $FeSO_4$. 7 H_2O: 0.1 - 1.5 mM was used as a standard. 3.6 ml of FRAP reagent were added to 0.4 ml of deionized water and incubated at 37°C for 5 minutes then mixed with a definite concentration of the plant extract and retained for 10 minutes at 37°C. The absorbance was estimated at 593 nm.

2.5. Gas chromatography (GC)/EIMS analysis (pet.ether)

The analysis of gas chromatography (GC/EIMS) was carried out on Agilent Technologies 7890A GC System (5975C inert MSD with Triple-Axis Detector) equipped with an HP-5MS capillary column coated with 5% phenyl-methyl silox (30 m x 250 µm; coating thickness 0.25 µm). Helium at a flow rate 1 ml/minute and a split ratio of 1:20

was used as a carrier gas. 2 µL of the extract was injected. A temperature program of 40°C for 5 minutes.; rising at 3 °C/ minutes to 200 °C and held for 1 minute; rising at 15°C/ minutes to 280°C and held for 10 minutes. The injector and detector were held at 250 and 300°C, correspondingly. The retention times of the identified compounds were compared with those of the authenticated samples and their linear retention indices were compared relative to the *n*-hydrocarbons series and computer matching against commercial (NIST11.L and demo.1). Phytochemical Screening by FTIR analysis of the sample was done at the infrared area between 400-4000 nm to identify the presence of certain functional groups in inorganic molecules.

2.6. Antimicrobial Activity

2.6.1. Pathogenic strains

Antimicrobial potential of the petroleum ether extract was tested against four pathogenic bacterial strains (*Bacillus subtilis* DMS 1088, *Klebsiella pneumoniae* ATCC 10031, *Erwinia carotovora, Escherichia coli* ATCC 10536*)* and one fungal strain (*Candida albicans* EMCC number 105*)* by the disk diffusion assay. The bacterial and fungal strains were acquired from the Genetic Engineering and Biotechnology Unit at Mansoura University.

2.6.2. Disc diffusion assay

The antimicrobial potential of essential oils presents in the petroleum ether extract and dissolved in DMSO were estimated by disc diffusion performance [29] using inoculums of 10^6 bacterial and 10^8 yeast cells / ml to spread on nutrient agar and Sabouraud agar plates, correspondingly. The sterilized discs of Whatman no.1filter paper with 6mm diameter was soaked in the tested extract until saturation. The discs were placed on the agar plates surface seeded with the tested organisms. The seeded plates with bacteria were incubated at 37 ^{o}C for 18-24 hours and those seeded with yeast were incubated at 30 for 24-48 hours then the diameters of inhibition zone (mm) were measured [30]. Paper discs impregnated in a solution of 10 µg/mL of Streptomycin which was used for bacteria and fungi as a standard antibiotic.

2.6.3. Determination of MIC

Several tubes of sterile nutrient broth (for bacteria) and Sabouraud broth (for yeast) containing different dilutions of the essential oil (5µg to 1000 µg/ml) were inoculated with 0.1 ml of standardized inoculum. The tubes were incubated at 37°C for 18-24 h for bacteria and at 30^0C for 24-48 h for yeast. Two control tubes for each organism were maintained. The lowest concentration of the extract that produced no visible growth when compared with the control tubes was observed as the minimum concentration (µg / mL) of the extract necessary for inhibition of the growth of the test organism (MIC) [31].

2.6.4. Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC)

The MBC and MFC were determined by subculturing the tested dilutions that inhibited the growth of the tested organisms in the MIC assay on to a fresh extract-free solid medium suitable for the tested organisms used, and incubated further for 18–24 h for bacteria and for 24-48 h for yeast. The lowest concentration of the plant extract that provide no bacterial growth was the MBC and lowest concentration of the plant extract that provide no fungal growth was the MFC [32].

3. RESULTS AND DISCUSSION

3.1. Phytochemical analysis

The phytochemical analysis of the light petroleum ether extract elucidated the presence of considerable amounts of phenolics, flavonoids, tannins, alkaloids and saponins that are considered as active constituents responsible for the medicinal importance, antioxidant activity and the biological activity of the studied extract of *Capparis spinosa* as illustrated in Table 1.

Table 1. Phytochemical analysis of petroleum ether extract of *Capparis spinosa*.

Extraction yield	2.568%
Alkaloids	0.064 gm/ 100 gm dried plant
Flavonoids	0.0224 gm catechine / 100 gm dried plant
Phenolics	0.148 gm gallic acid / 100 gm dried plant
Saponines	0.088 gm/ 100 gm dried plant
Tannins	0.1 gm gallic acid /100 gm dried plant

Table 2. Antioxidant activity of petroleum ether extract of *Capparis spinosa*.

DPPH assay	EC_{50}= 17.66 mg extract/ mg DPPH; AE= 0.056
FIC assay	IC_{50}= 0.662 mg extract/ mL
ABTS assay	27.54%
FRAP assay	225.0 (mmol Fe(II)/ gm extract)

3.2. Antioxidant scavenging activity of the extract

The antioxidant scavenging activity of the petroleum ether extract was estimated by many methods as illustrated in Table 2. The DPPH$^{\bullet}$ method estimates the antiradical power of extract. The antioxidant scavenging activity of the petroleum ether extract was good with an EC_{50} of 17.66 mg extract/ mg DPPH and antiradical efficacy of 0.056. The ferrous ion chelating **(**FIC) assay was used to estimate the capability of the sample to chelate ferrous ions and the IC_{50} value of the sample was 0.662 mg extract/ml. The radical scavenging activity of *C. spinosa* extracts expressed as a percent of inhibition against ABTS radical showed inhibition percent of 27.54% indicating a low total antioxidant activity. FRAP assay measures the reducing potential of an antioxidant reacting with a Fe^{3+}-TPTZ complex and producing a colored Fe^{2+}-TPTZ. The FRAP value of 225 mmol Fe (II)/1g dried extract revealed its good FRAP activity.

3.3. Chemical composition of the essential oils and fatty content

Agreeing to gas chromatography (GC)/EIMS analysis, forty compounds (99.71%), in which Thirty-three are identified and seven compounds are unidentified in *C. spinosa* essential oil (Table 3).

Among the established constituents, tetracosane (14.368%), cyclopentane, methyl- (11.979%), heptadecane (11.794%), heptacosane (10.098%), vitamin E (4.28%), eicosane (3.481%), butane,2,2,3-trimethyl- (3.311%) and α-Cadinol (3.079), stigmasterol (2.172%), Palmitic acid (1.132%), phytol (1.727%), α-Curcumene (1.188%), Muurolol (0.917%), Spathulenol (0.868%) and trans geranyl geraniol (0.862%) are the major ones. These are followed by nonadecane, octatriacontadiene, oxirane, tridecyl-, 4,8,12,16-tetramethylheptadecan-4-olide, hexadecyl-, *bis*(2-ethylhexyl) phthalate, octasiloxane and 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl in small quantities (Table 3 & **Fig. 1**).

Several studies were done regarding the essential oils' composition in the hydrodistillate of *C. spinosa*. The Iranian caper was found to contain thymol, 2-hexenal, isopropyl and butyl iso-thiocyanate as the main constituents in the leaves while methyl and isopropyl isothiocyanate were found to be the main constituents in fruits and roots. [33]. methyl iso-thiocyanate was found to be the main component in the essential oils of *C. spinosa* from Croatia and the Eolian-Archipelago [34]. Isopropyl, methyl and butyl iso-thiocyanate were the main constituents in the Jordanian caper [35]. Consequently, isothiocyanato-methane and 2-isothiocyanatopropane are principally found as constituents of in fruits and roots, in which 1-isothiocyanatobutane is tissue-specific that was found in leaves but not found in fruits and roots [36]. Thiocyanate and isothiocyanate were not identified in the

petroleum ether extract of the studied caper. Syrian caper found to contain thymol, Octanoic acid, methyl isothiocyanate and 2-hexenal, palmitic acid, oleic acid and linoleic acid[37]. Out of this we could conclude that new valuable compounds have been identified in the studied extract of *C. spinosa*.

Many applications were reported for many of the identified compounds, for example diterpene alcohols like geranyl geraniol are inhibitors of *Mycobacterium tuberculosis* [33] and phytol is utilized as a originator for manufacturing of vitamin E [34] and vitamin K1[35]. Also the unsaturated phytosterols stigmasterol is utilized as a originator for manufacturing of the semisynthetic hormone progesterone [36-38], that contributes to regulatory and tissue rebuilding mechanisms in addition to its role as an intermediate in the biosynthesis of androgens, estrogens, corticoids and is also used as the originator of vitamin D3[39]. Vitamin E is an important constituent that has many biological functions, including its anticancer activity and its role as a fat-soluble antioxidant that is capable of disabling the production of dangerous free radicals in tissues [40-42]. α-Cadinol was reported to have anti-fungal [43] and hepatoprotective activity [44] in addition to acting as a remedy for drug-resistant tuberculosis [45].

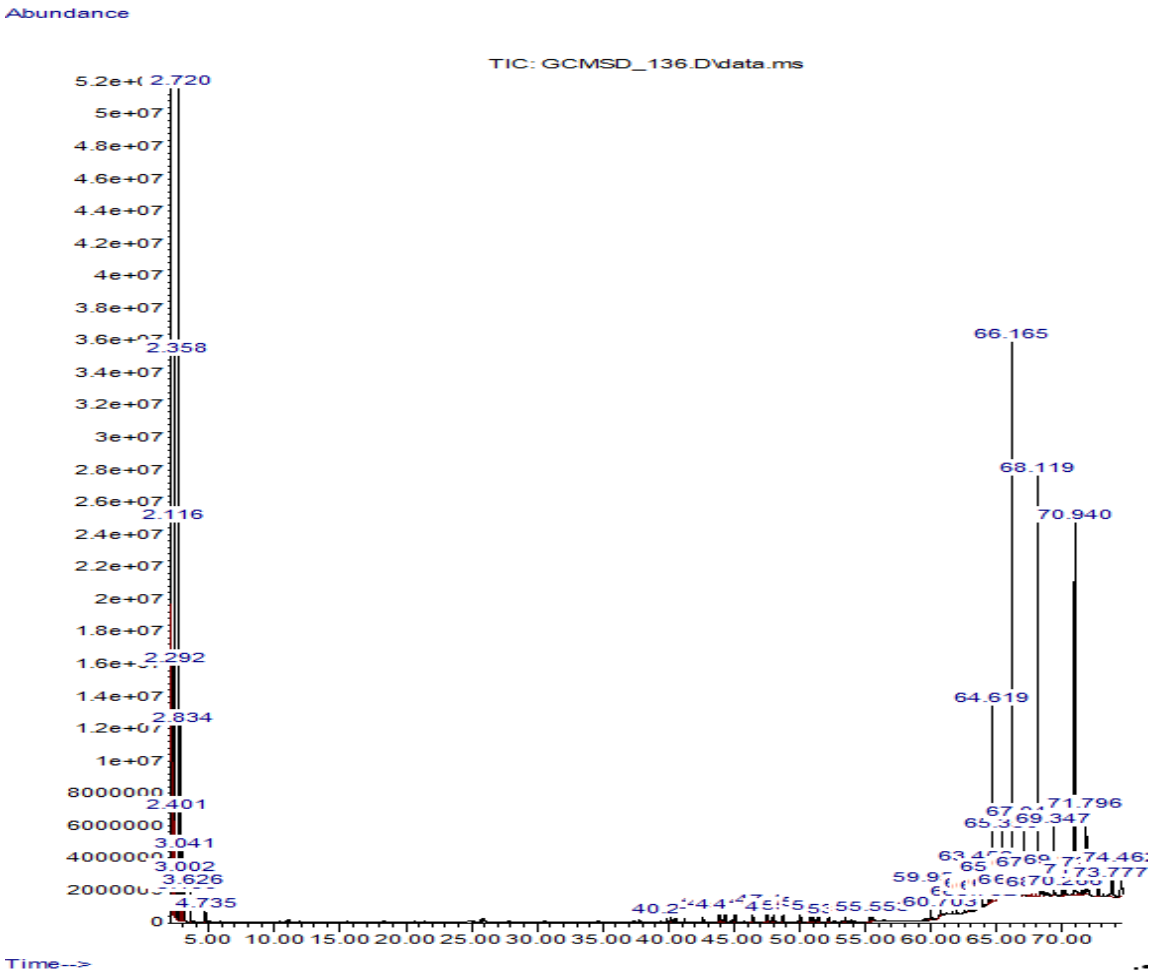

Fig.1. Chromatogram of *C. spinosa* leaves extract by GC-MS.

Table.3. The elucidated chemical composition of the extract leaves of *C. spinosa*

Entry	Chemical name	Classification	Retention time (min)	Molecular Weight	Molecular formula	Composition %
1	Butan,2,2,3-trimethyl-	alkane	2.292	100.2	C_7H_{16}	3.311
2	Cyclopentane,methyl-	cycloalkane	2.358	84.16	C_6H_{12}	11.979
3	Hexane,3,4-dimethyl-	alkane	2.401	114.23	C_8H_{18}	1.599
4	Pentane,3,3-dimethyl-	alkane	2.593	100.2	C_7H_{16}	1.042
5	Hexane,3-methyl-	alkane	2.834	100.2	C_7H_{16}	2.932
6	Cyclopentane,1,3-dimethyl-	cycloalkane	2.959	98.19	C_7H_{14}	1.957
7	Cyclopentane,1,2-dimethyl-, trans-	cycloalkane	3.041	98.19	C_7H_{14}	1.515
8	Heptane	alkane	3.188	100.2	C_7H_{16}	1.043
9	Cyclohexane, methyl-	cycloalkane	3.626	98.19	C_7H_{14}	1.183
10	Spathulenol	tricyclic sesquiterpenoid	40.219	220.36	$C_{15}H_{24}O$	0.868
11	α-Curcumene	sesquiterpene	47.991	202.34	$C_{15}H_{22}$	1.188
12	Unidentified		51.456			0.336
13	Muurolol	cadinane sesquiterpenoid	55.281	222.37	$C_{15}H_{26}O$	0.917
14	Palmitic acid	Fatty acid	55.553	256.42	$C_{16}H_{32}NO_2$	1.132
15	Phytol	acyclic diterpene alcohol	59.951	298.55	$C_{20}H_{42}O$	1.727
16	9,12-Octadecadienoic acid (Z, Z)-	Fatty acid	60.703	280.45	$C_{18}H_{32}O_2$	0.889
17	α-Cadinol	sesquiterpenoid alcohol	62.876	222.37	$C_{15}H_{26}O$	3.079
18	4,8,12,16-Tetramethylheptadecan-4-olide	terpenoid	63.458	324.54	$C_{21}H_{40}O_2$	1.401
19	Trans-Geranylgeraniol	diterpenoid	63.732	290.48	$C_{20}H_{34}O$	0.862
20	1-Nonadecene	alkene	64.432	280	$C_{19}H_{38}$	0.857
21	Nonadecane	alkane	64.619	268.52	$C_{19}H_{40}$	2.928
22	Hexadecane, 1-(ethenyloxy)-	alkane	64.861	268.48	$C_{18}H_{36}O$	1.031
23	Bis(2-ethylhexyl) phthalate	phthalates	65.026	388.54	$C_{24}H_{32}O_4$	1.248
24	Unidentified		65.151			0.107
25	Eicosane	alkane	65.350	282.55	$C_{20}H_{42}$	3.481
26	Unidentified		65.954			0.075
27	Heptacosane	alkane	66.165	380.73	$C_{27}H_{56}$	10.098
28	Z-11(13-methyl) tetradecen-1-ol acetate	ester	66.484	268.44	$C_{17}H_{32}O_2$	0.968
29	Oxirane,hexadecyl-	hexadecyloxirane	67.460	268.48	$C_{18}H_{36}O$	1.255
30	Unidentified		67.673			0.188
31	Unidentified		67.782			1.034
32	Heptadecane	alkane	68.119	240.47	$C_{17}H_{36}$	11.794
33	Unidentified		68.606			0.100
34	Oxirane,tridecyl-	tridecyloxirane	69.997	226.4	$C_{15}H_{30}O$	1.537
35	Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	Silicon compound	70.266	579.25	$C_{16}H_{50}O_7Si_8$	0.928
36	Tetracosane	alkane hydrocarbon	70.940	338.65	$C_{24}H_{50}$	14.368
37	Unidentified		71.470			0.462
38	Vitamin E	alpha tocopherol	71.796	430.71	$C_{29}H_{50}O_2$	4.28
39	1,37-Octatriacontadiene	alkene	73.777	530.99	$C_{38}H_{74}$	1.839
40	Stigmasterol	plant sterol	74.462	412.7	$C_{29}H_{48}O$	2.172
	Σ					Σ = 99.71

3.4. FTIR analysis:

The functional chemical groups were determined by Fourier transform infrared spectroscopy. The FTIR spectrum showed the characteristic absorption bands at ν (cm-1) = 3380 (O-H, stretching), 2919 (CH2, asymmetric stretching), 2855 (CH2, asymmetric stretching), 1714 (carboxylic acid C=O stretching), 1653 (C=O, ketone), 1452 (aromatic C=C bending), 1153, 1090 (C-O, stretch) and 854 (aromatic C-H, bending) (**Fig. 2**).

3.5. Evaluation of the antimicrobial activity

There is a demand for the discovery of new antimicrobial drugs from natural sources like plants. There are several studies that have been done regarding the antimicrobial activity of *C. spinosa*. The antimicrobial potentials of *C. spinosa* plant extracted by petroleum ether were examined using disc diffusion assay method against several pathogenic species of antibiotic resistant bacteria including *Bacillus subtilis*, *Klebsiella pneumoniae*, *Erwinia carotovora* and *Escherichia coli* and against the pathogenic fungal species *Candida albicans*.

The obtained results elucidate that the extract of the investigated plant, generally, possess variable degrees of activities against the tested microorganisms. *C. spinosa* extract showed inhibitory activity against the growth of all tested microorganisms as indicated in Table 4, while the cidal activity was exhibited only against *C. albicans, Bacillus subtilis* and *Klebsiella pneumoniae*.

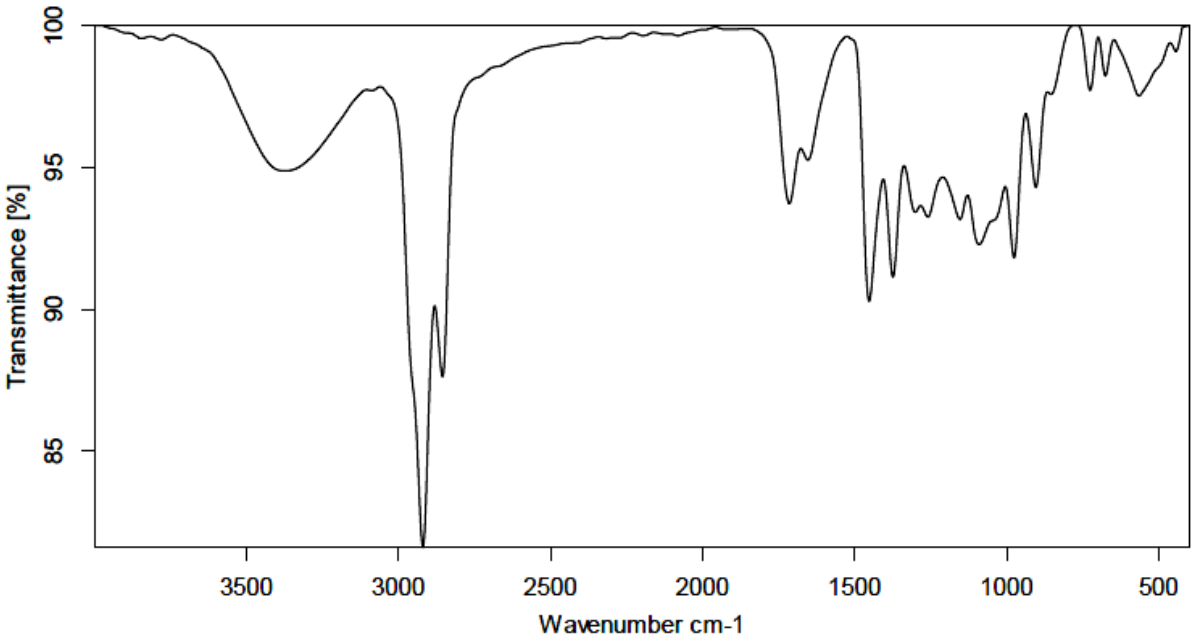

Fig. 2: FTIR analysis for petroleum ether extract of *Capparis spinosa*.

Table 4. Antimicrobial and antifungal potential of the petroleum ether extract of *C. spinosa* utilizing agar disc diffusion, MIC and MBC assays.

Microbial strains	DD [a]	Streptomycin [b]	MIC [d]	MBC [e]	MFC [f]
Bacillus subtilis	8	15	0.470	0.625	-
Klebsiella pneumoniae	8	-ve	0.470	0.625	-
Erwinia carotovora	8.5	18	0.625	-	-
Escherichia coli	9.5	-ve	0.312	-	-
Candida albicans	9	13	0.312	-	0.470

(*a*: DD, agar disc diffusion method. Diameter of inhibition zone (mm); including disk diameter of 6 mm; *b*: Streptomycin (antibacterial and antifungal); *d*: Minimal Inhibitory Concentrations (MIC) (mg/ml). *e*: Minimal Bactericidal Concentrations (MBC) (mg/ml)). *f* Minimal Fungicidal Concentrations (MFC) (mg/ml)).

Conclusion

The present work reported a rational justification on the essential oils and fatty constituents of the petroleum ether extract from the leaves of *C. spinosa*. The study of the antioxidant and antimicrobial activity of this extract showed that it moderate radical scavenging activity and high antimicrobial potential. From the antioxidant results, it can be concluded that the petroleum ether extract has a moderate activity, in which the obtained results by different assays are compatible with each other. In addition, the extract has potent antimicrobial activity against *K. pneumoniae* and *E. coli* than the antibiotic standard, while moderate activities were noticed against *B. subtilis* and *E. carotovora*. Furthermore, a moderate antifungal activity of the extract against *C. albicans* was found with an inhibition zone = 9 mm. The petroleum ether extract has the least MIC at 0.312 mg/ml against *E. coli* and *C. albicans*. Additional studies will be desirable to isolate, and purify other various constituents that are liable for many known therapeutic and nutritional virtues from literature for this plant.

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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