

Micropropagation of lemongrass *Cymbopogon citratus* L. and evaluation the effectiveness of methanolic leaves extract and its antibacterial activity in inhibiting three species of bacteria that cause bacterial gill disease in common carp fish

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Abstract

The current study was conducted in the laboratories of the Marine Science Center at the University of Basrah. In order to propagate lemongrass using plant tissue culture technology and test the effectiveness of leaves methanolic extract in concentrations (2.5%, 5%, 7.5%, and 10%), to demonstrate its effect in inhibiting three species of bacteria (*Aeromonas sobria*, *Sphingomonas paucimobilis*, and *Sphingomonas paucimobilis* Isolated from common carp (*Cyprinus carpio*) infected with bacterial gill disease, The bacterial types were collected from infected fish at a local fish farm in Basrah Governorate, southern Iraq, The bacterial isolates were identified chemically using VITEK II system cards, as well as genetically using 16s rRNA. The results showed that the 10% extract exhibited the highest antibacterial efficacy, while the 2.5% concentration showed the weakest activity. Notably, *Aeromonas sobria* strain demonstrated the greatest sensitivity to the lemongrass extract, whereas *Sphingomonas paucimobilis* showed complete resistance across all tested concentrations. The results of GC MS analysis indicated that the lemongrass extract contained many active compounds, including Itaconate and Eugenol, which have an antibacterial effect, Citral, Curlone, hydrazine, saponin, and all concentrations of the extracts showed varying degrees of activity against these tested bacterial species, comparable to their effectiveness with the standard antibiotic oxytetracycline.

Keywords: lemongrass, Micropropagation, Phytochemical, methanolic leaves extract, antibacterial activity



Introduction

Lemongrass (*Cymbopogon citratus*), a tropical species native to southern India and Sri Lanka, is now extensively cultivated in various tropical and subtropical regions around the world (Myriama *et al.*, 2018). This aromatic grass is highly regarded for its culinary, medicinal, and pesticidal properties. Taxonomically, *C. citratus* belongs to the Kingdom Plantae, Phylum Spermatophyta, Class Liliopsida, Order Poales, Family Poaceae, Genus *Cymbopogon*, and species *citratus* (Thomas, 2016; Myriama *et al.*, 2018). The propagation of *C. citratus* through *in vitro* techniques particularly micropropagation has proven efficient for the rapid production of genetically same, disease-free plantlets. These tissue culture methods consist of many well defined stages designed to ensure the conservation of genetic fidelity and sustainable production of biomass for both research and commercial use. Phytochemically, *C. citratus* is rich in bioactive constituents, including essential oils and phenolic compounds, many of which show important antimicrobial and antioxidant activities. One of the primary components, demonstrating nearly double the antimicrobial efficacy compared to the crude essential oil itself, is citral. Interestingly, the plant's leaves contain a broad spectrum of secondary metabolites such as flavonoids, alkaloids, tannins, saponins, and phenolic acids. Notable flavonoids identified include quercetin, luteolin, apigenin, iso-orientin-2'-O-rhamnoside, and kaempferol (Kulus and Miler, 2021). Other studies confirmed the presence of alkaloids, tannins, flavonoids, and phenolic compounds, while reporting the absence of saponins and cardiac glycosides (Naqqash and Al-Bazaz, 2019). Due to these diverse chemical constituents, *C. citratus* has found extensive applications in the pharmaceutical and food industries (Nambiar and Matela, 2012).

The widespread use of synthetic antibiotics has led to the emergence of multidrug-resistant bacterial strains, in addition to their potential adverse effects on fish tissues—an essential dietary protein source for many populations. As a result, there is a pressing need to identify eco-friendly and sustainable antimicrobial alternatives. One promising application of lemongrass is its use as a natural antimicrobial agent against fish pathogens. Several species of *Aeromonas* (family Aeromonadaceae) are known to infect both fish and humans. These bacteria are widely distributed in aquatic environments and have been isolated from various sources, including freshwater, estuarine and surface waters, sewage, food, and both healthy and diseased fish (Narvaez *et al.*, 2021). Pathogenesis in fish is largely driven by virulence factors such as aerolysin and extracellular hemolysin, which contribute to the onset of diseases such as bacterial gill disease (Rajeswari Shome *et al.*, 2005). Additionally, *Aeromonas* species are recognized for their ability to degrade fish and meat at ambient temperatures, especially under stress conditions such as overcrowding, poor water quality, and rough handling (Noga, 2010; Mailafia *et al.*, 2021). Quite notably, Taxonomically, *Aeromonas* are Gram-negative, facultatively anaerobic, chemoorganotrophic rods that are typically oxidase-positive and capable of growing in environments containing 0% NaCl but not in 6% NaCl (Narvaez *et al.*, 2021). It's worth mentioning that In both humans and ectothermic animals, these

bacteria can cause severe septicemia (Abbott *et al.*, 2003). The motile *Aeromonas* species—particularly *A. hydrophila*, *A. sobria*, and *A. caviae*—are frequently implicated in disease outbreaks. Furthermore, *Sphingomonas paucimobilis*, previously known as *Pseudomonas paucimobilis* when first isolated as a human pathogen in 1977, is another opportunistic Gram-negative bacterium of concern. It is non-fermentative, strictly aerobic, oxidase-positive, and produces distinct yellow-pigmented colonies on blood agar (Chowdhary *et al.*, 2016; Hajam *et al.*, 2022; Zaheen *et al.*, 2022;). Although generally of low pathogenicity, it can cause infections in immune-compromised individuals. It is worth noting that many of these bacteria naturally inhabit the gastrointestinal tracts of fish without causing disease. However, under environmental or physiological stress, these microorganisms can become opportunistic pathogens capable of causing significant morbidity and mortality in aquaculture systems (Iorizzo *et al.*, 2022).

Quite notably, The present study aimed to develop an efficient *in vitro* propagation procedure for *Cymbopogon citratus*, and to judge the antibacterial activity of its leaf extract against three pathogenic bacteria associated with bacterial gill disease in common carp (*Cyprinus carpio*). This research seeks to give a sustainable source of medicinal plants while exploring eco-friendly alternatives for disease management in aquaculture.

Materials And Methods

Micropropagation Techniques

Interestingly, The composition of the growth medium plays a vital role in the success of micropropagation. In real world terms, A well balanced medium must include essential nutrients, vitamins, and plant growth regulators in carefully adjusted proportions to support optimal plant development. It's worth mentioning that One of the most widely used media is that of Murashige and Skoog (1962), which can be modified particularly in the concentrations of auxins and cytokinins depending on the desired outcome, such as shoot proliferation or root induction. In this study, the medium formulated by Al-Aradi *et al.* (2017). was used for the *in vitro* propagation of *Cymbopogon citratus* (lemongrass).

Procedure for Extraction and Preparation of Plant Material

The extraction of bioactive compounds from plant tissues was conducted using the Soxhlet extraction method, a standard and effective technique that relies on the principle of continuous solvent circulation. Interestingly, In this method, the plant sample is placed in a thimble inside a Soxhlet apparatus, and methanol solvent repeatedly passes through the sample to extract the desired components. Quite notably, before extraction, the plant material was air-dried and finely ground into a powder to increase the surface area for better solvent penetration and improved extraction efficiency (Liu *et al.*, 2012; Alara *et al.*, 2021).

Determination of Total Chemical Constituents of *C. citratus*

The chemical composition of lemongrass (*C. citratus*) leaf methanolic extract was analyzed using Gas Chromatography–Mass Spectrometry (GC-MS), performed with a

Shimadzu GC-QP 2010 system. Chemical compounds were identified by comparing the mass spectra of the detected peaks with those available in the National Institute of Standards and Technology (NIST, 2005) library.

Assessment of Antibacterial Activity

The antibacterial properties of the lemongrass methanolic extract of the leaves was evaluated against three bacterial isolates: *Sphingomonas paucimobilis*, *Aeromonas sobria*, and *Sphingomonas paucimobilis*. These isolates were obtained from common carp (*Cyprinus carpio*). The infected fish were collected from a local fish farm in the Basrah region of southern Iraq and promptly transferred to the laboratory of the Marine Science Center at the University of Basrah.

Identification of Bacterial isolates

Infected gills were squished after being cleaned with regular saline to get rid of the bacteria in mucus. The homogenate solutions were serially diluted from 10⁻¹ to 10⁻⁷ milliliters. Nutrient agar (Hi media-India) was inoculated with 0.1 ml of the successive dilutions. For twenty-four hours, the plates were incubated at 37°C. At the same temperature and time as before, one colony expansion was chosen and moved to new media. *Aeromonas sobria* produced smooth, round, semitranslucent, greyish-white colonies on culture media, whereas *Sphingomonas paucimobilis* produced smooth, round, flat colonies with deep yellow pigmentation. Gram stain was used to discriminate between positive and negative bacteria and to identify the isolated bacteria morphologically. Growths were purified by being cultivated for 24 hours at 37°C on nutrient agar.

then used the VITEK 2 system to identify the bacteria. (Biomerieux- USA). (Tables 1, 2, and 3), Molecular identification was based on 16S rRNA gene Forward 5'- AGAG TTGGA TCCTG GCTCAG3' Reverse 5'GGTT ACCTTGT TACGA CTT- 3' The universal primer 16Sr RNA of the aforementioned gene was used for PCR amplification, and the mixture was a combination of the specific interaction for diagnosis gene.

Table (1): Identified species of *Sphingomonas paucimobilis* by Vitek 2 system.

Test	Result	Test	Result	Test	Result	Test	Result	Test	Result	Test	Result	Test	Result	Test	Result
APPA	-	APPA	-	PyrA	-	IARL	-	XCEL	-	BGAL	-	BNAG	-	AGLTp	-
dGLU	-	dGLU	-	OFF	-	BGLU	+	dMAL	+	dMAN	-	dMNE	-	BXYL	-
BALap	-	BALap	-	PLE	-	TyrA	-	URE	-	dSOR	-	SAC	-	dTAG	-
dTRE	-	dTRE	-	MNT	-	5KG	-	ILATk	-	AGLU	-	SUCT	-	NAGA	-
AGAL	-	AGAL	-	GlyA	-	ODC	-	LDC	-	uHS	-	CMT	-	BGUR	-
O129R	-	O129R	-	ILMLTa	-	ELLM	-	ILATa	-	-	-	-	-	-	-

Table (2): Identified species of *Sphingomonas paucimobilis* by Vitek 2 system.

Test	Result	Test	Result	Test	Result	Test	Result	Test	Result	Test	Result	Test	Result	Test	Result
APPA	-	ADO	-	PyrA	+	IARL	-	XCEL	-	BGAL	-	BNAG	-	AGLTp	-
dGLU	-	GGT	-	OFF	-	BGLU	+	dMAL	+	dMAN	-	dMNE	-	BXYL	-
BALap	-	LIP	-	PLE	-	TyrA	-	URE	-	dSOR	-	SAC	-	dTAG	-
dTRE	-	CIT	-	MNT	-	5KG	-	ILATk	-	AGLU	-	SUCT	-	NAGA	-
AGAL	-	PHOS	-	GlyA	-	ODC	-	LDC	-	uHS	-	CMT	-	BGUR	-
O129R	-	DGGA	-	ILMLTa	-	ELLM	-	ILATa	-	-	-	-	-	-	-

Table (3): Identified species of *Aeromonas sorbia* by Vitek 2 system

Test	Result	Test	Result	Test	Result	Test	Result	Test	Result	Test	Result	Test	Result	Test	Result
APPA	+	ADO	-	PyrA	-	IARL	-	dCEL	-	BGAL	+	H2S	-	BNAG	+
BGLU	-	dMAL	+	dMAN	+	dMNE	+	BXYL	-	BALap	-	ProA	+	LIP	+
PLE	-	TyrA	+	URE	-	dSOR	-	SAC	-	dTAG	-	dTRE	+	CIT	+
MNT	-	5KG	-	ILATk	-	AGLU	-	SUCT	+	NAGA	+	AGAL	-	PHOS	-
GlyA	-	ODC	-	LDC	-	IHISe	+	CMT	+	BGUR	-	O129R	-	GGAA	+
IMLTa	+	ELLM	+	ILATa	-										

Parts Taq PCR PreMix Concentration 5µl Forward primer One microliter (10 picomols/µl) 10 picomols/µl (1 µl) reverse primer 1.5µl of DNA 16.5 µl of distilled water 25µl is the final volume. The ideal detecting condition was the cycling condition. No. Phase Tm (C) Cycle Time No. 1. First Denaturation 5 minutes at 94°C for one cycle 2. Denaturation -2 94°C 45 seconds 35 cycles 3. Annealing at 56°C for one minute; 4. Extension-1 at 72°C for one minute; and 5. Extension-2 at 72°C for seven minutes. One cycle was carried out utilizing a thermal cycler (GTC-96). Gel electrophoresis was used to identify the PCR products, and the Nanodrop (Nabi/Korea) 260/280 column was used to evaluate purity. Using the BigDye Terminator v3.1 Cycle Sequencing kit on an ABI 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA), PCR products were purified and sequenced in both directions. Macrogen Korea carried out the gene sequencing. The Basic

Local Alignment Search Tool (BLAST) tool, which is accessible online at the National Center for Biotechnology Information (NCBI) at <http://www.ncbi.nlm.nih.gov>, and the BioEdit program were used for the homology search. The NCBI's nucleotide databases were used to analyze the sequence:(Jassim *et al.*, 2026).

Antibacterial Testing Procedure

According to the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS, 1993), the well diffusion method was employed to assess the antibacterial activity. Three to five similar colonies from each isolate were transferred using a sterile loop into test tubes containing 5 mL of Tryptic Soy Broth (TSB). The turbidity of each bacterial suspension was adjusted to match the 0.5 McFarland standard, corresponding to a bacterial concentration of approximately $1-2 \times 10^8$ CFU/mL.

The McFarland standard was prepared by mixing 995.5 mL of 1% sulfuric acid with 5 mL of 1% barium chloride. Müller-Hinton agar plates were inoculated using sterile swabs, streaked across the entire surface in three directions each time rotating the plate by nearly 60° to ensure even distribution. Also swabbed. were the edges of the agar After allowing the inoculum to dry at room temperature, 6 mm wells were created in the agar. Each well was loaded with 50 μ L of lemongrass extract at different concentrations (2.5%, 5%, 7.5%, and 10%), with each concentration tested in copy. The plates were left at room temperature for one hour to allow for diffusion of the extract, and then incubated at 37°C for 18 hours. Following incubation, the plates were examined for zones of bacterial growth inhibition, and the diameters of these inhibition zones were measured in millimeters to judge antibacterial efficacy.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics version 27. Data were expressed as mean \pm standard division (SD). The effects of different concentrations and bacterial groups on the measured outcome were evaluated using two-way analysis of variance (ANOVA) to determine the significance of main effects and their interaction. When significant differences were detected, post hoc multiple comparisons were conducted using the Least Significant Difference (LSD) test to identify pairwise differences between groups. Statistical significance was considered at $p < 0.05$.

Results and discussion

1- Micropropagation Techniques:

Lemongrass, can be quickly multiplied through tissue culture by using micropropagation techniques that entail multiple separate phases. The procedure is intended to preserve genetic integrity and disease-free status while guaranteeing the effective development of plantlets from tiny tissue samples. The shoots and roots can arise either directly from the explant tissue . This process is vital for the establishment of the plantlets Depending on (Al-Aradi *et al.*, 2017), Figure 1 show the stages of plant propagation *in vitro*.

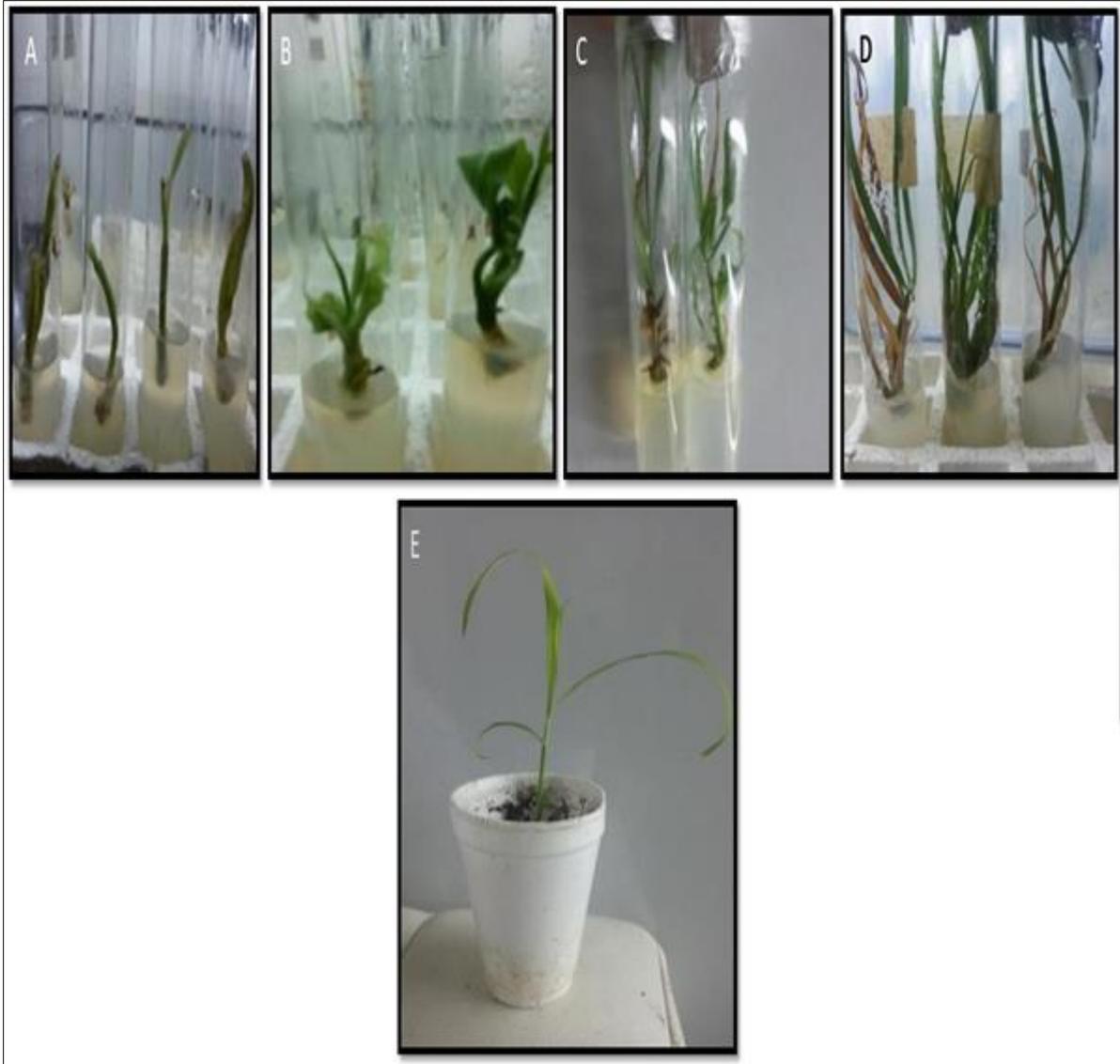


Figure (1): stages of (*Cymbopogon citratus*), propagation *in vitro* (A-Apical buds grown in nutrient media. B- Growth of buds two months after planting. C-D The vegetative multiplication stage. E- Plants that have passed the acclimatization stage.

2-Identification and Analysis of Secondary Metabolites of *C. citratus*, L.

The GC-MS analysis of *Cymbopogon citratus* (lemongrass) leaf extract revealed the presence of 30 distinct chemical constituents (Figure 2; Table 4). Among the most prominent compounds: Citral, a natural mixture of the isomers geranial (citral A) and neral (citral B), has been well-documented for its insect-repelling and antimicrobial activities (Bard *et al.*, 1988). Additionally, several studies have demonstrated its *in vitro* and *in vivo* antitumor potential against leukemia, hepatoma, and melanoma cell lines (Suzanne *et al.*, 1995; Yu *et al.*, 1995; Burke *et al.*, 1997). Geraniol, a key component of

citral, exhibits inhibitory activity against *Escherichia coli* (BA₅₀= 0.15), *Listeria monocytogenes* (BA₅₀= 0.028), and *Salmonella enterica* (BA₅₀= 0.15) (Si et al., 2006).

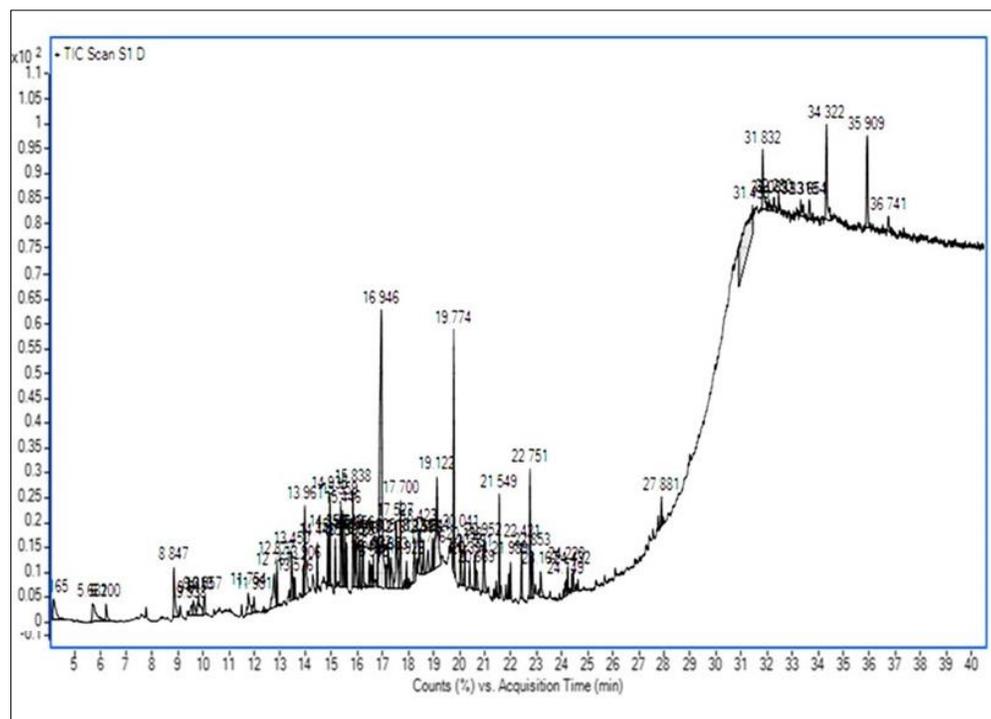


Figure 2: Chromatogram of lemongrass methanolic leaves extract.

Table (4): Compounds identified in the methanolic extract of lemongrass leaves In GC-MS.

Peak	R.T.	Area	Pct Total	Area Pct	Library/ID
5	9.617	143892	1.356	1.3561	Trimethylphosphine
6	9.813	119585	1.127	1.127	3,5-Dithiahexanol 5,5-dioxide
7	10.057	81375	0.767	0.7669	Cyclotetrasiloxane, octamethyl-
8	10.63	57093	0.538	0.5381	Ethanol, 2-(methoxyethylthio)-
9	11.754	161183	1.519	1.519	Butane, 1-isothiocyanato-
10	11.981	76300	0.719	0.7191	2-Furanmethanamine
11	12.767	243332	2.293	2.2932	1,3-Disilacyclobutane, 1,1,3,3-tetramethyl-
12	13.348	55743	0.525	0.5253	Glutaric acid, decyl tetrahydrofurfuryl ester
13	13.45	140177	1.321	1.321	4-(2-Hydroxyethyl) piperazin-2-one
14	13.576	120751	1.138	1.138	iso-Pentanal N-methyl-N-formylhydrazone
15	13.961	452455	4.264	4.264	4-Vinylphenol
16	14.275	88203	0.831	0.8312	Carbonic acid, but-2-yn-1-yl eicosyl ester

17	14.448	119582	1.127	1.127	2,6-Octadien-1-ol, 3,7-dimethyl-
18	14.699	96843	0.913	0.9127	Sulfurous acid, 2-methyl-4-methoxybutyl octyl ester
19	14.856	97500	0.919	0.9189	Hydrazine, 1-(5-hexenyl)-1-methyl-
22	15.359	465256	4.385	4.3846	2-Methoxy-4-vinylphenol
23	15.838	284976	2.686	2.6857	2,6-Octadienoic acid, 3,7-dimethyl-, (E)-
24	15.956	134102	1.264	1.2638	Eugenol
25	16.098	102972	0.97	0.9704	2,4,6-Octatriene, 2,6-dimethyl-, (E,Z)-
27	16.475	69040	0.651	0.6506	5,6-Epoxy-6-methyl-2-heptanone
28	16.946	2E+06	15.667	15.6672	6,8-Dioxa-3-thiabicyclo(3,2,1)octane 3,3-dioxide
30	17.323	81483	0.768	0.7679	2-Propanol, 1,1,1-trichloro-2-methyl-
31	17.527	236819	2.232	2.2318	.beta.-D-Glucopyranose, 1,6-anhydro-
32	17.7	269131	2.536	2.5363	Butyldimethylsilane
36	18.596	77953	0.735	0.7346	Phenol, 4-ethenyl-2,6-dimethoxy-
41	19.774	441074	4.157	4.1567	aR-Turmerone
43	20.198	94626	0.892	0.8918	Curlone
46	20.952	216200	2.038	2.0375	Loliolide
47	21.549	168900	1.592	1.5917	Neophytadiene
53	24.22	67739	0.638	0.6384	Phytol

Furthermore, its vapor has been shown to possess antibacterial effects against respiratory pathogens such as *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, and *Staphylococcus aureus*, geraniol also effectively inhibits *Erwinia amylovora*, the causative agent of fire blight in rosaceous plants, at concentrations ranging from 600 to 1500 µg/ml within 24 hours, and reduces germ counts by 64% in air washer systems (Sato *et al.*, 2007). Other related compounds such as citronellol and nerol also demonstrated antitubercular activity against *Mycobacterium tuberculosis*, with mean inhibitory concentrations ranging from 64 to 128 µg/ml (Cantrell *et al.*, 2001).

Eugenol, a colorless phenylpropanoid found in various essential oils including lemongrass, is commonly used as a flavoring agent. Recent studies have revealed its anti-inflammatory, antioxidant, and analgesic properties, suggesting potential therapeutic applications in the treatment of neoplasms, inflammatory bowel disease, renal and pulmonary injuries, and osteoporosis (Damasceno *et al.*, 2024). Curlone (also known as turmerone) is another bioactive compound exhibiting anti-inflammatory and antioxidant

properties, and has shown potential in improving insulin sensitivity and regulating blood glucose levels (Raina *et al.*, 2024). The extract also contains PYT derivatives, a broad class of compounds known for diverse pharmacological activities, including antimicrobial, cytotoxic, antitumor, antimutagenic, anti-teratogenic, chemotherapeutic, antidiabetic, lipid-lowering, antispasmodic, anticonvulsant, antinociceptive, antioxidant, anti-inflammatory, anxiolytic, antidepressant, and immunoadjuvant effects. Among them, phytanic acid (PA), an important biometabolite, has been reported to possess cytotoxic, anticancer, antidiabetic, lipid-lowering, and anti-teratogenic effects, and also serves as a biomarker for diseases such as Refsum's Disease, Sjögren-Larsson syndrome, and Zellweger's disease (Islam *et al.*, 2015; 2020). Accordingly, phytol emerges as a promising new therapeutic candidate. Lemongrass also contains loliolide, a monoterpene hydroxylactone known for its anti-aging and anti-inflammatory properties (Park *et al.*, 2019). In addition, aromatic turmerone, a sesquiterpene found in the extract, possesses anti-inflammatory, anticonvulsant, antifungal, anticancer, and anti-metastatic activities. It has been reported to suppress T-cell production of IFN- γ and IL-2, inhibit seizure activity in animal models, and prevent dermatophyte growth in various experimental systems (Oh *et al.*, 2014). Moreover, the presence of neophytadiene, a diterpene used traditionally to treat headaches, rheumatism, and certain skin disorders, has been linked to analgesic, antipyretic, anti-inflammatory, and antioxidant effects (Gonzalez-Rivera *et al.*, 2023). Taken together, these bioactive constituents identified in Table 4 and Figure 2 may significantly contribute to inhibiting the bacterial pathogens responsible for bacterial gill disease in common carp (*Cyprinus carpio*). These findings are consistent with earlier research confirming the antibacterial potential of lemongrass against many microbial species.

3- Antibacterial Efficacy of Extracts:

Lemongrass (*Cymbopogon citratus*) has attracted growing scientific attention due to its potent antimicrobial activity, particularly against various pathogenic bacterial strains. Numerous studies have reported that alcoholic extracts of *C. citratus* exhibit notable inhibitory effects on the growth of harmful bacteria. Lemongrass (*Cymbopogon citratus*) has attracted growing scientific attention due to its potent antimicrobial activity, particularly against many pathogenic bacterial strains. Many studies have reported that alcoholic extracts of *C. citratus* show notable inhibitory effects on the growth of harmful bacteria. Judged, and the results are summarized in Table (5) and shown in Figures (3) and (4), was in the present study, the antibacterial activity of lemongrass extracts. Interestingly, Among the tested concentrations, the 10% extract showed the highest antibacterial efficacy, while the 2.5% concentration showed the weakest activity. Notably, *Aeromonas sobria* showed the greatest sensitivity to the lemongrass extract, while *Sphingomonas paucimobilis* showed finish resistance across all tested concentrations. Consistent with findings by Pathirana et al. are these results (2019), who reported that

lemongrass essential oil (LGO) possesses promising antibacterial potential and could serve as a basis for developing novel treatments targeting fish pathogens.

Table 5: Antibacterial activity of methanolic extract of lemongrass leaves against the tested bacterial strains.

Bacterial species	Concentration							
	2.5		5		7.5		10	
	L	O	L	O	L	O	L	O
<i>Aeromonas sobria</i> strain L10	7	17	7	20	9	26	12	30
<i>Oxytetracycline</i>								
<i>Sphingomonas paucimobilis</i>	R	17	7	15	7	26	8	30
<i>Oxytetracycline</i>								
<i>Sphingomonas paucimobilis</i>	R	17	R	20	R	26	R	30
Std. Deviation	29.3106		30.2563		41.4742		69.5613	

L: Lemongrass leave extract, O: Oxytetracycline

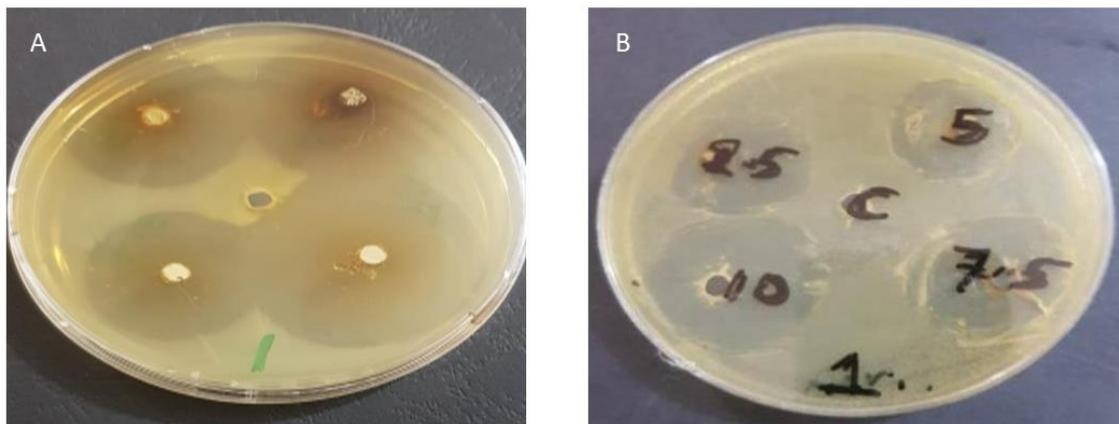


Fig (3):A/ Results of the antibiotic oxytetracycline against *Aeromonas sobria* and B/ antibiotic activity of *Aeromonas sobria* against lemongrass alcoholic methanolic extract (replace it).

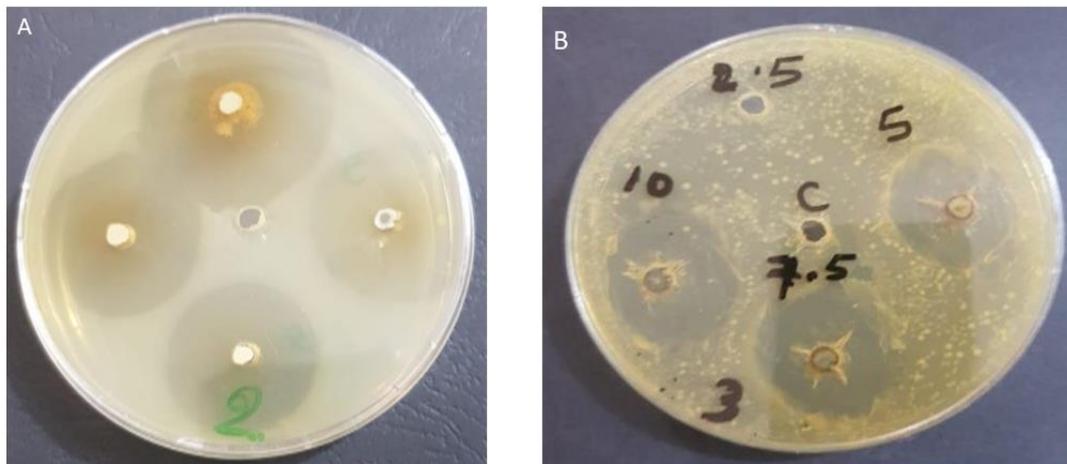


Fig 4:A. Results of the antibiotic oxytetracycline against *Sphingomonas paucimobilis* and B. antibiotic activity of *Sphingomonas paucimobilis* against lemongrass alcoholic methanolic extract

It's worth mentioning that This highlights the possibility that the method of extract preparation and potentially even the plant's propagation technique may significantly influence its antibacterial effectiveness. In line with the ends drawn by Mandalakis et al. are also, these observations (2021), who emphasized the importance of evaluating natural plant-derived products in aquaculture. Their study suggested that to judge the curative and preventive benefits of essential oil (EO) blends in farmed fish, experimental fish feeds enriched with various concentrations of the most effective EO formulations should be prepared and administered under controlled conditions.

Statistical analysis confirmed that the differences observed in antibacterial activity were significant at the 0.01 level of probability. Shendurse et al. (2021) identified a diverse array of bioactive phytochemicals in lemongrass (*Cymbopogon citratus*) essential oils, including flavonoids, tannins, saponins, steroids, terpenoids, and coumarins all of which give significantly to its antimicrobial potential. When judged using the agar well diffusion method, the oil showed notable inhibition zones against Gram-positive bacteria: *Staphylococcus aureus* (32.0 ± 0.75 mm), *Bacillus subtilis* (48.0 ± 1.05 mm), and *Bacillus cereus* (21.0 ± 0.64 mm). Among the Gram-negative strains, only *Proteus vulgaris* was susceptible, with a zone of inhibition measuring 23.0 ± 0.73 mm, while *Pseudomonas aeruginosa* and *Escherichia coli* showed no response to the treatment. Interestingly, Statistically, lemongrass oil significantly ($P < 0.05$) improved the inhibition zones of *B. subtilis*, *B. cereus*, *P. vulgaris*, and *S. aureus*. Generally speaking, *S. aureus*, and its activity against *P. vulgaris*, *S. aureus*, and *B. subtilis* exceeded that of the antibiotic azithromycin. Further supporting its broad-spectrum activity, lemongrass oil and its main component, citral, were found to effectively disrupt dual-species biofilms of *Staphylococcus aureus* and *Candida spp. in vitro*. Microscopic and viability assays showed that both compounds bigly reduced biofilm biomass and cellular viability. From a broader view, These effects were attributed to disruption of the extracellular biofilm matrix, particularly its protein, carbohydrate, and nucleic acid components, as well as interference with the adhesive mechanisms of the microbial species (Gao et al., 2020; Wickramanayake, 2023). Often tolerant to conventional antibiotics, indicating its potential as a natural alternative for combating resistant infections, lemongrass essential oil has also shown important efficacy against gram-negative bacteria (Naik et al., 2010). Specifically, the oil inhibited the growth of *Aeromonas hydrophila*, *A. caviae*, *Citrobacter freundii*, *Salmonella enterica*, *Edwardsiella tarda*, and *Proteus mirabilis*, though *Pseudomonas aeruginosa* remained unaffected. Reported minimum inhibitory concentrations (MICs) ranged from 0.016 to 0.5% (v/v), with *E. coli* being the most sensitive. Interestingly, Despite high many antimicrobial resistance (MAR) indices among the tested strains (0.36–0.91), they remained susceptible to lemongrass oil (De Silva et al., 2017). also, both ethanolic and aqueous extracts derived from lemongrass residues showed inhibitory effects against *Pseudomonas aeruginosa* and other pathogenic microorganisms. Phytochemical analysis

of the ethanolic extract confirmed the presence of alkaloids, flavonoids, glycosides, phenols, saponins, terpenes, tannins, fatty acids, and coumarins, while steroids, volatile oils, and emodins were absent. Determined to be 12.5 $\mu\text{L}/\text{mL}$ for *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *P.* were the mic values of the extract Quite notably, *aeruginosa*, and 25 $\mu\text{L}/\text{mL}$ for *Candida albicans* (Mohammed *et al.*, 2020). Generally speaking, These findings highlight the potential of lemongrass residue extracts in treating infections, particularly inflammatory skin conditions, due to their rich phytochemical profile and associated pharmacological effects. Phenolic compounds and tannins, in particular, were highlighted as key agents in the suppression of bacterial and fungal growth (Marya, 2022).

Conclusion

The present findings reveal that tissue culture propagation of lemongrass is a promising strategy for producing pathogen-free plant material enriched with biologically active compounds. Extracts derived from these cultured plants were found to contain several potent antibacterial constituents, including itaconate, eugenol, citral, curlone, hydrazine, and saponins. These compounds were particularly effective in inhibiting the growth of two major pathogens responsible for bacterial gill disease in common carp (*Cyprinus carpio*), highlighting the therapeutic potential of lemongrass-derived extracts in aquaculture applications.

Recommendations

Based on the results of the study, we recommend using lemongrass extract in the biological control of some diseases that affect fish, as well as conducting experiments on other plant extracts because it is a safe and environmentally friendly method.

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التكاثر الدقيق لعشبة الليمون *Cymbopogon citratus* L. وفعاليتها في تثبيط ثلاثة أنواع من البكتيريا المسببة لمرض الغلاصم البكتيري

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المستخلص

أجريت الدراسة الحالية في مختبرات مركز علوم البحار في جامعة البصرة. بهدف إكثار عشبة الليمون باستخدام تقنية زراعة الأنسجة النباتية واختبار فعالية مستخلص الأوراق الميثانولي في تثبيط ثلاثة أنواع من البكتيريا *Aeromonas sobria* سلالة L10، *Sphingomonas paucimobilis* سلالة K3، و *Sphingomonas paucimobilis* سلالة E6-5 المعزولة من سمك الكارب الشائع (*Cyprinus carpio*) المصاب بمرض الغلاصم البكتيري، تم جمعها من الأسماك المصابة في مزرعة سمكية محلية في البصرة تم تشخيص العزلات البكتيرية الثلاث في محافظة جنوب العراق كيموحيويا باستخدام بطاقات نظام التشخيص VITEK II، وكذلك شخصت وراثيا باستخدام srRNA16 وأشار تحليل GCMS إلى أن مستخلص عشبة الليمون يحتوي على العديد من المركبات النشطة، بما في ذلك Itaconate و Eugenol الذي له تأثير مضاد للجراثيم، Citral، Curlone، hydrazine، saponin، وأظهرت جميع تراكيز المستخلصات درجات متفاوتة من النشاط ضد هذه الكائنات التي تم اختبارها، مقارنة بفعاليتها مع المضاد الحيوي القياسي oxytetracycline.

الكلمات المفتاحية: عشبة الليمون، التكاثر الدقيق، الكيمياء النباتية، الزيوت العطرية، مضادات حيوي.