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Original article

Comprehensive Phytochemical and GC-MS Analysis of Leptadenia pyrotechnica: Unveiling Antioxidant and Antibacterial Potential.

Sura Goma Mohamud[1], Safaa M ismaiel[1], Samia Ali Haroun[2], Ghada A. El-Sherbeny[2], Asmaa M Radwan[1*]

[1] Botany and Microbiology Department, Faculty of Science, Girls Branch, Al-Azhar University, Nasr City, Cairo 11884 Egypt.

[2] Botany Department, Faculty of Science, Mansoura University, Mansoura 35516, Egypt.

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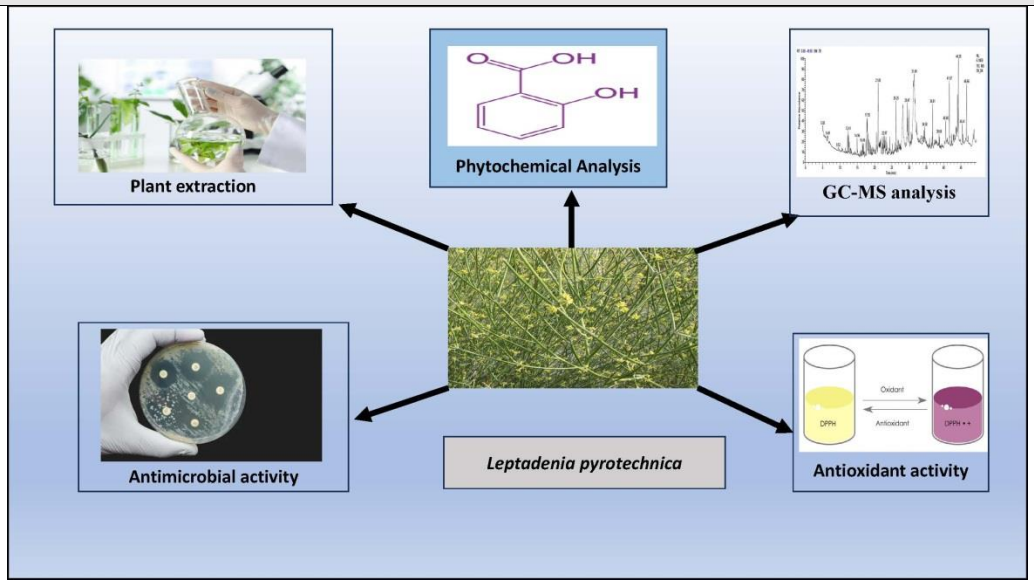
ABSTRACT

The increasing global popularity of wild plants is linked to their potential as sources of antioxidants and phytochemical components that could be used in medicine as antibacterial agents against antibiotic-resistant bacteria. This study aims to determine and compare the phytochemical secondary metabolites, essential oils, fatty constituents, free radical scavenging activity, and antibacterial potential of water, methanol, and petroleum ether extracts from the aerial parts of *Leptadenia pyrotechnica* (Forssk) Decne growing in Egypt. The results indicated that the extracts showed variable content of total phenolics, flavonoids, tannins, alkaloids, and saponins. The water extract recorded the highest secondary metabolite content and radical scavenging effect, as measured by the DPPH assay (IC50 = 0.16 mg extract/ml). GC-MS analysis revealed fifty-six volatile constituents, including thirteen non-oxygenated hydrocarbons, fourteen oxygenated hydrocarbons, eight terpenes, seventeen fatty acids and lipids, and four steroidal components. Regarding antibacterial properties, the studied extracts demonstrated antibacterial activity against *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus cereus*, and *Staphylococcus epidermidis*. The extracts of *L. pyrotechnica* could be utilized as antioxidant resources, drugs, and antibacterial botanicals to combat antibiotic-resistant bacterial strains.

Graphical abstract



∗ *Corresponding author*
E-mail address: asmaamahmoud.59@azhar.edu.eg

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1. Introduction

Medicinal plants are invaluable in a traditional context and modern healthcare systems, serving as a reservoir of bioactive compounds with diverse therapeutic applications. The increasing global challenges of antibiotic resistance and oxidative stress-related diseases necessitate the exploration of natural alternatives to synthetic drugs. Among these, xerophytic plants, known for their adaptation to harsh environments, are exciting due to their rich and unique phytochemical profiles—Decne., a perennial shrub, renowned for its medicinal and ecological significance.

Leptadania pyrotechnica (Forssk.) Decne, famous by Khimp or Khip, is a xerophytic plant that belongs to the family Asclepiadaceae [1, 2]. It is a perennial, ascendingly growing, leafless, erect, and evergreen shrub of 0.5 to 3 meters in height [3]. It possesses a glabrous stem, green to pale yellow with watery sap, and yellow flowers which are actinomorphic and bisexual. The plant sepals are free at the top and joined together at the base [4]. *L. pyrotechnica* has fruits that resemble pods, which could be used as a vegetable for cooking. Besides, its seeds have tufts of hair on them. The plant roots elongated and extended, acting as soil binders, stabilizing the dune habitat [3]. It possesses frantic economic potential as a nutritional and medicinal value, and it is utilized by inhabitants of desert zones [5]. It is instinctive to the Mediterranean region and the semi-arid deserts of Africa and Asia [6].

L. pyrotechnica is used in folklore medicine for anti-constipation, treatment of fatness, and dysmenorrhea [7]. Its decoction treats upper gastrointestinal tract syndromes, spermatorrhea, flu, lactagogue, and tussive [8, 9]. *L. pyrotechnica* extracts comprise diverse biologically active categories of molecules, for instance, polyoxypregnanes, sterols, flavonoids, triterpenes, cardenolides, and alkaloids [10]. The bioactivity of the natural constituents was found to be antitumor, anti-inflammatory, diuretic, and anti-analgesic [11-13].

Traditionally, it has been used to treat gastrointestinal disorders, respiratory ailments, obesity, and other health issues, reflecting its significant role in ethnomedicine [7]. Beyond its medicinal applications, the plant's roots, seeds, and aerial parts are also valued for their nutritional properties, particularly in resource-scarce desert communities [4].

L. pyrotechnics has antimicrobial activity against several pathogenic microbial strains, including fungi such *as A. flavus and F. moniliformis [14]* and bacteria *such as M. flavus, S. aureus, P. aeruginosa, E. coli, and B. subtilis [15]*. The plant also exhibited anticancer action against the human MCF-7 breast cancer cell line *and* expressed significant anti-inflammatory and antioxidant potential [16, 17].

Phytochemical investigations reveal that *L. pyrotechnica* is a rich source of secondary metabolites, including alkaloids, flavonoids, sterols, terpenes, saponins, and fatty acids, and these compounds exhibit diverse biological activities that contribute to the plant's pharmacological potential [18]. Notably, flavonoids and polyphenols in *L. pyrotechnica* are reported to possess potent antioxidant properties, protecting against oxidative stress and cellular damage [16, 19]. Steroidal components such as stigmasterol and β-sitosterol have anti-inflammatory and cholesterol-lowering effects, further highlighting the plant's medicinal relevance [10, 17].

The antimicrobial properties of *L. pyrotechnica* are equally noteworthy. Its extracts have demonstrated activity against a broad spectrum of pathogens, including fungal species like *Aspergillus flavus* and *Fusarium moniliforme* and bacterial strains such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Bacillus subtilis* [14, 15, 20]. These antimicrobial effects are attributed to the presence of bioactive terpenoids, alkaloids, and fatty acid derivatives, which disrupt microbial cell membranes and inhibit key enzymatic processes [7,11]. The plant's ability to combat antibiotic-resistant pathogens underscores its potential as a natural antimicrobial agent. Previous studies have focused mainly on isolated compounds, with limited exploration of whole-plant extracts and their bioactivity profiles. Additionally, the stability and bioavailability of these compounds in pharmaceutical formulations are poorly understood.

This study aims to investigate the phytochemical composition and biological activities of *Leptadenia pyrotechnica* (*L. pyrotechnica*). The research identifies key bioactive compounds and evaluates their antioxidant and antimicrobial potentials by employing a multi-solvent extraction strategy (water, methanol, and petroleum ether) and advanced analytical techniques, such as Gas Chromatography-Mass Spectrometry (GC-MS). The study establishes a link between the phytochemical profile and its observed biological effects, providing a strong foundation for the sustainable use of *L. pyrotechnica* in drug discovery and development.

Furthermore, this research contributes to the growing literature on *L. pyrotechnica* by exploring its pharmacological significance, particularly in combating oxidative stress and antibiotic-resistant infections. The findings underscore the potential applications of *L. pyrotechnica* as a natural resource for nutraceuticals, antimicrobial agents, and therapeutic compounds.

2. Materials and methods

Plant Materials

Leptadania pyrotechnica aerial parts were gathered from Wadi Hagool, Egypt. Prof. Ghada El-Sherbeny taxonomically ascertained the plant and authenticated it according to [21].

Phytochemical Investigations

Preparation of plant extracts: Three polar and nonpolar solvents (water, methanol, and petroleum ether) were used to extract the naturally active constituents in the aerial parts of Leptadania pyrotechnics.

Water extract: 10 grams of *L. pyrotechnica* dried aerial parts were combined with 100 mL of distilled water and

kept at 70 ºC for 30 minutes at 200 rpm in a water bath shaker. Using a Buchner funnel and Whatman filter paper No. 1 (Whatman Int. Ltd., Kent, UK), the mixtures were separated for further analysis and stored at 4°C.

Methanol extract: 10 grams of *L. pyrotechnica* dried aerial parts were combined with 100 mL of methanol and kept at room temperature in a water bath shaker for 4 hrs. at 200 rpm. Using a Buchner funnel and Whatman filter paper No. 1 (Whatman Int. Ltd., Kent, UK), the mixtures were separated for further analysis and stored at 4º C.

Petroleum ether extract: Petroleum ether extract was made using the Soxhlet extraction method [22]. A Soxhlet thimble linked to a Soxhlet extractor was filled with 10 grams of dried plant aerial parts, and 200 milliliters of petroleum ether were added to a flask with a flat bottom (500 mL), and the extraction process was held for 5 hours. The resulting extract was then concentrated to 50% of its original volume. For further analysis, the obtained extract was filtered by Whatman filter paper No. 1 (Whatman Int. Ltd., Kent, UK) and kept at 4º C.

Total Tannin Contents

The vanillin-hydrochloride assay was used to quantify condensed tannins (proanthocyanidins) in plant extracts. The vanillin reagent was prepared by dissolving 4% vanillin in methanol, and the HCl reagent was made by mixing concentrated HCl to create an 8% solution in methanol. For the analysis, 0.5 mL of the plant extract was combined with 2.5 mL of the vanillin reagent and 2.5 mL of the HCl solution. The mixture was incubated at room temperature for 20 minutes. The absorbance was measured at 500 nm using a UV-Vis spectrophotometer. A standard curve was constructed using tannic acid solutions. The plant extract containing tannin was measured and represented as grams of tannic acid equivalents /100 grams of dried sample. The amount of tannins present in the samples was calculated through a standard curve for tannic acid ($y = 0.0009x$; $r2 = 0.955$) [23].

The vanillin-hydrochloride assay was applied to analyze the tannin concentration, which involved measuring the absorbance of samples following processing with newly generated vanillin-hydrochloride.

Phenolics content

The total phenolic content (TPC) was determined using the Folin-Ciocalteu method described by [24]. Apple peel extracts were prepared by sonicating 1 g of freeze-dried, powdered peels in 10 mL of 80% methanol for 30 minutes at room temperature. The mixture was centrifuged at 10,000 × g for 15 minutes, and the supernatant was collected. This extraction was repeated twice, and the supernatants were pooled and filtered. For TPC analysis, 0.5 mL of the extract was mixed with 2.5 mL of diluted Folin-Ciocalteu reagent and added 2 mL of 7.5% sodium carbonate solution. The mixture was incubated for 30 minutes in the dark, and absorbance was measured at 765 nm**.** The obtained values were calculated by the standard curve of Gallic acid ($y = 0.0062x$, $r2 = 0.987$) and represented as milligram Gallic acid equivalents/grams of the dried sample [24, 25].

Flavonoids content

The flavonoid content was determined using an aluminum chloride colorimetric assay, which was represented as milligrams of catechin equivalent per gram of the plant's dry weight. Flavonoid extracts are prepared by dissolving 1 g of the sample in methanol and extracting with reflux for 1 hour. The extract is then filtered and evaporated to dryness. For the assay, 0.5 mL of the extract is mixed with 1.5 mL of methanol, followed by the addition of 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water. The mixture was allowed to react for 30 minutes, and the absorbance was measured at 415 nm. The total flavonoids were determined through the standard curve of catechin ($y = 0.0028$ x, $r2= 0.988$) [26].

Saponins content

Using the technique described by [27], saponin content was calculated. In this procedure, a dry plant sample that had been weighed was added to 100 mL of the preferred extraction solvent. The solution was shaken for two hours at 200 rpm and 30 °C. Using Whatman filter paper No. 1, the prepared extract was filtered out, and the leftover plant material was then again extracted using the extraction solvent and filtered. Over a water bath, the produced extract was concentrated to a set volume (40 mL). Therefore, 40 mL of the extracted sample was placed into a separate funnel, followed by 20 mL of diethyl ether; finally, the blend was vigorously agitated. The organic ether layer was separated from the aqueous layer. Multiple times, the separation process was emphasized. The aqueous layer was treated twice with sodium chloride solution (10 mL, 5%) before being treated once with n-butanol (60 mL). To evaporate the solvent over a water bath, the residual solution was heated. The sample was dried in the oven and weighed after the solvent had evaporated entirely. One gram of dried extract of the saponin contains mg of saponins.

Total Alkaloid contents

Using an ammonium hydroxide solution, the total content of alkaloid was determined. As a result, 1 ml of the sample and 1 ml of a concentrated ammonium hydroxide solution were combined. After allowing the solution to settle, 2 ml of diluted ammonium hydroxide solution was used to wash the precipitate that had formed. After that, the weighted filter paper was used to filter the precipitate. The weighed filter paper was dried with the precipitate after the filtration procedure and then weighed once more with the precipitate. As reported by [28], the alkaloid contents were estimated as a weighted value.

Potential DPPH antioxidant assay

The antioxidant aptitude of the analyzed extract samples was examined by DPPH• colorimetric approach utilizing ascorbic acid as a standard [29]. A serial dilution for each sample was carried out by adding an equal quantity of methanol. In a serial dilution, an equivalent volume of DPPH• solution was generated at a fixed concentration (0.135 mM), then it was mixed with the examined sample. After adding the DPPH solution, he prepared samples that were maintained for 30 minutes at room temperature in the dark. The absorbance of samples (517 nm) was measured. The percentage of remaining DPPH in the solution was calculated:

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

The exponential curve was designed for the % remaining DPPH• versus the different concentrations (mg/ml) to estimate the values of the effective concentration "IC_{50}." The strength of the sample for capturing the free radicals in the solution of DPPH• by 50% was identified as the IC_{50} value. The IC_{50} results establish an opposite correlation between the tested sample's antioxidant aptitude.

Gas chromatography–electron ionization mass spectroscopy (GC/EIMS)

GC/EIMS analysis was performed on a 7890A GC System (5975C inert MSD with Triple-Axis Detector) equipped with an HP-5MS capillary column coated with 5% phenyl-methyl siloxane. Helium was used as the carrier gas at a 1 mL/min flow rate. The temperature program was as follows: the initial temperature was maintained at 40°C for 5 minutes, increased by 3.0°C/min to 200°C and held for 1 minute, then further increased by 15°C/min to 280°C and held for 10 minutes. The injector and detector temperatures were set at 250°C and 300°C, respectively. Diluted extracts (0.2 µL) of the samples were injected in split mode. The mass spectra were obtained using electron ionization at 70 eV with a scanning range of m/z 50–550. Compound identification was achieved through comparison with commercially available databases (NIST11.L and demo.1), which rely on spectral matching and corroboration with literature data. Quantification was performed based on the relative abundance of the compounds, calculated using peak areas in the chromatograms. Efforts were made to ensure accuracy by analyzing known standards for comparison, where available, and evaluating potential co-elution of peaks in the complex plant chemical profile.

Determination of antimicrobial efficiency

The antimicrobial efficiency of the aerial parts extract in petroleum ether was assessed versus nine pathogenic bacterial species (*Bacillus subtilis*, *Klebsiella pneunomonia*, *Enterobacter colacae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella typhi)* by agar well diffusion assay. The investigated microbial species were from the genetic engineering and biotechnology unit (Mansoura University).

Agar well diffusion assay: A volume of the microbial species was distributed over the whole surface of the agar plates. Subsequently, a 7 mm hole diameter was made with a sterile tip or cork borer, and a known amount (40 µL) of the tested sample extract was placed into the pore. Gentamycin (10 µg/disc) was used as a control. The antimicrobial efficiency was determined by measuring the inhibition zone around the made pore injected by the tested sample [30, 31].

3. Results and discussion

The components of the aerial portions extracted from *L. pyrotechnica* were identified using a Gas Chromatography-Mass Spectrometry (GC-MS) assay. The sample components were identified based on the molecular structure, molecular weight, and the calculated fragments that were recorded at a definite retention time. The interpretation of the volatile components was constructed based on the conducted mass spectrum GC-MS using the database of NIST11.L and demo.1. The components' names, classification, retention time, molecular weight, molecular formula, and composition percentage of the compounds of *L. pyrotechnica* aerial portions were ascertained. The amount of the relative percentage of each volatile compound was determined by matching its average peak area to the total areas. This describes whether this plant species comprises any specific molecule or group of molecules, which might demonstrate its standing commercial and traditional usage as an herbal medicine. Furthermore, it supports investigating the most fitting approaches to extracting these constituents. These results will subsequently be deliberated in the light of their recognized biological or therapeutic significance.

Phytochemical screening revealed the presence of a considerable level of essential oils and fatty constituents as indicated in Table (1). In addition, there were significant secondary metabolites, including phenolics, flavonoids, tannins, alkaloids, and saponins, with notable differences across the extracts (Table 2).

GC-MS analysis identified fifty-six volatile compounds across categories such as hydrocarbons, terpenes, fatty acids, and steroids (Table 1). The relative abundance of a definite volatile component was recorded at different retention times as shown in **Figure 1**. The hydrocarbons heptacosane (12.69%) and dotriacontane (5.41%) were the most abundant, comprising a significant portion of the non-oxygenated hydrocarbons. The oxygenated hydrocarbons included 2-methylhexadecan-1-ol (7.46%), while terpenoid compounds such as (S)-2-methyl-6-(p-tolyl) hept-2-en-4-one (4.91%) were also prominent. These findings highlight the diverse phytochemical profile of *L. pyrotechnica* and its potential bioactivity. (Table 1).

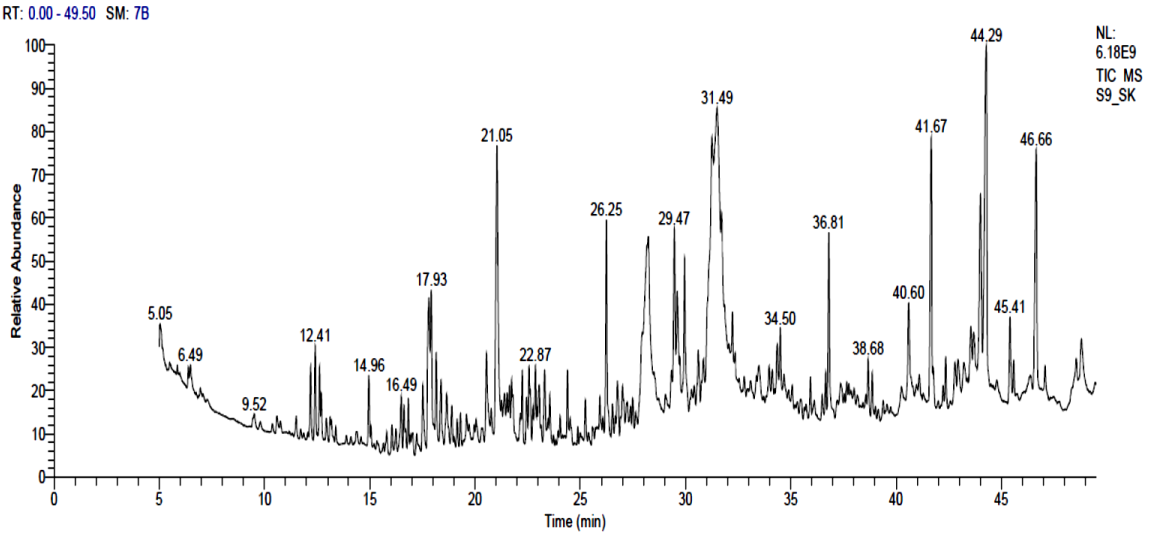

Figure 1. GC-MS Chromatogram of the relative abundance versus retention time of the prime molecules extracted from *L. pyrotechnica*.

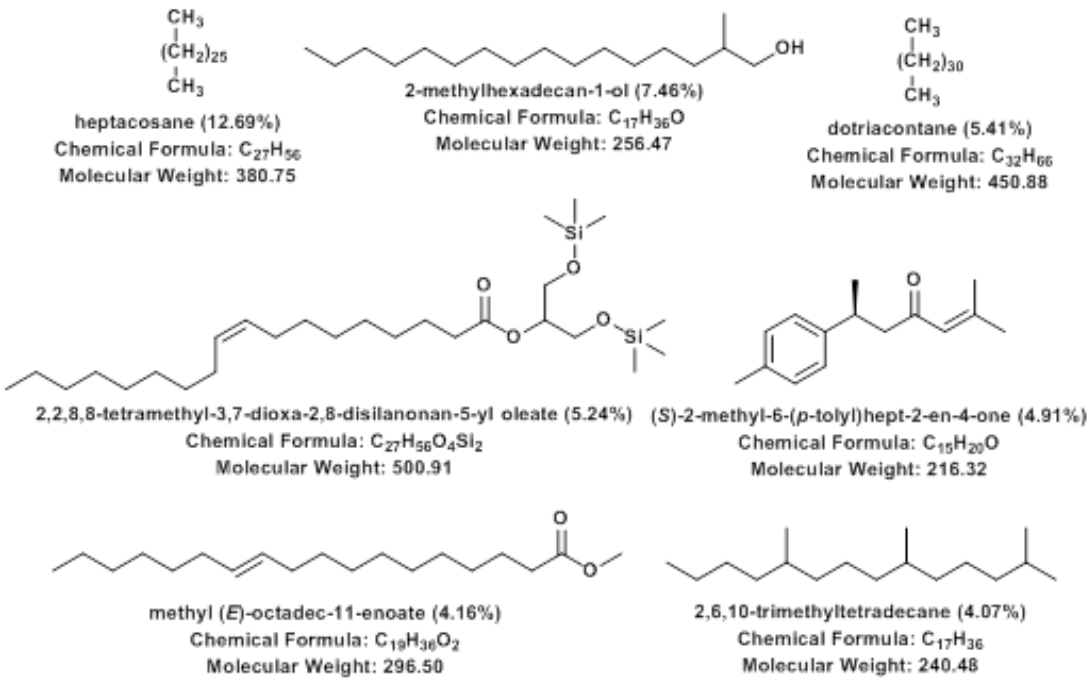

Figure 2. The structures of the major abundant volatile constituents of the extracted *L. pyrotechnica* were interpreted by GC/MS analysis.

Overall, thirteen compounds were classified as non-oxygenated hydrocarbons, fourteen as oxygenated hydrocarbons, eight as terpenes, seventeen as fatty acids and lipids, and four as steroids. The relative abundance of these volatile components was assessed by comparing their average peak area to the total peak areas. These findings suggest that *L. pyrotechnica* contains diverse bioactive molecules, supporting its traditional and potential commercial use as an herbal medicine. Furthermore, this study provides a foundation for investigating effective extraction methods for these compounds and understanding their biological or therapeutic significance. Key findings showed that the major constituents included oxygenated hydrocarbons like 2-methylhexadecan-1-ol (7.46%), non-oxygenated hydrocarbons such as heptacosane (12.69%), and dotriacontane (5.41%), terpenes like (S)-2-methyl-6-(p-tolyl) hept-2-en-4-one (4.91%), and esters of fatty acids such as 2,2,8,8-tetramethyl-3,7-dioxa-2,8-disilanonan-5-yl oleate (5.24%). These major components accounted for 43.94% of the total area of identified compounds.

Table 1. The interpreted components of the extracted *Leptadenia pyrotechnica*.

Entry	Chemical name	Classification	Retention time (min)	Molecular Weight	Molecular formula	Area %
	Non-Oxygenated Hydrocarbons					
1	3,4-dimethylhex-1-ene	Hydrocarbon	5.05	112.22	C_8H_{16}	0.61
2	8-methylenepentadecane	Hydrocarbon	6.49	224.43	$C_{16}H_{32}$	0.36
3	Hexadecane	Hydrocarbon	11.50	226.45	$C_{16}H_{34}$	0.29
4	Dodecane	Hydrocarbon	12.70	170.34	$C_{12}H_{26}$	0.66
5	2,6,10-trimethyltetradecane	Hydrocarbon	12.94	240.48	$C_{17}H_{36}$	4.07
6	Tetradecane	Hydrocarbon	13.18	198.39	$C_{14}H_{30}$	0.18
7	(1*R*,9*S*,*E*)-4,11,11-trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene	Hydrocarbon	14.96	204.36	$C_{15}H_{24}$	0.84
8	2,3,5,8-tetramethyldecane	Hydrocarbon	16.06	198.39	$C_{14}H_{30}$	0.35
9	Docosane	Hydrocarbon	22.57	310.61	$C_{22}H_{46}$	1.10
10	2-methylpentadecane-2-thiol	Hydrocarbon	27.23	258.51	$C_{16}H_{34}S$	0.36
11	heptacosane	Hydrocarbon	38.86	380.75	$C_{27}H_{56}$	12.69
12	nonacosane	Hydrocarbon	41.67	408.80	$C_{29}H_{60}$	3.98
13	Dotriacontane	Hydrocarbon	48.80	450.88	$C_{32}H_{66}$	5.41
	Oxygenated Hydrocarbons					
14	1-hydroperoxyhexane	Hydrocarbon	6.39	118.18	$C_6H_{14}O_2$	0.24
15	2-methyldecan-1-ol	Hydrocarbon	12.19	172.31	$C_{11}H_{24}O$	2.08
16	2-methylhexadecan-1-ol	Hydrocarbon	17.52	256.47	$C_{17}H_{36}O$	7.46
17	2-allyl-5-(*tert*-butyl) benzene-1,4-diol	Hydrocarbon	17.80	206.29	$C_{13}H_{18}O_2$	2.84
18	3,7,11-trimethyldodecan-1-ol	Hydrocarbon	18.16	228.42	$C_{15}H_{32}O$	2.03
19	5-allyl-1,2,3-trimethoxybenzene	Hydrocarbon	18.65	208.26	$C_{12}H_{16}O_3$	1.0
20	6-(3-hydroxyprop-1-en-2-yl)-4-methyl-1,2,4a,5,6,7,8,8a-octahydronaphthalen-2-ol	Hydrocarbon	20.78	222.33	$C_{14}H_{22}O_2$	0.25
21	tetradecan-1-ol	Hydrocarbon	23.04	214.39	$C_{14}H_{30}O$	1.8
22	hexadecan-2-ol	Hydrocarbon	23.31	242.45	$C_{16}H_{34}O$	1.5
23	(*E*)-3,7,11,15-tetramethylhexadec-2-en-1-ol	Hydrocarbon	24.39	296.54	$C_{20}H_{40}O$	0.85
24	stearaldehyde	Hydrocarbon	30.61	268.49	$C_{18}H_{36}O$	1.2
25	(*Z*)-hexadec-7-enal	Hydrocarbon	33.48	238.42	$C_{16}H_{30}O$	0.70
26	*bis*(6-methylheptyl) phthalate	Hydrocarbon	36.81	390.56	$C_{24}H_{38}O_4$	2.27
27	2-(3,4-dimethoxyphenyl)-3,5-dihydroxy-7-methoxy-4*H*-chromen-4-one	Hydrocarbon	47.08	344.32	$C_{18}H_{16}O_7$	0.93
	Terpenes					
28	(3*R*,3a*R*,7*R*,8a*S*)-3,8,8-trimethyl-6-methyleneoctahydro-1*H*-3a,7-methanoazulene	Sesquiterpene	16.49	204.36	$C_{15}H_{24}$	0.70
29	((3*R*,3a*S*,7*S*,8a*R*)-3,6,8-trimethyl-2,3,4,7,8,8a-hexahydro-1*H*-3a,7-methanoazulen-8-yl) methanol	Sesquiterpene	16.63	220.36	$C_{15}H_{24}O$	0.67
30	(*R*)-1-methyl-4-(6-methylhept-5-en-2-yl) cyclohexa-1,4-diene	Sesquiterpene	17.23	204.36	$C_{15}H_{24}$	0.41
31	1,1,7-trimethyl-4-methylenedecahydro-1*H*-cyclopropa[*e*]azulen-7-ol	Sesquiterpene	18.90	220.36	$C_{15}H_{24}O$	0.54

Entry	Chemical name	Classification	Retention time (min)	Molecular Weight	Molecular formula	Area %
	Non-Oxygenated Hydrocarbons					
32	2-(4a-methyl-8-methylenedecahydronaphthalen-2-yl) propan-2-ol	Sesquiterpene	20.54	222.37	$C_{15}H_{26}O$	1.30
33	(*S*)-2-methyl-6-(p-tolyl) hept-2-en-4-one	Sesquiterpene	21.04	216.32	$C_{15}H_{20}O$	4.91
34	(2*E*,4*E*,6*E*,8*E*)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-en-1-yl) nona-2,4,6,8-tetraenal "Retinal"	Diterpene	21.40	284.44	$C_{20}H_{28}O$	0.32
35	2-methyl-6-(4-methylenecyclohex-2-en-1-yl) hept-2-en-4-one	Sesquiterpene	21.74	218.34	$C_{15}H_{22}O$	0.48
	Fatty acids and esters of fatty acids					
36	(*E*)-3,7-dimethylocta-2,6-dien-1-yl 3-methylbutanoate	Ester of fatty acid	20.05	238.37	$C_{15}H_{26}O_2$	1.92
37	methyl octadeca-12,15-diynoate	Ester of fatty acid	22.16	290.45	$C_{19}H_{30}O_2$	0.72
38	octadec-17-ynoic acid	Fatty acid	25.24	280.45	$C_{18}H_{32}O_2$	0.54
39	methyl palmitate	Ester of fatty acid	26.25	270.46	$C_{17}H_{34}O_2$	3.11
40	palmitic acid	Fatty acid	28.23	256.43	$C_{16}H_{32}O_2$	3.89
41	oleic acid	Fatty acid	29.33	282.47	$C_{18}H_{34}O_2$	1.93
42	methyl (*E*)-octadec-11-enoate	Ester of fatty acid	29.61	296.50	$C_{19}H_{36}O_2$	4.16
43	methyl stearate	Ester of fatty acid	29.96	298.51	$C_{19}H_{38}O_2$	2.27
44	2,3-dihydroxypropyl palmitate	Ester of fatty acid	30.28	330.51	$C_{19}H_{38}O_4$	0.43
45	(*E*)-octadec-13-enoic acid	Fatty acid	31.02	282.47	$C_{18}H_{34}O_2$	0.31
46	(*Z*)-octadec-11-enoic acid	Fatty acid	31.50	282.47	$C_{18}H_{34}O_2$	2.87
47	2-(tetradecyloxy)ethyl palmitate	Ester of fatty acid	32.23	496.86	$C_{32}H_{64}O_3$	0.63
48	methyl 11-((2*R*,3*R*)-3-pentyloxiran-2-yl) undecanoate	Ester of fatty acid	33.37	312.49	$C_{19}H_{36}O_3$	0.31
49	methyl docosanoate	Ester of fatty acid	36.49	354.62	$C_{23}H_{46}O_2$	0.29
50	(*Z*)-icos-11-enoic acid	Fatty acid	37.37	310.52	$C_{20}H_{38}O_2$	1.06
51	2,2,8,8-tetramethyl-3,7-dioxa-2,8-disilanonan-5-yl oleate	Ester of fatty acid	42.36	500.91	$C_{27}H_{56}O_4Si_2$	5.24
52	methyl tricosa-10,12-diynoate	Ester of fatty acid	42.80	360.58	$C_{24}H_{40}O_2$	0.48
	Steroids					
53	Cholestan-3-ol, 2-methylene-, (3α,5α)-	Steroid	42.23	400.69	$C_{28}H_{48}O$	0.29
54	Cholesta-8,24-dien-3-ol, 4-methyl-, (3α,4α)-	Steroid	43.22	398.68	$C_{28}H_{46}O$	0.50
55	Ethyl *iso*-allocholate	Steroid	43.69	436.0	$C_{26}H_{44}O_5$	0.69
56	Stigmastan-3,5-diene	Steroid	44.0	396.70	$C_{29}H_{48}$	2.98
						Σ= 100.0

Table 3 shows the antioxidant capacity of plant extracts, with significant differences ($p < 0.05$) in % DPPH remaining, % scavenging activity, and IC50 values. The water extract had strong activity with an IC50 of 0.16 mg/ml and 35.75% scavenging at 0.104 mg/ml. The methanol extract showed 84.34% scavenging at 5.97 mg/ml but required a higher IC50 (1.95 mg/ml). Petroleum ether extract had the weakest activity, with 13.36% scavenging at 10.45 mg/ml and an IC50 of 4.44 mg/ml. Ascorbic acid, as a standard, exhibited the highest activity with 85.19% scavenging at 0.062 mg/ml and the lowest IC50 (0.0222 mg/ml). The water extract demonstrated the strongest antioxidant activity (IC50 = 0.16 mg/ml), followed by methanol (IC50 = 1.95 mg/ml) and petroleum ether extracts (IC50 = 4.44 mg/ml). This superior activity is attributable to the high phenolic and flavonoid content in the water extract. Polyphenolic compounds such as catechins and quercetin derivatives, previously identified in *L. pyrotechnica*, likely contribute to this activity by neutralizing free radicals and reducing oxidative stress [4]. The results in Table 4 indicate significant differences in antibacterial activity ($p < 0.05$) among *L. pyrotechnica* extracts and gentamycin. The methanol extract exhibited the highest inhibition zones for *S. aureus* (13.6 mm, significantly higher than gentamycin's 10 mm) and *B. cereus* (11.5 mm), also significantly higher than gentamycin's 9 mm. For *L. monocytogenes* (9 mm) and *S. epidermidis* (9 mm), the methanol extract was less effective compared to gentamycin (11 mm and 8.6 mm, respectively). Petroleum ether extract showed activity only against *S. aureus* and *B. cereus* (10 mm each), with no significant difference compared to gentamycin. Water extract was inactive for all bacterial species, showing statistically significant differences from the active extracts. Gentamycin's inhibition zones ranged from 8 mm (*P. aeruginosa*) to 14.3 mm (*E. coli*), with significant differences observed among bacterial species ($p < 0.05$). The methanol extract exhibited significant antibacterial activity, inhibiting four of nine tested strains. This activity is likely associated with the presence of bioactive terpenoids, fatty acids, and alkaloids, which are known to disrupt microbial cell membranes and inhibit enzymatic processes. The GC-MS identified terpenoids such as (S)-2-methyl-6-(p-tolyl) hept-2-en-4-one and fatty acid esters, such as 2,2, 8,8-tetramethyl-3,7-dioxa-2,8-disilanonan-5-yl oleate, which are strong candidates for the observed antibacterial effects.

Table 2. The phytochemical analysis of the investigated extracts.

	Phytochemical Analysis				
Extract	**Phenolics**	**Flavonoids**	**Tannins**	**Alkaloids**	**Saponins**
Water	276.47	76.64	81.08	10.95	6.89
Methanol	60.09	23.91	19.66	2.06	1.94
Petroleum ether	3.35	1.29	0.22	0.26	0.13

Phenolics Content “mg gallic acid/ gm dry extract”; Flavonoids Content “mg catechin/gm dry extract”; Tannins Content “mg tannic acid/gm dry extract”; Alkaloids Content “mg alkaloids/gm dry extract”; Saponins Content “mg saponins/gm dry extract”

The water extract exhibited the highest concentration of these bioactive compounds, correlating with its superior antioxidant capacity. This aligns with previous research highlighting the efficacy of polar solvents in extracting antioxidant-rich phytochemicals [16, 24]. However, despite its rich phytochemical profile, the water extract's unexpectedly low antibacterial activity suggests that compounds contributing to antibacterial effects might be less extractable in water or exist in insufficient concentrations. The phytochemical composition and bioactivity results of *L. pyrotechnica* are consistent with earlier studies [7, 14]. The high content of phenolics and flavonoids in the water extract corroborates the findings of [16], who reported the antioxidant potential of *L. pyrotechnica* extracts. The observed antibacterial activity against *Staphylococcus aureus* and *Bacillus cereus* agrees with the results of [20], who documented similar inhibitory effects in methanolic extracts of desert plants.

The high antioxidant activity of the water extract can be attributed to its elevated levels of phenolic and flavonoid compounds. Phenolic compounds are known to donate hydrogen atoms, thereby neutralizing free radicals and preventing oxidative damage [24]. These findings are consistent with previous reports by [19] and [29], who highlighted the role of polyphenols and flavonoids in enhancing antioxidant capacity in desert plants. The robust antioxidant activity observed in the water extract can be attributed to the high concentrations of phenolics and flavonoids. These compounds act as electron donors, neutralizing reactive oxygen species (ROS) and preventing oxidative damage. Specifically, phenolic hydroxyl groups are crucial in scavenging free radicals, while flavonoids stabilize oxidized species. The presence of vanillic acid and quercetin-3-β-D-glucoside, identified in earlier studies, supports this mechanism [32].

The antibacterial activity of the methanol extract suggests the involvement of bioactive compounds with lipophilic properties, facilitating interaction with microbial membranes. Terpenoids such as (S)-2-methyl-6-(p-tolyl) hept-2-en-4-one and fatty acids like methyl (E)-octadec-11-enoate are likely contributors due to their ability to disrupt bacterial cell walls and inhibit vital enzymes. Alkaloids may also play a role by interfering with nucleic acid synthesis [33]. The variation in antibacterial efficacy

can be attributed to the solvent polarity, which influences the extraction of different classes of bioactive compounds. Terpenoids and alkaloids present in the methanol extract are known to possess antimicrobial properties, as described by [31].

Table 3. The antioxidant capacity of the investigated plant extracts.

Sample	Concentrations (mg/ml)	% Remaining DPPH	% Scavenging activity	IC_{50} (mg/ml)
Water	0.104	64.25 b	35.75 c	0.16
	0.052	90.5 c	9.501 a	
	0.026	93.24 d	6.763 a	
	0.013	99.03 d	0.966 a	
methanol	5.97	15.66 a	84.34 d	1.95
	2.985	33.89 a	66.11 d	
	1.493	57.23 b	42.77 c	
	0.746	75.15 b	24.85 b	
Petroleum ether	10.45	86.64 c	13.36 a	4.44
	5.223	92.66 d	7.342 a	
	2.611	98.09 d	1.909 a	
	1.306	99.85 d	0.147 a	
Ascorbic acid	0.062	15.267 a	85.19 d	0.0222
	0.031	39.084 a	62.07 d	
	0.016	61.069 b	40.74 c	
	0.008	74.809 b	27.41 b	

Different letters denote significant differences between means at the $p < 0.05$ level according to two-way ANOVA followed by post-hoc tests

Table 4: The antibacterial efficiency of *L. pyrotechnica* extracts against the various bacterial species.

Microbial species	Gentamycin	Water	Methanol	Petroleum ether
Staphylococcus aureus	10 a	-ve	13.6 b	10 a
Bacillus subtilis	13b	-ve	-ve	-ve
Enterobacter cloacae	9.3a	-ve	-ve	-ve
Listeria monocytogenes	11b	-ve	9a	-ve
Salmonella typhi	8.3 a	-ve	-ve	-ve
Bacillus cereus	9a	-ve	11.5b	10 a
Staphylococcus epidermidis	8.6a	-ve	9 a	-ve
Pseudomonas aeruginosa	8a	-ve	-ve	-ve
E. coli	14.3b	-ve	-ve	-ve

Different letters denote significant differences between means at the $p < 0.05$ level according to one-way ANOVA followed by post-hoc tests.

Leptadenia pyrotechnica exhibits significant therapeutic potential due to its polyphenolic constituents, demonstrating potent antioxidant, anti-lipoxygenase, and cytotoxic activities. These properties suggest applications in managing oxidative stress-related disorders such as cardiovascular and neurodegenerative diseases, reducing inflammation in conditions like arthritis and asthma, and supporting anticancer therapies by targeting specific cancer cells. Additionally, its antioxidant and anti-inflammatory effects highlight its potential in wound healing, skin health, and possibly hepatoprotection. These findings reinforce the traditional medicinal use of *L. pyrotechnica* and provide a foundation for its integration into modern therapeutic strategies [16]. Its antibacterial efficacy has been demonstrated against resistant strains like *Staphylococcus aureus*, underscoring its relevance in addressing antibiotic resistance [19]. Additionally, bioactive terpenoids and fatty acids support its use in wound healing and as an anti-inflammatory agent [34].

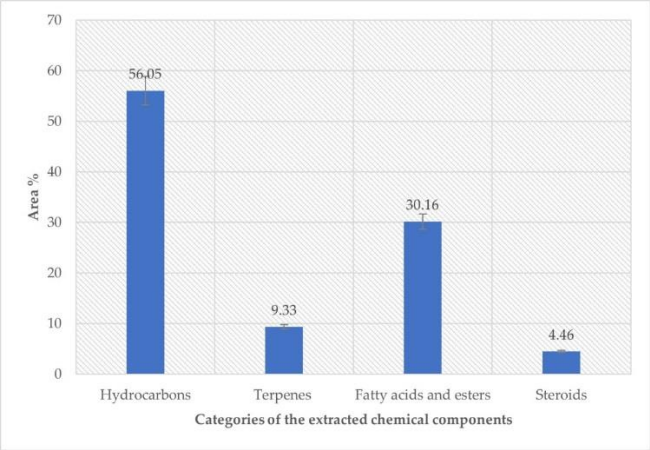

Figure 3. The categorized volatile constituents were deduced from the methanol extract of *Leptadenia pyrotechnica* by GC-MS mass spectroscopy assay.

These findings emphasize the importance of further exploring the plant's phytochemicals and pharmacological applications. The analysis highlights the distinctive phytochemical composition of *L. pyrotechnica* from the studied region, on its bioactive profile. The data also support the plant's commercial and therapeutic relevance, with potential applications in natural medicine, nutraceuticals, and industrial sectors. Future studies should optimize extraction methods to isolate and characterize these valuable compounds, particularly terpenoids and fatty acids, to maximize their medicinal and commercial potential.

4. Conclusion

Leptadenia pyrotechnica (Forssk.) Decne., a wild plant native to Egypt, possesses a remarkable array of phytochemical constituents, essential oils, fatty acids, and secondary metabolites that contribute to its promising medicinal properties. The water extract of *L. pyrotechnica* showed the highest concentration of secondary metabolites. It demonstrated the most potent free radical scavenging activity, with an IC50 value of 0.16 mg/ml in the DPPH assay, highlighting its potent antioxidant potential. GC-MS analysis revealed fifty-six volatile compounds, including hydrocarbons, terpenes, fatty acids, lipids, and steroids, suggesting a diverse phytochemical profile that may contribute to its therapeutic effects. The extracts also exhibited significant antibacterial activity against several antibiotic-resistant bacterial strains, including *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus cereus*, and *Staphylococcus epidermidis*. These findings underscore the plant's potential as a source of natural antioxidants and antibacterial agents, with possible applications in developing novel botanical drugs to combat antibiotic-resistant bacteria. The antibacterial efficacy, particularly against resistant strains, is attributed to the bioactive compounds identified in the extracts, including terpenoids, fatty acids, and other secondary metabolites. Despite the promising bioactivity, the lower antibacterial activity of the water extract, despite its rich phytochemical content, suggests that alternative extraction methods may enhance compound recovery. The study also provides a foundation for further research into the isolation and characterization of active constituents and investigations into potential synergistic effects among the extract components to maximize their antioxidant and antimicrobial properties. These findings confirm the traditional medicinal use of *L. pyrotechnica* and suggest its integration into modern pharmaceutical and nutraceutical applications. Further optimization of extraction techniques and in-depth evaluations of the bioavailability, stability, and synergistic interactions of its bioactive compounds will be essential for fully harnessing its medicinal and commercial potential.

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