



Phytochemical screening and antibacterial activity of *Portulaca oleracea* L. extracts against antibiotic-resistant bacteria

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ABSTRACT

The rise of antibiotic resistance to newly developed antibiotics supports the need for innovation, monitoring antibiotic use, prevention, diagnosis, and a quick decrease in drug abuse. The utilization of medicinal plants can be effective as natural antimicrobial treatments due to the presence of many phytochemical compounds. In this context, the antibacterial activity of ethanol and aqueous extracts of *Portulaca oleracea* L. were evaluated against antibiotic-resistant pathogenic bacteria that were isolated from wound and urine samples. The pathogenic bacterial isolates were identified by Kirby-Bauer disc diffusion method. The antibacterial activity of aqueous and ethanolic extract as well as commercial antibiotics were evaluated by agar well diffusion assay and minimum inhibitory concentration. *Klebsiella spp.* was the common isolate in both urine and wound samples, followed by *Escherichia coli* and *Staphylococcus aureus* in urine and wound samples, respectively. While *Pseudomonas spp.* and *Proteus Spp.* were the lowest frequent isolates in wound samples. The inhibition effect of the *P. oleracea* extraction is mostly greater than that of antibiotics against *Klebsiella Spp.* Minimum inhibitory concentration (MIC) of ethanolic extract for *Staphylococcus Aureus* was 0.35 gm/ml, for *E. coli* was 0.15 gm/ml, for *Proteus spp.*, *Pseudomonas spp.* and *Klebsiella spp.* was 0.25 mg/ml. For aqueous extract, MIC was 0.5 gm/ml for *Staphylococcus aureus* and *Klebsiella spp.*, 0.4 gm/ml for *Escherichia coli*, *Pseudomonas spp.* and *Proteus spp.* The GC-MS analysis of the extracts revealed the presence of several phytochemical compounds. The results of inhibition activity and phytochemical analysis suggested that *P. oleracea* ethanol and aqueous extracts can be effective as antibacterial agents against some pathogenic bacteria.

Key Words:

Antibacterial activity, GC Mass, Medicinal plants

1. INTRODUCTION

Urinary tract and nosocomial infections are mainly caused by opportunistic and pathogenic bacteria which have been documented to be multidrug resistant in the recent years [1, 2, 3]. These include *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus saprophyticus*, *Enterococcus faecalis*, and *Proteus mirabilis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* [4, 5]. The resistance of these bacteria to the available commercial antibiotics is currently increasing due to their inappropriate use at optimal doses and duration. Methicillin-resistant *Staphylococcus aureus*, *Streptococcus pyogenes*, *Enterococci*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Streptococcus epidermidis* are the main causes of wound infections [6]. The clinical efficacy of antibiotics gradually declines due to the widespread of multidrug resistance bacteria that threatening human health [7, 8]. On the other hand, the treatment by the chemical antibacterial drugs are costly and have many side effects. Such conditions support the need for innovation, monitoring antibiotic use, prevention, diagnosis, and a quick decrease in drug abuse of these antibiotics. Antimicrobial use protocols will need to be changed to ensure that these medications are only administered when all other therapeutic options have failed [9]. In this respect, medicinal plants can be utilized as new alternative antibacterial agents for pathogenic multidrug resistant bacteria [10].

Since early times, humans have been interested in the usage of medicinal plants to recover from a disease as they have a wide variety of bioactive substances, which can be utilized to create new drug manufacturing techniques [10]. The effects of these medicinal plants on microorganisms have been found to be due to the presence of phytochemical compounds such as flavonoids, alkaloids, volatile oils, tannins, glycosides and other secondary metabolites [11]. Therefore, medicinal plants have played a major role in the treatment of various diseases including bacterial and fungal infections [12].

The World Health Organization lists *Portulaca oleracea* L. as one of the most popular therapeutic plants, which has been assigned the name “Global Panacea” [13]. *Portulaca* has been applied in a wide range of research due to its effective benefits [14, 15]. It has been used as a food source, spice and medicine since the ancient Egyptians’ era, and in England during the medieval ages [16]. It is entirely considered to have antiphlogistic, bactericidal, anti-diabetic, emollient, calming, diuretic, and refreshing properties [17]. Moreover, it is particularly useful as a dietary supplement and as an alternative in cases of scurvy and liver illness [18]. *P. oleracea* had a wide variety of chemical substances, such as alkaloids, terpenoids, organic acids, coumarins, flavonoids, volatile oil, and polysaccharides [19].

The present study aims to evaluate the antibacterial effect of the ethanol and aqueous extracts of *P. oleracea* against some multidrug resistant pathogenic bacterial isolates. Furthermore, the GC/MS analysis was carried out to explore the bioactive compounds of both ethanol and aqueous extracts of *P. oleracea*.

2. METHODS AND MATERIALS

2.1 Samples collection

2.1.1 Plant collection

Portulaca oleracea samples were collected from natural habitats of 'Al-Sharqya Governorate, Egypt. They were transferred to the laboratory where air dried at room temperature for 3 months. Then, the air-dried plant samples were ground with electric blender to a fine powder and stored for further experiments.

2.1.2 Bacterial strains collection

A total number of 60 samples (40 urine and 20 wound) were collected from 50 patients at the outpatient of Gastroenterology Center (GEC), Mansoura University, Egypt. Bacterial strains were isolated from the positive wound and urine specimens. The wound samples were collected with sterile swabs, while the urine samples were collected in sterile plastic containers under complete aseptic conditions. Morning samples were preferred. All the samples were kept in an ice box and were transferred to the laboratory of the Faculty of Science, Port Said University for the experimental study.

2.2 Strain isolation and identification

Informed consents were obtained from all contributed patients who were fully informed by the diagnostic procedures and disease nature. The study protocol conformed to ethical guide-lines of 1975 Helsinki Declaration. Patients who received antibiotic treatment systemically within the previous 72 hr. were excluded from the study. Swabs were collected from patients wounds under aseptic precautions and transported to the microbiology laboratory in ice box within two hours.

Purulent material, wound swab specimens, and urine samples have been inoculated directly or with the help of a sterile inoculating loop onto MacConkey agar and blood agar medium by continuous streaking method. They also have been incubated at 37°C for 24 hours in aerobic condition. Bacterial growth were identified according to the colony characters, microscopic examination by Gram's stain and biochemically identified by VITEK 2 compact 15 (Biomerieux, France). The identification of bacterial isolates was done according to Bergey's Manual of systemic bacteriology and standard microbiological techniques [20].

2.3 Antibiotic susceptibility

The antibiotic susceptibility of bacterial isolates were carried out on Mueller Hinton Agar plates using Kirby-Bauer disc diffusion method according to CLSI guidelines [21]. The antibiotic used were Gentamicin(CN), Pefloxacin (PEF), Amikacin (AK), Cefepime (FEP), Amoxicillen / Clavulanic acid 2:1(AMC), Meropenen (MEM), Cephalexin (CL), Nitrofurantoun (F), Cefoperazone (CEP), Piperacillin (PRL), Rifampin (RA), Clindamycin (DA), Vancomycin (VA) and Cefoperazone\ Sulbactam (CES). The results have been expressed as diameter of inhibition zones as recommended by National Committee for Clinical Laboratory standards (CLSI guidelines 2010) [22].

2.4 Preparation of ethanolic and aqueous extracts of *P. oleracea*

For preparation of ethanolic and aqueous extracts, 125 gm of air dried powdered of *P. oleracea* shoot has been soaked in 250 ml of ethanol and distilled water, respectively (ratio 1: 2). The methods of extraction were carried out according to Nostro et al. [23]. The extraction has been sieved through mesh cloth and it has been concentrated at 60 °C using rotary evaporator (Stuart RE300DB, England). The residue has been re-dissolved in 10 ml of Dimethyl sulfoxide (DMSO) solution and it has been stored at 4 °C for further procedures.

2.5 Antibacterial Activity of *Portulaca oleracea* extracts

Ethanolic and aqueous extracts of *P. oleracea* were serially diluted by DMSO to obtain diluted concentrations 0.5, 0.35, 0.25, 0.15 and 0.1 gm/ml. Antibacterial activity of each extracts has been carried out using the well diffusion method according to NCCLS [24]. Petri plates that contain 25 ml of nutrient agar medium have been inoculated with 1ml standard inoculums (20×10^8 CFU/ml) of each bacterial isolate. Agar wells have been made by using a sterile cork borer (7 mm diameter). Each well has been filled with 100 µl of the tested plant extract and the plates have been incubated at 37 °C for 24 h. All tests have been performed in triplicate and the antibacterial activity has expressed as the mean diameter of inhibition zones (mm). Minimum inhibitory concentration (MIC) (minimum inhibition concentration) value was taken as the lowest concentration of each extract which inhibit the bacterial growth.

Diameters of inhibition zone of <10 mm zone has been considered as low sensitivity, 10-14 mm as medium sensitivity, 15-19 mm as high sensitivity, and 20 mm as extreme sensitivity, following the standard for pharmacology of traditional Chinese medicine [25]. The diameters of inhibition zone of <7 mm zone has been considered as resistant, 7-14 mm as low sensitivity, 15-20 mm as medium sensitivity, 21-29 mm as high sensitivity, and 30 mm as extreme sensitivity (Table 1).

Table 1. Diameters of inhibition zone for used commercial antibiotics according to CLSI & EUCAST (2021/2022) .

Antibiotic		Disk Potency	Inhibition zone diameter (mm)		
			R	I	S
1	Gentamicin(CN) [16][18]	10 µg	≤ 12	13-14	≥ 15
2	Pefloxacin (PEF) [16][18]	5 µg	≤ 23	-	≥ 24
3	Amikacin (AK) [16][18]	30 µg	≤14	15-16	≥17
4	Cefepime (FEP) [16][18]	30 µg	≤18	19-24	≥25
5	Amoxicillen / Clavulanic acid 2:1 (AMC) [17][19][20]	30 µg	≤18	19-23	≥24
6	Meropenen (MEM) [16][18]	10 µg	≤ 19	20-22	≥ 23
7	Cephalexin (CL) [16][18]	30 µg	≤ 14	-	≥ 15
8	Nitrofurantoun (F) [21][21]	300 µg	≤14	15-16	≥ 17
9	Cefoperazone (CEP) [16][18]	75 µg	≤15	16-20	≥21
10	Piperacillin (PRL)[21] [22]	100 µg	≤ 17	20-18	≥ 21
11	Rifampin (RA)	5 µg	≤ 16	17-19	≥20
12	Clindamycin (DA) [22] [21]	2 µg	≤ 14	20-15	≥21
13	Vancomycin (VA) [21] [22]	30 µg	≤14	16-15	≥17
14	Cefoperazone\ Sulbactam (CES)	75\30 µg	≤ 24	25-31	≥ 32

S = Susceptible, I = Intermediate, R = Resistant

2.6 GC-MS analysis

The GC-MS analysis were carried out using gas chromatography-mass spectrometry (THERMO Scientific Corp., USA), coupled with a thermo mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The identification of the chemical constituents of the essential oil was de-convoluted using AMDIS software (www.amdis.net) and identified by its retention indices and by utilization of Wiley spectral library collection and NSIT library database.

2.7 Statistical analysis

Data was statistically analyzed for comparing the significance difference between paired means by student t-test with SPSS software 16.0.

3. RESULTS AND DISCUSSION

3.1 Pathogenic bacterial growth

Five different bacterial species were isolated from the tested patients (Table 2). The isolates of *Klebsiella spp.* and *E. coli* were recoded with 17 and 15, respectively from urine samples. In wounds samples, *Klebsiella spp.* recorded the highest isolates number (6), followed by *S. aureus* (4), *Pseudomonas spp.* (2) and *Proteus spp.* with only one isolate. These results are aligned with those of Raza et al. [26] who reported that *S. aureus* was the most commonly isolated species with surgical wound

infections, followed by *Klebsiella pneumoniae*. Furthermore, they are in consistent with the reported data for surgical wound infections in Eastern Nigeria [27].

Table (2). Pathogenic isolated bacteria from urine and wound samples.

Pathogenic bacteria	Urine		Wounds	
	No.	%	No.	%
<i>Klebsiella spp.</i>	17	42.5	6	30
<i>Staphylococcus aureus</i>	-	-	4	20
<i>Escherichia coli</i>	15	37.5	-	-
<i>Pseudomonas spp.</i>	-	-	2	10
<i>Proteus spp.</i>	-	-	1	5
Total	32	80	13	65

Pathogenic bacteria associated with surgical wounds may be found in normal flora, in sick people, or in hospital environments [28, 29]. *S. aureus* is the common source of wound infection because it is a component of the skin's natural microbial flora [30]. Post-operative surgical site infections continue to rank among the most common nosocomial infections in countries with limited resources [31]. Identification of the pathogenic organism types and selection of an antibiotic that is effective against those organisms are necessary for the efficient treatment of patients with bacterial diseases. One of the pillars of current medicine, antibiotics are essential for both the prevention and treatment of infectious diseases. Given this, the global community at large faces critical issues regarding the choice, availability, and appropriate use of these resources [32].

3.2. Antibiotic susceptibility

Generally, all the tested bacterial isolates in the current study were drug resistant with more than six antibiotics for each isolate (Table 3). The most effective antibiotic for *E. coli*, was Meropenem with clear zone diameter of 30 mm, while Clindamycin was the lowest effective one with inhibition zone of 10 mm. The maximum inhibition zone of 27 mm for each of Amikacin and Meropenem was recorded on *S. aureus* which had the minimum inhibition zone (12 mm) was for each of Nitrofurantoin and Pefloxacin. For *Proteus* isolates, the maximum inhibition zone (30 mm) was demonstrated for each of Amikacin and Gentamicin, while the minimum one (11 mm) was reported for each of Pepracillin and Rifamycin SV. For *Pseudomonas*, the maximum and minimum inhibition zones were 30 mm and 13 mm for Meropenem and Pefloxacin, respectively. For *Klebsiella*, only Amikacin was reported to exhibit a poor inhibition effect with 13 mm diameter of inhibition zone.

Atef et al. [33] documented high antibiotic resistance of his tested gram-positive and gram-negative bacterial isolates to ampicillin, amoxycillin, and erythromycin. The results of the current study showed that *S. aureus* isolates were sensitive to Vancomycin and varied (sensitive or resistant) against Tetracycline and Salmonella. *E. coli*, and *Pseudomonas* isolates have shown great resistance against both antibiotics. β -lactams, such as penicillin group (Ampicillin, Amoxicillin) and cephalosporins (Cefaclor), prevent the formation of the cell wall by inhibiting peptidoglycan polymerization, while glycopeptides (Vancomycin) combine with the cell wall. The behavior of *E. coli*, and the gram-positive isolates showed some resistance against Gentamycin. Amikacin was discovered to be able to exert an antibacterial effect on all tested bacterial isolates. Quinolones (Nalidixic acid and Ciprofloxacin) bind to a bacterial complex of DNA and DNA gyrase, and blocking DNA replication is effective on both Gram-negative and some Gram-positive bacteria. Ribosome function is affected by aminoglycosides (Amikacin, Gentamycin, and Tobramycin), tetracyclines (Tetracycline), and macrolides (Erythromycin). These results are in agreement with those reported by [34, 35, 36]. The variations in the antibiotic sensitivity patterns of the isolated organisms may be related to a variety of factors, such as pH value variations, conditions and timing of

incubation, composition, the nature of the culture media, the size of the inoculum, the source of the isolated organism, and potential strain activity variations [37]. Variations in antibiotic sensitivity may be influenced by the composition of the bacterial cell wall and the permeability of the cell membranes to different antibiotics [38].

Table 3. Effect of commercial antibiotics on growth of some bacterial isolates (inhibition zone in mm).

Antibiotic type	Ak	CN	AMC	PRL	CL	FEP	CEP	MEM	VA	DA	F	PEF	RA	CES
Bacteria isolates														
<i>Staphylococcus spp.</i>	27 (S)	15 (S)	20 (I)	R	R	20 (I)	18 (I)	27 (S)	21 (S)	R	R	R	R	R
<i>Escherichia coli</i>	20 (S)	23 (S)	R	R	R	R	27 (S)	30 (S)	R	R	20 (S)	R	R	26 (I)
<i>Proteus Spp.</i>	30 (S)	30 (S)	R	R	R	20 (I)	R	21 (S)	R	R	R	R	R	26 (I)
<i>Pseudomonas Spp.</i>	19 (S)	17 (S)	R	R	R	20 (I)	R	30 (S)	R	R	R	R	R	R
<i>Klebsiella Spp.</i>	R	R	R	R	R	R	R	R	R	R	R	R	R	R

Susceptible (S), Intermediate (I), Resistant (R). R ≤ 7 mm, L.S: ≥7 to ≤ 14, I: ≥14 to ≤ 20 mm, H.S: ≥20 to ≤ 25 mm, E.S: ≥ 25 to ≤ 30.

3.3 Antibacterial activity of *Portulaca oleracea* extracts

Both the aqueous and ethanolic extracts of *P. oleracea* displayed antibacterial activity against the tested pathogenic isolates with different responses for each extract as indicated by various zones of inhibition (Table 4). Such antibacterial effect was shown to be concentration dependent (Table 4). Each tested isolate showed different pattern of zone of inhibition for each extract and the antibacterial activity of extracts decreased with decrease in concentration (Table 4). Furthermore, the isolates are showed significant more sensitivity to the ethanolic extract concentrations than those of aqueous one ($P \leq 0.05$). The ethanolic extract of *P. oleracea* had a significant effect on *E. coli*, *Klebsiella spp.*, *Proteus spp.* and *Pseudomonas spp.* with zone of inhibition (30 mm) at the 0.5 g/ml which recorded of 25 mm for *S. aureus* (Table 4). The inhibition zone of 15 mm was recorded for *E. coli* at concentration level of 0.15 mg/ml. The same inhibition zone was recorded at concentration 0.25 g/ml for each of *Klebsiella spp.*, *Pseudomonas spp.* and *Proteus spp.*, while it was at concentration of 0.35 g/ml for *S. aureus*.

In aqueous extract, the inhibition zone (20 mm) was reported for *E. coli*, *Proteus spp.*, and *Pseudomonas spp.* isolates at concentration of 0.5 g/ml (Table 4). The inhibition zone of 15 mm was reported for *E. coli* at concentration of 0.4 mg/ml. Other tested concentrations were recorded to have no effect on all bacterial strains. This means that the resistance of bacterial strains for the aqueous extract at concentration level is smaller than 0.35 g/ml.

These results are aligned with those of [39, 40] who found the ethanolic extract of *P. oleracea* exhibits high inhibitory activity against *S. aureus* and *P. aeruginosa* at concentration of 0.1 g/ml. Sun et al. [39] have reported that the ethanolic extract of *P. oleracea* showed higher antibacterial activity than the aqueous extract. The current study also explored that the ethanolic extract of *P. oleracea* had antibacterial activity ranging from 0.25 to 0.5 g/ml against all tested multidrug resistant bacterial isolates. Moreover, our results showed that both ethanolic and aqueous extracts with 0.5 concentration had greater effect on pathogenic bacteria than commercial antibiotics.

Table 4. Zone of inhibition of alcoholic and aqueous *Portulaca oleracea* extracts against tested pathogenic bacterial isolates. The mean of each isolate with similar letters are significantly different between ethanolic and aqueous extracts (Student t-test, paired samples).

Bacterial isolates	Alcoholic extract (g/ml)						Mean \pm SE
	0.50	0.40	0.35	0.25	0.15	0.10	
<i>Staphylococcus spp.</i>	25	20	15	7	7	7	13.5 ^a \pm 7.79
<i>Escherichia coli</i>	30	25	20	15	7	7	17.33 ^b \pm 9.44
<i>Proteus spp.</i>	30	25	20	15	7	7	17.33 ^c \pm 9.44
<i>Pseudomonas spp.</i>	30	25	20	15	7	7	17.33 ^d \pm 9.44
<i>Klebsiella spp.</i>	30	25	20	15	7	7	17.33 ^e \pm 9.44
	Aqueous extract (g/ml)						
	0.50	0.40	0.35	0.25	0.15	0.10	
<i>Staphylococcus spp.</i>	15	7	7	7	7	7	8.33 ^a \pm 3.27
<i>Escherichia coli</i>	20	15	7	7	7	7	10.5 ^b \pm 5.65
<i>Proteus spp.</i>	20	15	7	7	7	7	10.5 ^c \pm 5.65
<i>Pseudomonas spp.</i>	20	15	7	7	7	7	10.5 ^d \pm 5.65
<i>Klebsiella spp.</i>	15	7	7	7	7	7	8.33 ^e \pm 3.27

3.4 Phytochemistry of ethanolic and aqueous extracts

The antibacterial activity of *P. oleracea* ethanolic and aqueous extracts could be due to the phytochemical components. The results of GC/MS analysis revealed that thirty-two and thirty-six compounds were detected in the ethanolic and aqueous extracts, respectively (Tables 5 and 6). The data revealed the presence of fatty acids such as linoleic acid, octadecanoic acid, phthalic acid, fumaric acid and erucic acid. These fatty acids have been found to inhibit bacterial activity and serve as antibacterial drugs [41]. Polysaccharides found in *P. oleracea* extract are potential therapeutic agents for the treatment of diabetes mellitus owing to their modulation of blood lipids, metabolism, and decrease of blood glucose. Furthermore, *P. oleracea* contains monoterpenes such as portulosides A and B, diterpenes such as portulene, and β - amyrin type triterpenoids [40, 42]. Diterpene and terpenoid groups have been reported as antimicrobial activity [43, 44].

4. CONCLUSION

Both ethanolic and aqueous extracts of *P. oleracea* showed inhibitory effects against some pathogenic bacteria isolates especially for *E. coli*, *Pseudomonas*, *Proteus* and *Klebsiella*. The inhibition activity of the alcoholic extract was higher than aqueous one. The inhibition activities for both ethanolic and aqueous extracts were more significant compared to those of antibiotic drugs especially for *Klebsiella Spp.* The phytochemical analysis of *P. oleracea* extracts revealed the existence of many effective antimicrobial compounds. The results of the current study suggest the utilization of *P. oleracea* as promised antibacterial agent controlling some pathogenic multidrug resistant bacteria.

Table 5. Phytochemical screening of *Potulaca oleracea* alcoholic extract identified by GC-MS.

Phytochemical compounds	R.T (min)	Molecular formula	Molecular weight (g/mol)	peak area %
2-PENTANONE, 4-HYDROXY-4-METHYL-	4.41	C ₆ H ₁₂ O ₂	116	1.36
Benzyl chloride	7.04	C ₇ H ₇ Cl	126	1.83
Diacetone alcohol, TMS derivative	7.77	C ₉ H ₂₀ O ₂ Si	188	3.26
(2-FURYL) METHYLPHENYLCHL OROSILANE	8.54	C ₁₁ H ₁₁ ClOSi	222	0.32
Benzaldehyde, 3-benzyloxy-2-fluoro-4-methoxy-	8.99	C ₁₅ H ₁₃ FO ₃	260	0.24
Cyclotetrasiloxane, octamethyl-	10.75	C ₈ H ₂₄ O ₄ Si ₄	296	0.32
Benzene, (iodomethyl)-	11.45	C ₇ H ₇ I	218	0.39
1-Dodecanamine, N,N-dimethyl-	19.62	C ₁₄ H ₃₁ N	213	9.39
Octadecanoic acid, 2-(octadecyloxy)ethyl ester	22.56	C ₃₈ H ₇₆ O ₃	580	0.17
Nizatidine	24.29	C ₁₂ H ₂₁ N ₅ O ₂ S ₂	331	3.99
2H-Pyran-3-ol, tetrahydro-2,2,6-trimethyl-6-(4-methyl-3-cyclohexen-1-yl)-, [3S-[3à,6à(R*)]]-	25.21	C ₁₅ H ₂₆ O ₂	238	0.38
9-OCTADECENOIC ACID (Z)-	25.72	C ₁₈ H ₃₄ O ₂	282	0.36
TETRADECANOIC ACID, ETHYL ESTER	26.24	C ₁₆ H ₃₂ O ₂	256	0.28
Neophytadiene	27.17	C ₂₀ H ₃₈	278	5.88
2-PENTADECANONE, 6,10,14-TRIMETHYL-	27.30	C ₁₈ H ₃₆ O	268	1.75
Neophytadiene	27.69	C ₂₀ H ₃₈	278	1.89
2,6,10-TRIMETHYL,14-ETHYLENE -14-PENTADECNE	28.05	C ₂₀ H ₃₈	278	2.63
Hexadecanoic acid, methyl ester	28.95	C ₁₇ H ₃₄ O ₂	270	0.69
Phthalic acid, isobutyl octadecyl ester	29.66	C ₃₀ H ₅₀ O ₄	474	0.15
HEXADECANOIC ACID	29.86	C ₁₆ H ₃₂ O ₂	256	1.9
Hexadecanoic acid, ethyl ester	30.32	C ₁₈ H ₃₆ O ₂	284	12.29
cis-13-Eicosenoic acid	31.91	C ₂₀ H ₃₈ O ₂	310	0.12
Phytol	32.54	C ₂₀ H ₄₀ O	296	7.97
Linoleic acid ethyl ester	33.42	C ₂₀ H ₃₆ O ₂	308	11.56
Ethyl Oleate	33.55	C ₂₀ H ₃₈ O ₂	310	13.64
Octadecanoic acid, ethyl ester	34.01	C ₂₀ H ₄₀ O ₂	312	4.06
1-Heptatriacotanol	34.30	C ₃₇ H ₇₆ O	536	0.20
Fumaric acid, myrtenyl octyl ester	36.16	C ₂₂ H ₃₄ O ₄	362	0.34
ISOCHIAPIN B	36.52	C ₁₉ H ₂₂ O ₆	346	0.29
cis-Vaccenic acid	36.71	C ₁₈ H ₃₄ O ₂	282	0.22
Diisooctyl phthalate	39.84	C ₂₄ H ₃₈ O ₄	390	4.54
Erucic acid	42.13	C ₂₂ H ₄₂ O ₂	338	0.29

Table 6. Phytochemical screening of *Potulaca oleracea* aqueous extract identified by GC-MS.

Phytochemical compound	R.T (min)	Molecular formula	Molecular weight (g/mol)	Peak area %
Glycolic acid, 2TMS derivative	5.38	C ₈ H ₂₀ O ₃ Si ₂	220	1.35
ACETAMIDE, 2,2,2-TRIFLUORO-N-METHYL-N-(TRIMETHYLSILYL)-	6.46	C ₆ H ₁₂ F ₃ NOSi	199	0.26
Diacetone alcohol, TMS derivative	7.67	C ₉ H ₂₀ O ₂ Si	188	15.18
Propanoic acid, 3-(trimethylsilyl)-	8.13	C ₆ H ₁₄ O ₂ Si	146	0.82
Propanoic acid, 3-(trimethylsilyl)-	8.41	C ₆ H ₁₄ O ₂ Si	146	5.68
Ethylene glycol, TMS derivative	9.10	C ₅ H ₁₄ O ₂ Si	134	0.43
Tris(trimethylsilyl)amine	9.35	C ₉ H ₂₇ NSi ₃	233	1.58
Pyruvic acid, TMS derivative	10.18	C ₆ H ₁₂ O ₃ Si	160	0.52
Mercaptoethanol, 2TMS derivative	11.44	C ₈ H ₂₂ OSSi ₂	222	5.97
Pinacol, 2TMS derivative	11.66	C ₁₂ H ₃₀ O ₂ Si ₂	262	3.03
D-Carvone	12.84	C ₁₀ H ₁₄ O	150	0.17
Propylene glycol, 2TMS derivative	13.91	C ₉ H ₂₄ O ₂ Si ₂	220	1.01
Glycerol, 3TMS derivative	14.24	C ₁₂ H ₃₂ O ₃ Si ₃	308	4.37
Pinacol, 2TMS derivative	16.39	C ₁₂ H ₃₀ O ₂ Si ₂	262	7.77
Propane, 2-methyl-1,2-is(trimethylsiloxy)-	16.6	C ₁₀ H ₂₆ O ₂ Si ₂	234	2.64
PYRIMIDINE, 2-(TRIMETHYLSILOXY)-4-[(TRIMETHYLSILYL)THIO]-	16.95	C ₁₀ H ₂₀ N ₂ OSSi ₂	272	0.41
10,12-Tricosadiynoic acid, TMS Derivative	18.48	C ₂₆ H ₄₆ O ₂ Si	418	0.15
Isospathulenol	18.48	C ₁₅ H ₂₄ O	220	0.15
Decanoic acid, TMS derivative	18.55	C ₁₃ H ₂₈ O ₂ Si	244	0.12
Methyl 3-hydroxybenzoate, TMS Derivative	19.40	C ₁₁ H ₁₆ O ₃ Si	224	2.52
Linolool oxide, TMS derivative	19.52	C ₁₃ H ₂₆ O ₂ Si	242	0.22
Butylated Hydroxytoluene	19.8	C ₁₅ H ₂₄ O	220	1.32
Triethanolamine, 2TMS derivative	20.74	C ₁₂ H ₃₁ NO ₃ Si ₂	293	1.06
2-Butenedioic acid, (E)-, 2TBDMS Derivative	22.16	C ₁₆ H ₃₂ O ₄ Si ₂	344	1.29
Triethanolamine, 3TMS derivative	23.04	C ₁₅ H ₃₉ NO ₃ Si ₃	365	5.03
BENZOIC ACID, 4-[(TRIMETHYLSILYL)OXY]-, PROPYL ESTER	23.43	C ₁₃ H ₂₀ O ₃ Si	252	1.83
Succinic acid, 3-pentyl tridec-2-ynyl Ester	26.14	C ₂₂ H ₃₈ O ₄	366	0.33
Undec-10-ynoic acid, tetradecyl ester	26.31	C ₂₅ H ₄₆ O ₂	378	0.16
Linolool oxide, TMS derivative	26.73	C ₁₃ H ₂₆ O ₂ Si	242	0.56
1,2-BENZENEDICARBOXYLIC ACID, BIS(2-METHYLPROPYL) ESTER	27.78	C ₁₆ H ₂₂ O ₄	278	3.22
1-Hexadecanol, TMS derivative	29.77	C ₁₉ H ₄₂ OSi	314	7.85
Palmitic Acid, TMS derivative	31.40	C ₁₉ H ₄₀ O ₂ Si	328	4.21
OCTADECANOIC ACID	33.41	C ₁₈ H ₃₆ O ₂	284	0.56
Stearic acid, TMS derivative	34.88	C ₂₁ H ₄₄ O ₂ Si	356	2.30
1-Monopalmitin, 2TMS derivative	40.68	C ₂₅ H ₅₄ O ₄ Si ₂	474	1.19
DOCOSANE	42.09	C ₂₂ H ₄₆	310	0.19

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