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COMPARATIVE ANTIBACTERIAL ACTIVITY OF FRUITING BODY EXTRACTS FROM PLEUROTUS OSTREATUS GROWN ON SUBSTRATES SUPPLEMENTED WITH CAROXYLON CYCLOPHYLLA AND ATRIPLEX TATARICA AGAINST PATHOGENIC BACTERIA AND COMMON ANTIBIOTICS

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Abstract

This study investigated the effect of the active compounds in *Pleurotus ostreatus* fruiting body extracts on substrates enriched by two desert weeds containing 10% *Caroxylon cyclophyllum* and *Atriplex tatarica* and 2% urea as a control. Two extraction methods using ethanol and methanol were done for three treatments and applied as antibiotics against three pathogenic bacteria (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*). Results showed that the ethanolic extract of the urea-supplemented substrate fruiting bodies produced the best inhibition zones at 18mm against *P. aeruginosa* and 16mm against *E. coli*, and a low inhibition rate of 10mm for *S. aureus*. Also, the ethanolic extract of the fruiting bodies of the *Caroxylon cyclophylla*-supplemented substrate recorded a higher inhibition diameter of 17mm against *S. aureus* and a lower area of 12mm against *P. aeruginosa*. In contrast, the inhibitory effect decreased with the ethanolic extract of

fruiting bodies of the *Atriplex tatarica*-supplemented substrate at 10-14mm for both *P. aeruginosa* and *E. coli*. However, the methanolic extract of fruiting bodies of the urea-supplemented substrate showed apparent inhibition (10-16mm) against all bacteria. The methanolic extract of fruiting bodies produced substrates enriched by *Atriplex tatarica*, and *Caroxylon cyclophyllum* showed inhibitory effects ranging between 13-14mm and 10-12 mm, respectively. The reason for the inhibitory effect is related to the identified active compounds in these extracts, especially *Caroxylon cyclophyllum*. As such, these extracts can be used as drugs to eliminate these bacteria isolates.

Keywords: Atriplex tatarica, Antibacterial Activity, GC-MS, Desert weeds, Oyster mushroom, Pathogenic bacteria. Chenopodiaceae.

مقارنة النشاط المضاد للبكتيريا لمستخلصات الجسم الثمرية من Pleurotus ostreatus النامية على ركائز مضاف إليها Caroxylon cyclophylla و Atriplex tatarica ضد البكتيريا المسببة للأمراض والمضادات الحيوية الشائعة

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الخلاصة

أجريت هذه الدراسة لمعرفة تأثير المركبات الفعالة في مستخلصات الأجسام الثمرية للفطر المحاري Pleurotus osteratus المنتجة على ركائز مخصبة بنوعين من النباتات الصحراوية بنسبة 10% (Caroxylon cyclophyllum and Atriplex tatarica) أو مخصبة بنسبة 2% يوريا كمجموعة قياسية. تم إجراء طريقتي استخلاص باستخدام الإيثانول والميثانول لثلاث معاملات وتطبيقها كمضادات حيوية ضد ثلاث اجناس من البكتيريا مسببة للأمراض (Staphylococcus aureus و Pseudomonas aeruginosa و Escherichia

coli). أظهرت النتائج أن المستخلص الإيثانولي للأجسام الثمرية للركيزة المكملة باليوريا حقق أفضل منطقة تثبيط 18 مم ضد Ps. aeruginosa و16 مم ضد E. coli، ووصل أدنى تثبيط إلى 10 مم تجاه S. aureus. في الوقت نفسه، سجل المستخلص الإيثانولي للأجسام الثمرية للركيزة المكملة بـ C. cyclophylla قطر التثبيط أعلى بمقدار 17 مم ضد S. aureus وتثبيط أقل بمقدار 12 مم ضد Ps. aeruginosa. في المقابل، انخفض التأثير المثبط مع المستخلص الإيثانولي للأجسام الثمرية للركيزة المكملة بـ A. tatarica إلى 10–14 مم لكل من Ps. aeruginosa وE. coli. ومع ذلك، أظهر المستخلص الميثانولي للأجسام الثمرية للركيزة المكملة باليوريا تثبيطًا واضحًا (10–16 مم) ضد جميع البكتيريا. أنتج المستخلص الميثانولي للأجسام الثمرية ركائز غنية بـ A. tatarica، وأظهر C. cyclophyllum تأثيرًا مثبطًا يتراوح بين 13–14 مم و10–12 مم على التوالي. يعود سبب التأثير المثبط للمستخلصات إلى المركبات النشطة المحددة في هذه المستخلصات، وخاصة المستخلصات الإيثانولية C. cyclophyllum، وبالتالي يمكن استخدام هذه المستخلصات كمضادات حيوية لهذه الميكروبات.

كلمات مفتاحية: رغل تتاري، فعالية المضادات الحيوية، كروماتوغرافيا الغاز، الأعشاب الصحراوية، الفطر المحار، البكتيريا المسببة للأمراض.

Introduction

The oyster mushroom (*Pleurotus osteratus*) ranks second after the white button mushroom in terms of production and the economic importance for global producers (19). Belonging to the Agaricales order (Basidiomycota) this species grows naturally on cellulose residues and lignin, and this species (24). It is characterized by the ease of preparing substrates, ease of cultivation and production, in addition to the speed of growth and low cost (18).

Its fruiting body contain flavonoids (4), various bioactive substances, carbohydrates, fibers, vitamins (35), and high protein averages of 23-25% (39). Moreover, SMS (spent mushroom substrate) is used to produce other mushrooms (30) or as an organic fertilizer for ornamental plants and vegetables (28 and 38) due to its suitable nutritional value (33) and resistance against fungal diseases (27). The composition of the substrate has a wide impact on mushroom growth (15), which affects the chemical composition of fruiting bodies (42) as well as on productivity and biological efficiency (41). These substrates also alter the composition of fruiting bodies, leading to varying antimicrobial efficacies (27).

Many studies have shown the cultivation of oyster mushrooms on organic substrates such as wheat straw, rice straw, bean straw, finger millet straw, Eucalyptus sawdust sp., water hyacinth, coconut fibers, banana fibers (25), corn cobs (7 and 21), cardboard (31), wood sawdust, fibers of date-palm (42), husks of sunflower (30), wastes of olive (13), and asparagus old stalks (46). Two other plants, *Caroxylon cyclophyllum* (17) and *Atriplex tatarica* (26) of the Chenopodiaceae family are found in Iraq, especially in the Anbar deserts. The two desert weeds, Cyclophyllum saltbush (*Caroxylon cyclophyllum*) (10) and Tatarian orache (*Atriplex tatarica*) (23) grow in saline soil under high temperatures and drought conditions. (2) used these two hay

plants as supplements with a wheat hay substrate to produce oyster mushrooms that can adapt better to desert environments.

The components of the oyster mushroom include a large number of substances such as polysaccharides (β-glucans), lipids, linoleic acid, phenolic compounds and flavonoids, which are being explored for anti-inflammatory and antimicrobial therapies. They differ in terms of species and their proportions (18 and 19). Their importance lies in their medical applications in formulating therapeutic medications. These bioactive substances have chemical, physical and biological properties that enable them to be used in the medical field (20). A spawn produced by white corn exhibited good growth and yield of oyster mushrooms compared to that of wheat hay and barley, and the *Atriplex tatarica* hay-supplemented substrate gave higher results for protein content, fruiting bodies number, biological efficiency, and yield (2). *S. aureus, P. aeruginosa,* and *E. coli* are clinically significant bacteria, representing major pathogens in healthcare. They are associated with a wide range of infections and antimicrobial resistance challenges, and are pivotal in studying pathogenesis, biofilm formation, and therapeutic interventions (3). This research investigated the active compounds of the oyster mushroom fruiting bodies extracts cultivated on substrates enriched by *Atriplex tatarica* and *Caroxylon cyclophyllum* and examined their antibacterial activities.

Materials and Methods

Microbial strains: Three species of pathogenic bacteria were acquired from the Microbiology Laboratory at the Center of Desert Research, University of Anbar for this assay (zone of inhibition), namely *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 11775, and *Pseudomonas aeruginosa* ATCC 27853 (22).

Oyster mushroom samples: Three fresh samples of the oyster mushrooms were obtained from the Microbiology Lab., Center of Desert Research, University of Anbar, cleaned of compost, and dried at 40 °C. These samples were obtained from 3 substrates supplemented by 10% straws of *Atriplex tatarica*, *Caroxylon cyclophyllum*, and 2% urea (46%) as a control (2).

Oyster alcoholic mushroom extracts: The alcoholic extracts of the mushrooms were prepared using ethanol and methanol as solvents. The dried mushrooms were ground into a fine powder, and 10 g was mixed with 100 mL of ethanol or methanol. The mixture was subjected to continuous shaking for 24 hours at room temperature to ensure the effective extraction of bioactive compounds. The extract was filtered with Whatman No. 1 filter paper, and the solvents evaporated under reduced pressure using a rotary evaporator (20). The resulting concentrated extract was stored at 4 °C for subsequent analysis.

Antibacterial activity: The antibacterial efficacy test utilized the diffusion method using 90mm Petri dishes. The mushroom extract was solubilized using a DMSO (dimethyl sulfoxide) solution to ensure complete dissolution. Sterilized fresh Muller-Hinton Agar plates and autoclaves were used to complete this work by their inculcation using three bacterial species separately involving *S. aureus, E. coli*, and *P. aeruginosa*. A cork borer was used to drill 6mm-wells in the plates. About 60 µl of each extract was placed in a well (6mm) under sterilization conditions in laminar

flow. All inoculated plates were incubated at 37 °C for 18 hr to determine the zone of inhibition (3). As a control, the bacteria were checked for their inhibition using different antibiotics (9), as shown in Table 3.

Phytochemical screening: The dried oyster mushroom samples were ground using a blender and then extracted with ethanol and methanol to obtain three ethanolic and three methanolic extracts. The active chemical compounds for both extracts of the fruiting bodies were identified using GC-MS as described by (6).

Statistical analysis: The experiment was designed according to the completely randomized design (CRD) with three replications. The averages were compared according to the least significant difference (LSD) $p \leq 0.05$ using the GenStat statistical program (41).

Results and Discussion

Table 1 shows the highest production and the protein content of the oyster mushrooms was 262.278 $g.Kg^{-1}$ (dry weight) and 24.87% on the substrate supplemented by *Atriplex tatarica*, respectively, while the lowest values were 124.348 $g.Kg^{-1}$ and 20.44% on the urea-supplemented substrate. Moreover, total production and protein content on the substrate supplemented by *Caroxylon cyclophylla* exhibited 194.5558 $g.Kg^{-1}$ and 22.56%, respectively. The best results on the substrate supplemented by *Atriplex tatarica* are due to the adequate nutritional value and the suitable physical properties of this substrate which encourage the growth of oyster mushroom (44). The total protein is relevant to its ability to inhibit bacterial growth from the presence of bioactive proteins and peptides in mushrooms (18, 19 and 35). These bioactive compounds often possess antimicrobial properties, which play a direct role in inhibiting the growth of pathogenic microorganisms.

Table 1: Effect of the used substrates on total production and protein content of oyster mushrooms.

Treatment	Substrates supplemented with		
	Atriplex tatarica	***Caroxylon cyclophylla***	**Urea**
Total production ($g.Kg^{-1}$)	262.278	194.558	124.348
Protein content (%)	.870402	022.560	.440002
LSD ($p< 0.05$)		8.143	

Table 2 shows the effect of the oyster mushroom fruiting body extracts on the inhibition of three bacterial species, namely *S. aureus*, *P. aeruginosa,* and *E. coli*. The ethanolic extract of the bodies of the urea-supplemented substrate showed the highest inhibition zone at 18mm against the growth of *P. aeruginosa*, followed by 16mm and 10mm against *E. coli* and *S. aureus*, respectively. The ethanolic extract of fruiting bodies cultivated on the *Caroxylon cyclophylla*-supplemented substrate recorded the highest inhibition zone at 17mm against *S. aureus* and 15mm against *E. coli.* In contrast, *P. aeruginosa* showed less sensitivity with an inhibition zone of 12mm. The *Atriplex tatarica*-supplemented substrate recorded a smaller inhibition zone of 14mm against *E. coli* while *S. aureus* and *P. aeruginosa* recorded 10 mm.

Table 2: Effect of oyster mushroom fruiting body extracts on the inhibition zones of some pathogenic bacteria.

Treatment		Inhibition zone (mm)		
Extract type	Substrates supplemented by	*P. aeruginosa*	*E. coli*	*S. aureus*
Ethanol	Urea	18	16	10
	Caroxylon cyclophylla	12	15	17
	Atriplex tatarica	10	14	10
Methanol	Urea	13	10	16
	Caroxylon cyclophylla	10	10	12
	Atriplex tatarica	13	13	14

On the other hand, the methanolic extract of fruiting bodies of the urea-supplemented substrate showed an inhibition zone ranging from 10 mm to 16 mm. The extract of the *Atriplex tatarica*-supplemented substrate recorded inhibition zones of 13-14 mm while those of *Caroxylon cyclophylla*-supplemented substrate were at 10-12 mm against all the studied bacterial species.

Table 3 shows the effect of seven antibiotics against pathogenic bacteria growth and the resistance and sensitivity of intermediate bacteria toward those antibiotics. Ciprofloxacin exhibited the best result in inhibiting all bacteria at 22-30, 30-40, 25-33 mm against *S. aureus*, *E. coli*, and *P. aeruginosa*, respectively. Cefotaxime and norfloxacin recorded the highest inhibition zone against the oyster mushroom reaching 22-29 mm for each. Moreover, tobramycin recorded a higher inhibition zone against *S. aureus* at 19-29 mm, and showed resistance between ≤10-≤15 for all antibiotic types in this work. In comparison, the resistance intermediates were 10-12 and 15-22mm for oxacillin and cefotaxime, respectively.

Table 3: Diameters of inhibition zones against pathogenic bacteria strains for control antibiotics.

Antimicrobial	Disc content (µg)	Diameter of inhibition zone (mm)					
		S. aureus	***E. coli***	***P. aeruginosa***	**Resistant**	**Intermediate**	**Susceptible**
Amikacin	30	20-26	19-26	18-26	≤14	15-16	≥17
Cefotaxime	30	16-20	25-32	22-29	≤14	15-22	≥23
Ciprofloxacin	5	22-30	30-40	25-33	≤15	16-20	≥21
Gentamicin	10	19-27	19-26	16-21	≤12	13-14	≥15
Tobramycin	10	19-29	18-26	19-25	≤12	13-14	≥15
Norfloxacin	10	17-28	28-35	22-29	≤12	13-16	≥17
Oxacillin	1	18-24	-	-	≤10	10-12	≥13

BSAC Methods for Antimicrobial Susceptibility Testing, Version 12 May 2013.

As shown in Table 3, the higher inhibition zones ranged between ≥21 and ≥23mm compared to the use of mushroom extracts, which did not show any inhibition effect against all species of bacteria. The ethanolic extract of fruiting bodies of the urea-supplemented substrate recorded a higher inhibition zone at 16mm, and 18mm for *E.*

coli and *P. aeruginosa*, respectively, consistent with (19 and 39). Hence, this extract can be used instead of the studied antibiotics except cefotaxime and ciprofloxacin to decrease the risk of these bacteria.

Tables 2 and 3 show that the ethanolic extract of fruiting bodies of the *Caroxylon cyclophylla*-supplemented substrate had an inhibition average of 15 and 17 mm against *E. coli* and *S. aureus*, respectively. Also, this extract can be used instead of gentamicin and tobramycin against *E. coli*, or amikacin, oxacillin, norfloxacin, gentamicin, and tobramycin against *S. aureus*, respectively. The ethanolic extract of the *Atriplex tatarica*-supplemented substrate registered an inhibition zone of 14mm against *E. coli*, and thus can be used instead of oxacillin, which recorded ≥13mm. These results agree with those results of (14 and 19).

The methanolic extract of the urea-supplemented substrate recorded an inhibition average that reached 13mm and 16mm against *P. aeruginosa* and *S. aureus*, respectively, allowing it to be used as a drug against the former bacteria instead of oxacillin which recorded ≥ 13 mm. Also, this extract can be used against *S. aureus* instead of gentamicin, tobramycin, and oxacillin, which recorded speed effects of ≥15, ≥15, and ≥13mm, respectively (Table 3). Tables 2 and 3 show that the methanolic extract of the fruiting bodies of the *Caroxylon cyclophylla*-supplemented substrate cannot be used as an antibiotic to inhibit pathogenic bacteria due to the low width of the inhibition zone of 10-12mm whereas the antibiotics exhibited an effect speed ranging from ≥13 and ≥23 mm. Moreover, the methanolic extract of the fruiting bodies of the *Atriplex tatarica*-supplemented substrate can be used instead of oxacillin against all bacteria species, as reported by (8 and 19).

The components of the oyster mushroom include a large number of substances that differ in terms of species and their proportions. These bioactive substances have chemical, physical and biological properties enabling their use in the medical field. They differ in composition and according to the extraction method, and may contain glycosides, alkaloids, phenols, resins, aromatic oils, salivary materials, stable oils, free materials, and antibiotics (20).

The ability of the active substances in inhibiting pathogens vary depending on their composition, properties, and concentrations. For instance, their amounts in the crude extract may be inadequate to give efficacy to the dosages, or there may be substances in the extract that have an opposite effect to the active ingredients (36).

Table 4 exhibits many bioactive substances in the ethanolic extract of fruiting bodies of the *Atriplex tatarica*-supplemented substrate. This extract has an inhibitory effect against pathogenic bacteria due to the presence of bioactive compounds such as alkaloids and toxic alcohols, which have an inhibitory effect on the growth of some species of bacteria (20). However, alkaloids have complete activity against microbes due to their impact on the nucleic acids and plasma membrane of bacteria or have adverse effects on their metabolic processes (36). Also, the inhibitory effect may be related to the role of these extracts in reducing the ability of bacterial species to adhere to these extracts and their content of compounds and active substances. The results showed $C_{15}H_{24}O$, which is used as an antioxidant, and $C_{19}H_{38}O_2$ and $C_{15}H_{30}O_2$, play an essential role in inhibiting the studied bacteria. Also, it contains the fatty acid $C_{14}H_{26}O_2$, which is an unsaturated acid and is believed to have toxic effects

and a useful role in treating some diseases affecting the prostate (45). These findings agree with those on the extracts of fruiting bodies of the *Atriplex tatarica*-supplemented substrate against *E. coli* (14 mm inhibition zone).

Table 4: Active substances in the ethanolic extract of the oyster mushroom fruiting bodies of the Atriplex tatarica-supplemented substrate.

No.	Peak	R.T.	Area %	Height %	Mol. weight	Name	Formula
1.	1	10.226	1.21	1.94	220	Butylated Hydroxytoluene (Antioxidant)	$C_{15}H_{24}O$
2.	2	15.399	9.96	11.28	298	Nonadecanoic acid	$C_{19}H_{38}O_2$
3.	3	17.204	29.74	21.35	226	E-9-Tetradecenoic acid	$C_{14}H_{26}O_2$
4.	4	17.370	4.30	6.31	242	Pentadecylic acid	$C_{15}H_{30}O_2$
5.	5	20.767	0.90	1.35	-	Unknown	-
6.	6	22.934	53.90	57.77	-	Unknown	-

Table 5 shows the 14 active substances and compounds in the ethanolic extract of oyster mushroom fruiting bodies of the *Caroxylon cyclophylla*-supplemented substrate. They are: (3Z)-3-dodecene; n-heptane; 2-ethyl-1-hexanethiol; butylated hydroxytoluene; 2-tetradecene, (e)-; hexane, 2,4-dimethyl-; 1h-benzocyclohepten-7-ol, 2,3,4,4a,5,6,7,8-octahydro-1,1,4a,7-tetramethyl-, cis-; 4-tetradecene, (e)-; nonadecanoic acid; ethyl tridecanoate; methyl (7e,10e)-7,10-hexadecadienoate; aspidofractinin-3-ylmethanol; 5-ethyl-1-nonene and unknown compound. The extract's active substances vary in their effects, which contributed to enhancing its role in inhibiting bacterial species. These compounds include the acid $C_{12}H_{24}$ and $C_7H_{16,}$ which inhibit their ability to enter the bacterial cell. Also, the inhibitory effect may be related to the presence of $C_8H_{15}S$ and $C_{15}H_{24}O$, which are considered antioxidants (16). The main active substances in the ethanolic extract of *P. ostreatus* fruits bodies of the urea-supplemented substrate (Table 6) includes 7 compounds, as follows: n-dodecyl thioglycolate; 1h-benzocyclohepten-7-ol, 2,3,4,4a,5,6,7,8-octahydro-1,1,4a,7-tetramethyl-, cis-; nonadecanoic acid; ethyl tridecanoate; 1,e-11,z-13-heptadecatriene; aspidofractinine-3-methanol, (2.alpha.,3.beta.,5.alph.); and four unknown compounds.

Table 5: Active substances in the ethanolic extract of oyster mushroom fruiting bodies of the Caroxylon cyclophylla-supplemented substrate.

No.	Peak	R.T.	Area %	Height %	Mol. weight	Name	Formula
1.	1	8.501	0.89	1.12	168	(3Z)-3-Dodecene	$C_{12}H_{24}$
2.	2	8.610	0.84	1.10	100	n-Heptane	C_7H_{16}
3.	3	9.087	2.29	2.69	146	2-Ethyl-1-hexanethiol	$C_8H_{18}S$
4.	4	10.225	3.10	4.64	220	Butylated Hydroxytoluene (Antioxidant)	$C_{15}H_{24}O$
5.	5	11.130	1.39	2.09	196	2-Tetradecene, (E)-	$C_{14}H_{28}$
6.	6	11.223	0.63	1.10	114	Hexane, 2,4-dimethyl-	C_8H_{18}
7.	7	11.630	2.06	2.53	222	1H-Benzocyclohepten-7-ol, 2,3,4,4a,5,6,7,8-octahydro-1,1,4a,7-tetramethyl-, cis-	$C_{15}H_{26}O$
8.	8	13.496	1.03	1.73	196	4-Tetradecene, (E)-	$C_{14}H_{28}$
9.	9	15.392	12.93	13.48	298	Nonadecanoic acid	$C_{19}H_{38}O_2$
10.	10	15.630	2.07	3.19	242	Ethyl tridecanoate	$C_{15}H_{30}O_2$
11.	11	17.209	46.23	29.68	-	Unknown	-
12.	12	17.311	1.33	2.78	266	Methyl (7E,10E)-7,10-hexadecadienoate	$C_{17}H_{30}O_2$
13.	13	20.777	16.97	24.29	310	Aspidofractinin-3-ylmethanol	$C_{20}H_{26}N_2O$
14.	14	22.890	8.24	9.58	154	5-Ethyl-1-nonene	$C_{11}H_{22}$

Also, $C_{14}H_{28}$ and C_8H_{18} (one of the volatile compounds) are believed to have anti-inflammatory and antimicrobial effects against staphylococci and the causes of pneumonia and bacilli (12). $C_{15}H_{26}O$ is an alcoholic compound with excellent biological control, anti-fungi, and insecticide properties. The compound $C_{17}H_{30}O_2$ (a fatty compound extracted from microalgae) may have a role in the inhibiting process (12). Moreover, the compounds $C_{19}H_{38}O_2$, $C_{15}H_{30}O_2$, and $C_{20}H_{26}N_2O$ may contribute to the inhibition of the studied bacteria. The compound $C_{11}H_{22}O_2$ (a stearic acid extracted from oily vegetables) inhibits some pathogenic fungi growth activity of the genus *Paracoccidioides* spp. and has an inhibitory strength ranging between 15.6 - 500 mg/ml (37). The ethanolic extract of *P. ostreatus* fruiting bodies of the urea-supplemented substrate achieved a significant antibiotic effect against *E. coli* and *P. aeruginosa* (Table 2).

Table 6: Active substances in the ethanolic extract of the oyster mushroom fruiting bodies of the urea-supplemented substrate.

No	Peak	R.T.	Area %	Height %	Mol. weight	Name	Formula
1.	1	9.084	1.60	2.43	260	n-Dodecyl thioglycolate	$C_{14}H_{28}O_2S$
2.	2	11.630	1.42	2.42	222	1H-Benzocyclohepten-7-ol, 2,3,4,4a,5,6,7,8-octahydro-1,1,4a,7-tetramethyl-, cis-	$C_{15}H_{26}O$
3.	3	15.429	6.39	8.57	298	Nonadecanoic acid	$C_{19}H_{38}O_2$
4.	4	15.632	1.08	1.96	242	Ethyl tridecanoate	$C_{15}H_{30}O_2$
5.	5	17.259	56.14	34.07	-	Unknown	-
6.	6	17.327	2.65	6.76	234	1, E-11,Z-13-Heptadecatriene	$C_{17}H_{30}$
7.	7	17.378	5.37	6.52	-	Unknown	-
8.	8	20.796	23.36	35.01	310	Aspidofractinine-3-methanol, (2. alpha.,3.beta.,5.alph.	$C_{20}H_{26}N_2O$
9.	9	22.300	1.60	1.68	-	Unknown	-
10.	10	22.885	0.38	0.58	-	Unknown	-

The active substances in the methanolic extract of the oyster mushroom fruiting bodies of the *Atriplex tatarica*-supplemented substrate showed 7,9-dimethyl-8-nitrobicyclo[4.3.1]decan-10-one; butyric acid, 2-methyl-, tetradecanoic acid, 12-methyl-, methyl ester; decanoic acid, methyl ester; palmitic acid, methyl ester; nonadecanoic acid; 9,11-octadecadienoic acid, methyl ester, (e,e)-; 12-octadecenoic acid, methyl ester; octadecanoic acid, 17-methyl-, methyl ester; nonadecanoic acid; 1,2-benzenedicarboxylic acid, bis(2-ethylhexyl) ester; e-11-hexadecenal and three unknown compounds (Table 7).

Table 7: Active substances in the methanolic extract of the oyster mushroom fruiting bodies of the Atriplex tatarica-supplemented substrate.

No	Peak	R.T.	Area %	Height %	Mol. weight	Name	Formula
1.	1	11.629	0.58	0.61	225	7,9-Dimethyl-8-nitrobicyclo [4.3.1]decan-10-one	$C_{12}H_{19}NO_3$
2.	2	13.441	0.17	0.22	102	Butyric acid, 2-methyl-	$C_5H_{10}O_2$
3.	3	13.536	0.25	0.36	256	Tetradecanoic acid, 12-methyl-, methyl ester	$C_{16}H_{32}O_2$
4.	4	13.849	0.93	1.19	186	Decanoic acid, methyl ester	$C_{11}H_{22}O2$
5.	5	14.945	13.97	16.93	270	Palmitic acid, methyl ester	$C_{17}H_{34}O_2$
6.	6	15.416	5.54	4.50	298	Nonadecanoic acid	$C_{19}H_{38}O_2$
7.	7	16.707	29.42	27.80	294	9,11-Octadecadienoic acid, methyl ester, (E,E)-	$C_{19}H_{34}O_2$
8.	8	16.759	8.63	17.47	296	12-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$
9.	9	16.953	1.90	2.48	312	Octadecanoic acid, 17-methyl-, methyl ester	$C_{20}H_{40}O_2$
10.	10	17.213	15.94	8.93	-	Unknown	-
11.	11	17.379	1.95	2.24	298	Nonadecanoic acid	$C_{19}H_{38}O_2$
12.	12	20.444	0.45	0.47	-	Unknown	-
13.	13	20.770	0.74	0.86	390	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	$C_{24}H_{38}O_4$
14.	14	22.301	2.20	1.53	238	E-11-Hexadecenal	$C_{16}H_{30}O$
15.	15	22.926	17.31	14.41	-	Unknown	-

Its use showed an inhibition zone of 13mm against *P. aeruginosa* and *E. coli* and 14mm against *S. aureus*. The results also showed that this extract could be used as a substitute for the antibiotic oxacillin against the above bacterial species. According to the results in Table 7, it should be noted that the extract contained 12 active substances that differed in their effect, and this variation may be due to the concentration of these active substances in the extract (20).

Table 8 shows the active substances and compounds in the methanolic extract of oyster mushroom fruiting bodies of the *Caroxylon cyclophylla*-supplemented substrate, which include 1-hexanethiol, 2-ethyl; 1h-benzocyclohepten-7-ol, 2,3,4,4a,5,6,7,8-octahydro-1,1,4a,7-tetramethyl-, cis-; cyclopentanetridecanoic acid, methyl ester; Dodecanoic acid, methyl ester (Lauric acid, methyl ester); 9-Dodecenoic acid, methyl ester, €-; hexadecanoic acid, methyl ester; 9,11-octadecadienoic acid, methyl ester, (e,e)-; 11-octadecenoic acid, methyl ester, (z)-(cis-vaccenic acid); octadecanoic acid, 17-methyl- methyl ester; e-9-tetradecenoic acid; octadecanoic acid; e-9-tetradecenal and an unknown compound.

Table 8: Active substances in the methanolic extract of the oyster mushroom fruiting bodies of the Caroxylon cyclophylla-supplemented substrate.

No	Peak	R.T.	Area %	Height %	Mol. Weight	Name	Formula
1.	1	9.090	2.17	1.78	146	1-Hexanethiol, 2-ethyl	$C_8H_{18}S$
2.	2	11.631	1.53	1.29	222	1H-Benzocyclohepten-7-ol, 2,3,4,4a,5,6,7,8-octahydro-1,1,4a,7-tetramethyl-, cis-	$C_{15}H_{26}O$
3.	3	13.535	0.67	0.76	296	Cyclopentanetridecanoic acid, methyl ester	$C_{19}H_{36}O_2$
4.	4	13.848	0.85	0.91	214	Dodecanoic acid, methyl ester (Lauric acid, methyl ester)	$C_{13}H_{26}O_2$
5.	5	14.727	0.44	0.54	212	9-Dodecenoic acid, methyl ester, €-	$C_{13}H_{24}O_2$
6.	6	14.939	13.38	14.39	270	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$
7.	7	16.697	27.96	30.90	294	9,11-Octadecadienoic acid, methyl ester, (E,E)-	$C_{19}H_{34}O_2$
8.	8	16.748	23.72	31.35	296	11-Octadecenoic acid, methyl ester, (Z)-(cis-Vaccenic acid)	$C_{19}H_{36}O_2$
9.	9	16.951	2.12	2.28	312	Octadecanoic acid, 17-methyl-, methyl ester	$C_{20}H_{40}O_2$
10.	10	17.195	17.24	8.23	226	E-9-Tetradecenoic acid	$C_{14}H_{26}O_2$
11.	11	17.360	1.57	1.65	284	Octadecanoic acid	$C_{18}H_{36}O_2$
12.	12	22.288	1.97	1.01	210	E-9-Tetradecenal	$C_{14}H_{26}O$
13.	13	22.894	6.39	4.92	-	Unknown	-

These compounds are similar to those in the previous extracts but did not give positive results in inhibiting the pathogenic bacteria. Hence, they are not recommended for use as a substitute for antibiotics due to their low concentrations in the extract or the presence of other compounds that work in contradiction to the other active substances against bacterial growth (20).

Table 9 exhibits the active substances and compounds in the methanolic extract of oyster mushroom fruiting bodies of the urea-supplemented substrate. They include: glyceraldehyde; butylated hydroxytoluene; 7,9-dimethyl-8-nitrobicyclo [4.3.1] decan-

10-one; ethylene lactic acid; nonadecanoic acid; (z)6,(z)9-pentadecadien-1-ol; aspidofractinin-3-ylmethanol; and four unknown compounds. This extract showed an inhibition zone of 13mm against *P. aeruginosa* and 16mm against *S. aureus;* thus, it can be used as an alternative to gentamicin, tobramycin, and oxacillin against the two bacteria.

Table 9: Active substances and compounds in the methanolic extract of the oyster mushroom fruiting bodies of the urea-supplemented substrate.

No.	Peak	R.T.	Area %	Height %	Mol. Weight	Name	Formula
1.	1	3.035	1.49	0.77	90	Glyceraldehyde	$C_3H_6O_3$
2.	2	10.229	0.95	1.70	220	Butylated Hydroxytoluene (Antioxidant)	$C_{15}H_{24}O$
3.	3	11.632	0.83	1.46	225	7,9-Dimethyl-8-nitrobicyclo [4.3.1] decan-10-one	$C_{12}H_{19}NO_3$
4.	4	13.910	0.41	0.76	90	Ethylene lactic acid	$C_3H_6O_3$
5.	5	14.005	0.63	1.19	-	Unknown	-
6.	6	15.395	15.30	16.61	298	Nonadecanoic acid	$C_{19}H_{38}O_2$
7.	7	17.208	51.22	37.98	-	Unknown	-
8.	8	17.315	1.64	3.89	224	(Z)6,(Z)9-Pentadecadien-1-ol	$C_{15}H_{28}O$
9.	9	20.779	8.68	14.11	310	Aspidofractinin-3-ylmethanol	$C_{20}H_{26}N_2O$
10.	10	22.293	4.74	4.03	-	Unknown	-
11.	11	22.902	14.12	17.51	-	Unknown	-

The results show that extraction, especially for *Caroxylon cyclophylla* using ethanol was more efficient than methanol extraction in inhibiting the growth of the studied bacterial species. This may be due to the types of compounds extracted, their quantity and concentration, and the presence of toxic alcohol compounds, alkalis, fatty acids, or ester compounds inhibiting the growth of microorganisms (20). These compounds damage the cytoplasmic membrane of the bacterial cell, protein materials, nucleic acids, and their influence on the metabolic activities of the microorganism growth (37).

The variations in bacterial inhibition may be due to the type of bacteria, the type of extract used, its concentration, and its extraction method. The results are consistent with (11) who used sumac extract, which contains active compounds similar to this study, that inhibited the bacteria at the inhibition zones of 0-14mm and 0-17mm for *P. aeruginosa*, and *E. coli*, respectively. These results also agree with (5 and 19) who found that the extracts contained the active compounds, n-docosane, hydrocoumarin, 1,2-benzenedicarboxylic acid, 9,12,15-octadecatrienoic acid, and methyl 3-(2-hydroxyphenyl) propionate, which had positive results against the studied bacteria. Generally, *A. tatarica* was used as a popular drug by Bedouins due to its volatile oils, saponin (1.13%), proteins (23%), fibers (25.21%), acidic lignin (15%), and nitrates (5.1%) (43). These results agree with (34). Finally, the variances found in the bioactivity of the methanolic and ethanolic extracts relate to the differences in the extracted active substances of the mushroom samples (29 and 40).

Conclusions

This study demonstrated the efficacy of the active compounds found in the extracts of the fruiting bodies of the oyster mushroom cultivated on substrates augmented with two desert weeds, *Caroxylon cyclophyllum* and *Atriplex tatarica*. The ethanolic extract of the urea-supplemented substrate's fruiting bodies had the most effective inhibitory zones against *P. aeruginosa* and *E. coli*. The ethanolic extract of the fruiting bodies from the *Caroxylon cyclophylla*-supplemented substrate exhibited a greater inhibitory zone against *S. aureus* while the methanolic extract from the urea-supplemented substrate had significant suppression properties against all the bacterial strains.

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Authors 1 and 3: methodology, writing—original draft preparation; Authors 2, 4 and 5 writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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The authors declare no conflict of interest.

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