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Antibacterial Activity of Three Algal Genera against some Pathogenic Bacteria

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Abstract:

In the current study, three types of algae namely Tetradesmus nygaardi (MZ801740), Scenedesmus quadricauda (MZ801741) and Coelastrella sp (MZ801742) were extracted by 95% ethanol and hexane against two types of gram positive and two types of gram negative bacteria by wells diffusion methods. Eleven concentrations from the extract of algae (2, 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 mg/ml) were utilized. It was noticed that ethanolic extraction was more effective than hexane in *Scenedesmus quadricauda* than the two other mentioned algal species against all pathogenic bacteria, *Acintobacter baumanii* (ATCC: 19606), *Klebsiella pneumonia* (ATCC: 13883) *Enterococcus faecalis* (ATCC: 29212) and *Staphylococcus aureus* (ATCC: 14028). In addition to that, extraction of *Tetradesmus nygaardi* by hexane was more effective than ethanol against all studied pathogenic bacteria. Extract of *Coelastrella* sp by Ethanol showed weak effect against all pathogenic bacteria compared with the other types of algae. Many chemical compounds which possess antibacterial activities were obtained through analyzing the extraction of algae by gas chromatography–mass spectrometry (GC-MS)

Keywords: Activity, Algae, Antibacterial, Genera, Pathogenic

Introduction:

Small living organisms that have the ability of giving rise to illness are named pathogens. Pathogenic bacteria can cause sickness through many mechanisms in hosts that found in human. The term “disease” point to circumstances that disorder ordinary tissue careers. The damage caused by pathogens to hosts through infection is named virulence, whose difference between species ranges from minimum to immediate of deaths[1, 2] .Bacteria giving rise to infection are deemed pathogenic bacteria, producing toxic materials named inner toxins and external toxins. These materials account for the symptoms of diseases returning to bacteria. The symptoms can vary from moderate to intense and could even be fatal[3]. Antibiotics, also defined as antibacterial, are drugs that murder or retard bacterial development. These cover a number of active drugs utilized to treat bacteria causing diseases. Antibiotics are strong drugs that resist some types of infections and can save a human life when used correctly; they block the bacteria from multiplicity or remove them[4]. [3] It has been noticed that there is a significant increase in the ratio and a number of resistant bacterial pathogens to many antibacterial factors over the last ten years. Nowadays, multiple medicines-resistance (MDR) bacteria are considered an emerging worldwide disease and a main public health issue. Bacterial types resistant to the inhibitory impact of antibiotics pose a universal menace to the potential of chemical therapies. In addition to that, the majority of antibiotics are differentiated by many side effects that may cause harm for normal human body cells[5]. The use of compounds of microalgae as hopeful or promising resources for antibiotics taken from nature and free from manufactured chemicals against human pathogens means using different substitution of natural compounds obtainable to take control of pathogenic bacteria, especially microalgae-derivative. They have the benefits of minimizing the negative or side effects of synthetic antibiotics as well as being considered of low cost [6]. Recently, there has been rising interesting in microalgae investigation or search for antibiotics and pharmacologically active compounds. A big number of compounds as used as antibiotic have

been insulated and differentiated, many with their newly structures. Microalgae are particularly attractive because they are made up naturally and have effective compounds because these algae have the potential to form or produce such substances that make it possible to produce complex materials that are considered very active against different pathogenic bacteria and fungi[7]. Due to their wide use in many life fields such as medicines, clean energy and many other industries serving humanity without side effects therefore microalgae have newly attracted great interest globally[7]. Active compounds extracted from microalgae supplied various chemical materials such as phenols, fatty acids, indoles, terpenes, acetogenins, and some volatile halogenated hydrocarbons have showed activity against pathogenic bacteria[8]. The dried biomass of green algae showed high antibacterial activity against gram-negative and gram-positive human pathogenic bacteria, like Klebsiella pneumonia, Proteus mirabilis, Vibrio cholera, Salmonella typhi, Escherichia coli, Staphylococcus aureus, Bacillus subtilis and Enterococcus sp. because it has phytochemicals such as phenol, tannins, flavonoids, terpenes, terpenoids, alkaloids, and saponins[7] . Much research indicates that some bioactive compounds in the freshwater green algae inhibit the growth of several representatives of Gram-positive and Gram-negative bacteria , exhibit anticancer, antimicrobial, antifungal or anti-inflammatory which contain proteins which 18 amino acids (including all the essential amino acids), lipid, vitamins and minerals.This study is considered the first attempt to use *Scenedesmus quadricauda*, *Tetradesmus nygaardi* and *Coelastrella* sp as an antibiotic against pathogenic bacteria in Iraq. The main aim of this study is to evaluate the activity of active compounds by extracting of three types of algae, as mentioned above against pathogenic bacteria obtained from Medya diagnostic Center.

Materials and Methods:

Collection of algae

Three algal species (*Tetradesmus nygaardi*, *Scenedesmus quadricauda* and *Coelastrella* sp) were collected or obtained from some springs and streams water resources in Shaqlawa district (Aquban village and Sarkand village) located 32Km northwest of Erbil city.

Identification of algal species

Algae books was used to identify and diagnose algal species [9, 10].

Isolation of algal species

Necrotic parts from algal Sample were removed. Then, algal sample was incubated in glass container. BG-11 media were utilized for growth of algae. Streaking technique plate methods were used to isolate and purified sample of algae. After that they were incubated at 25 ±2°C, light intensity 3000-5000 lux distributed for16 hr. of light and 8 hr. for dark, pH 8.2 for 14 days. This step was repeated many times until obtaining purified algal species, which transfer purified algal colony to a tube containing 25 ml of BG-11 media and incubated under the same conditions mentioned above for 14 days to obtain algal inoculum. This alga was isolated, by using Streak Plating Technique according to [11].

Biomass preparation and harvested

25ml of isolated algae was transferred to a flask containing of 100ml BG-11 media then incubated under the same conditions mentioned before for 14 days .After that, this culture media put to 500ml glass flask that contained 100 ml. The BG-11 media were also placed in an incubator for 14 days in the controlled conditions. These steps were repeated many times till the algal growth reached to 4 liter in the container covered by piece of cotton and the air was provided with rubber [12]. After day 20 culture of algal mass was harvested by using centrifuge device at 4000 rpm for a period of 10 minutes [13]. Then algal sample was washed with sterilized water and desiccated in the oven in temperature around 38-40 ° C. These algal samples were weighed and stored in the refrigerator [14].

Antimicrobial activity

Bacteria which have been utilized in the current study was received from Media Diagnostic Center Erbil which is sited in Iraq-Kurdistan Region, Erbil governorate were *Staphylococcus aureus*(ATCC:14028), *Acintobacter baumanii* (ATCC:19606), *Enterococcus Faecalis* (ATCC:29212) and *Klebsiella Pneumonia* (ATCC:13883). The bacterial culture incubated on Muller Hinton agar (24 hr. at 37C).

Determining minimum inhibitory concentration (MIC) of algal extraction

96-well microliter plates (This way had a benefit in examining plants for anti-bacterial activity and the insulate of anti-microbial substances) were utilized to assay various concentrations of algal extracts (0, 2, 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 mg/ml) by mixing up with nutrient broth. All wells were inoculated with 10µl of the activated culture of *Acintobacter baumanii*, *Enterococcus faecalis*, *Staphylococcus aureus* and *Klebsiella pneumonia* overnight incubated at 37 °C Then the MIC was estimated by Eliza instrument (EL 800 Biotek, Epison LQ-300+II) and the absorbency was read at wave length 490 nm before and after incubation[15].

Phytochemical examination of the algal species

Depending on [3], 25gram powder for each of *Tetradesmus nygaardi*, *Scenedesmus quadricauda* and *Coelastrella* sp was extracted with 250ml of hexane and 97% ethanol each separately by Soxhlet extraction at 76°C for 3-4 hours until these solvent become insoluble. Rotary evaporator was used to dry raw extract of algae at 40°C. Parts of the extracts were utilized for phytochemical screening carried out by gas chromatography–mass spectrometry (GC-MS) way while the remain was utilized for bacterial sensitivity test. Algal crude extracts were dissolved in dimethyl sulfoxide (DMSO) to a final concentration 300 mg/mL, sterilized by filtration and kept at 4°C [16].

Chemical Composition of Algae Extracts

The biochemical constituent of the algal extract was determined by using GC-MS analytical procedure (Agilent technologies, USA) equipped with a single quadrupole detector with an HP-5 capillary column (30 m×0.25 mm I.D., 1 µm film thickness). The oven temperature was set at three degrees including 40°C for two minute and to 150°C for five minutes, then to 300°C for fifteen minutes. The temperature of the injector port was kept at 280°C. Helium was utilized as a carrier gas and 1 µl of the sample was injected into the system (dissolved in 100% dimethyl sulfoxide) [3].

Microalgae Molecular Identification via amplification of ITS region:

DNA extraction and PCR amplification

Total genomic DNA form microalgae cells was extracted using Genomic DNA purification kit: thermo scientific/USA) depending on manufacturer's instructions. The target sequence 750bp of the Microalgae in the rDNA fragments were successfully amplified using universal primers designed by[17]. The total of 25 µl PCR master mix reaction volume was performed containing 3µl of genomic DNA, 12.5 µl of 2X GoTaqGreen Master Mix (Promega/USA) and 1µl was added for each of the forward and reverse primer for both ITS1 and ITS4, forward (ITS1, F´5-TCC GTA GGT GAA CCT GCG G-´3), reverse (ITS4, R-5´ TCC TCC GCT TAT TGA TAT GC-´3) then the mixture was completed by adding 7.5 µl of nuclease free water. The PCR amplification process was carried out with a Techne/UK thermocycler under the following conditions: an initial denaturation cycles at 95°C for 5 minutes, followed by 35 cycles at 94°C for 1 minute, 55°C for 1 minute, 72°C for 1 minute and a last extension at 72°C for 7 minutes. The size of PCR products was confirmed by using 2% agarose gel electrophoresis in 1XTBE buffer and PCR products of Candida isolates were sent to (Macrogen/South Korea) for sequencing[17].

The data sequence analysis of the target region:

The DNA target sequence analysis was done using MEGA5 and alignment to NCBI BLAST (http://www.ncbi.nlm.nih.gov/BLAST/). The PCR products sequenced on 3500 Genetic analyzer (Applied Biosystems).

Results and Discussions:

In the current study, antibacterial action of three species of algae *Tetradesmus nygaardi*, *Scenedesmus quadricauda* and *Coelastrella* sp was tested against four pathogenic gram^{+ve} and gram^{-ve} bacteria *Staphylococcus aureus*(ATCC:14028), *Acintobacter baumanii* (ATCC:19606), *Enterococcus Faecalis* (ATCC:29212) and *Klebsiella Pneumonia* (ATCC:13883) by well diffusion method. Eleven concentrations of extract of algae were used including (2, 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50mg/ml). The minimum inhibitory concentration (MIC) formed by the extracts at various concentrations against specific bacteria for testing were calculated (Tables 1, 2 and 3). Algae have great attractiveness as a natural resource of biological active substances with a broad extent of activities biologically, covering antifungal, antimicrobials, antivirus, antioxidants, and anti-inflammatories evidence from phytochemical and drugs studies. They form high amounts of metabolites such as amino acids, terpenoids, phlorotannins, hormones, phenolic compounds, halogenated ketones, alkenes, and cyclic polysulphides, which are some of the active substances derived from algae. The utilization of different organic solvents of increasing order of polarity has diagnosed much lipid compounds with antibacterial characteristics [18-20]. In the case of hexane extract of *Tetradesmus nygaardi* and *Coelastrella* sp they are considered both most effective against pathogenic bacteria; the minimum inhibitory concentration was highest (10mg/ml) against all pathogenic bacteria except *Staphylococcus aureus* which explained lower minimum inhibitory concentration than previous bacteria mentioned above with (20mg/ml). In this study, purified extract of *Tetradesmus nygaardi* and *Coelastrella* sp algae showed that bioactive compounds were more effectiveness against both gram+ve and gram-ve bacteria, the highest biological activity was reported. But the highest activity of algal extracts by hexan is more than ethanol against pathogenic bacteria and causes to kill or stop growth under this study. The findings of this investigation explained that green algae could be the best resource for antimicrobial substances[21]. Also the extract by ethanol explained the lowest discouragement development of the growth of

bacteria causing diseases in this study. This may be due to the quality and quantity of the chemical compounds isolated through the extract of algae and also the type of solvent used for algal extraction which plays an important role for isolating active compounds from algae by hexan in comparison to ethanol which is considered more effective against pathogenic bacteria and inhibit their growth or may kill them[18]. [22] It has been discovered that the hexan extract of some Chlorophyceae explained antibacterial action with the highest discouragement zone against bacteria causing diseases, while the ethanol extract showed lower inhibition to pathogenic bacteria. It was noticed that extract by ethanol of *Scenedesmus quadricauda* was more effective one against gram^{+ve} and gram^{-ve} bacteria with antibacterial action starting at concentration 2mg/ml for *Enterococcus Faecalis* and 5mg/ml for the remaining pathogenic bacteria. In addition to that, the minimum inhibitory concentration in hexane extract of *Scenedesmus quadricauda* was (20mg/ml) in all pathogenic bacteria except *Acintobacter baumanii* which was (15mg/g/ml). In the current investigation, *Scenedesmus quadricauda* showed the highest inhibition growth of all pathogenic gram^{+ve} and gram^{-ve} bacteria when using ethanol extract more than hexan. This may be due to phytochemical nature of algal sample which comprises utilized active compounds. These bioactive substances in *Scenedesmus quadricauda* play an important role in obtaining various bioactive compounds as a useful precursor. The drugs derived from these species of algae find some special uses to inhibit bacterial growth, which results in more control of vector infections without any harmful impacts [23].In addition to that, *Scenedesmus* spp. Was found to be a rich resource of new antibacterial and anticancer substances. Many studies concluded that the antimicrobial activity of the extract of Scenedesmus species is very efficient against various pathogenic bacteria[24].Most substances derivative from these species are likely to be impractical antibiotics for medical used as a result of their in vivo toxicity or inactivity[25]. During the extract of three algal genera in the current study many chemical compounds determined related to human health. (Tables 4, 5, 6, 7, 8 and 9). The current study discovered and utilized organic solvents in the preparation of *Tetradesmus*, *Scenedesmus* and *Coelastrella* extract and diagnosis many compounds by GC-MS. During GC-MS screening of solvent extract of *Tetradesmus*, it was noticed that ethanol and hexane extract showed sixteen and fourteen compounds respectively (Tables 4 and 5) and for *Scenedesmus* showed eight and fourteen compounds through extract by ethanol and hexan (Tables 6 and 7) respectively, finally extraction of *Coelastrella* by ethanol has nine compounds and eleven compounds by hexane extract (Tables 8 and 9). Most of these compounds have various antibiotic actions, chemical substances that may notice antioxidant and anticancer recorded in Tables (4-9).Some of these compounds were found in all or most of the extract by ethanol or hexane of three algal genera, such as Acetamide, Aldehyde, Alcohol, Dimethyl, Benzene, Aceter, Ketone, Heterocycle, Alkane, Furan, Propanoic acid, Tetrazole, Acetic acid, Allyl acid, Butanic acid as their surface area mentioned in (Tables 4 - 9). Acetamidoacetaldehyde; 2, 3-Hexanediol and 4, 5-Octanediol appear potent antibiotic activity against pathogenic gram^{+ve} and gram^{-ve} bacteria and fungi through screening by ethanol and hexane for algal genera in this study[26]. Also, Camphene possesses very high antioxidant, antibiotic and hypolipidenic activities these belong to thiosemicarbazone compounds[27]. Tetrazole compounds manifested antibiotic action that was more potent against all tested bacteria. Also, they were also more active against resistant bacterial species[28]. Furan heterocyclic compounds that isolated from algae in this study which contain many substances that are antibiotic active and considered antimicrobial and anti- inflammatory effect therefore showed high effect against most pathogenic bacteria under this study[29]. Previous investigations also reported that the compounds such as Octadecene, Heptadeceane present in both algae and higher plants are responsible for their anticancer, antioxidant and antimicrobial activities[28]. It has been suggested that the lipids and fatty acids present in the algal strains could also be responsible for the antimicrobial activity. The antimicrobial activity of two algae extracts used in this study results from the phytochemical compositions which were done using GC/MS device. The results showed the extraction of two algae represented by Acetamide, Aldehyde, Alcohol, Dimethyl, Benzene, Aceter, Ketone, Heterocycle, Alkane, Furan, Propanoic acid, Tetrazole, Acetic acid, Allyl acid, Butanic acid[29]. The algal identification molecularly by utilizing comprehensive or particular initial gene magnification was constant with phenotypic screening. Data for molecular sample from ITS nucleotide sequencing supplied minute properties and diagnosis of isolation. The data sequence of three genera of algae includes *Tetradesmus nygaardi*, *Scenedesmus quadricauda* and *Coelastrella* sp supplied Gene bank accession number nucleotide sequencing (Table 10).

Table 1. Minimum Inhibitory Concentration (MIC) of *Tetradesmus nygaardi* against some pathogenic bacteria

Concentration of extract Name of bacteria with solvent	Absorbency of bacterial culture (extract concentration mg/ml)											
	Control	2	5	10	15	20	25	30	35	40	45	**50**
Staphylococcus aureus(Ethanol)	0.970	0.632	0.611	0.504	0.466	0.397	0.284	0.208	0	0	0	**0**
Staphylococcus aureus(Hexane)	1.275	0.406	0.355	0.072	0	0	0	0	0	0	0	**0**
Acintobacter baumanii(Ethanol)	0.368	0.358	0.345	0.323	0.235	0	0	0	0	0	0	**0**
Acintobacter baumanii(Hexane)	0.275	0.232	0.198	0	0	0	0	0	0	0	0	**0**
Enterococcus Faecalis(Ethanol)	1.150	0.587	0.511	0.419	0.337	0	0	0	0	0	0	**0**
Enterococcus Faecalis(Hexane)	1.076	0.587	0.432	0	0	0	0	0	0	0	0	**0**
Klebsiella Pneumonia(Ethanol)	0.283	0.233	0.211	0.177	0.162	0.145	0.133	0.120	0	0	0	**0**
Klebsiella Pneumonia(Hexane)	0.363	0.233	0.170	0	0	0	0	0	0	0	0	**0**

0 means no growth observed

Table 2. Minimum Inhibitory Concentration (MIC) of *Scenedesmus quadricauda* against some pathogenic bacteria

Concentration of extract Name of bacteria with solvent	Absorbency of bacterial culture (extract concentration mg/ml)											
	Control	2	5	10	15	20	25	30	35	40	45	**50**
Staphylococcus aureus(Ethanol)	0.964	0.308	0	0	0	0	0	0	0	0	0	**0**
Staphylococcus aureus(Hexane)	1.075	0.421	0.392	0.264	0.052	0	0	0	0	0	0	**0**
Acintobacter baumanii(Ethanol)	0.992	0.0357	0	0	0	0	0	0	0	0	0	**0**
Acintobacter baumanii(Hexane)	0.953	0.758	0.328	0.268	0	0	0	0	0	0	0	**0**
Enterococcus Faecalis(Ethanol)	0.301	0	0	0	0	0	0	0	0	0	0	**0**
Enterococcus Faecalis(Hexane)	0.269	0.114	0.095	0.051	0.029	0	0	0	0	0	0	**0**
Klebsiella Pneumonia(Ethanol)	0.318	0.210	0	0	0	0	0	0	0	0	0	**0**
Klebsiella Pneumonia(Hexane)	0.359	0.109	0.100	0.057	0.022	0	0	0	0	0	0	**0**

0 means no growth observed

Table 3. Minimum Inhibitory Concentration (MIC) of *Coelastrella* sp against some pathogenic bacteria

Concentration of extract Name of bacteria with solvent	Absorbency of bacterial culture (extract concentration mg/ml)											
	Control	2	5	10	15	20	25	30	35	40	45	**50**
Staphylococcus aureus(Ethanol)	1.075	0.782	0.768	0.745	0.598	0.571	0.346	0.199	0.128	0.111	0	**0**
Staphylococcus aureus(Hexane)	1.192	0.438	0.410	0.358	0.206	0	0	0	0	0	0	**0**
Acintobacter baumanii(Ethanol)	0.925	0.916	0.843	0.716	0.622	0.512	0.434	0.390	0.210	0	0	**0**
Acintobacter baumanii(Hexane)	0.931	0.336	0.279	0.212	0	0	0	0	0	0	0	**0**
Enterococcus Faecalis(Ethanol)	1.211	1.008	0.911	0.788	0.711	0.632	0.487	0.367	0.323	0.211	0	**0**
Enterococcus Faecalis(Hexane)	1.201	0.508	0.286	0.109	0	0	0	0	0	0	0	**0**
Klebsiella Pneumonia(Ethanol)	0.493	0.402	0.340	0.321	0.288	0.265	0.211	0.145	0.111	0.098	0	**0**
Klebsiella Pneumonia(Hexane)	0.442	0.128	0.114	0.080	0.023	0	0	0	0	0	0	**0**

0 means no growth observed

Table 4. GC-MS screening of various substances in ethanol extract of *Tetradesmus nygaardi*

Peak	Retention Period	No.	Compounds	Chemical formula	%
1	15.235	1	Acetamidoacetaldehyde	$C_4H_7NO_2$	28.61
		2	2,3-Hexanediol	$C_6H_{14}O_2$	
		3	4,5-Octanediol	$C_8H_{18}O_2$	
2	16.267	4	N-Acetyl-N-(acetyloxy)acetamide	$C_6H_9NO_4$	13.67
		5	Di(1,2,5-oxadiazolo)[3,4-b;3,4-E]pyrazine, 4,8-diacetyl-	$C_8H_6N_6O_4$	
		6	Isobutane	C_4H_{10}	
		7	Isobutyl nitrite	$C_4H_9NO_2$	
		8	2,3-Pentanedione	$C_5H_8O_2$	
3	17.025	9	Acetamidoacetaldehyde	$C_4H_7NO_2$	28.72
		10	2,3-Hexanediol	$C_6H_{14}O_2$	
		11	4,5-Octanediol	$C_8H_{18}O_2$	
4	20.722	12	Propanoic acid, 2-methyl-, anhydride	$C_8H_{14}O_3$	29.00
		13	Butane, 2-iodo-3-methyl-	$C_5H_{11}I$	
		14	Tetrahydro-2-furancarbonyl chloride	$C_5H_7ClO_2$	
		15	Butanoic acid, 2-propenyl ester	$C_7H_{12}O_2$	
		16	Vinyl butyrate	$C_6H_{10}O_2$	

Table 5. GC-MS screening of various substances in hexane extract of *Tetradesmus nygaardi*

Peak	Retention Period	No.	Compounds	Chemical formula	%
1	10.810	1	Acetamidoacetaldehyde	$C_4H_7NO_2$	31.59
		2	2,3-Hexanediol	$C_6H_{14}O_2$	
		3	4,5-Octanediol	$C_8H_{18}O_2$	
2	13.155	4	Acetamidoacetaldehyde	$C_4H_7NO_2$	33.31
		5	2,3-Hexanediol	$C_6H_{14}O_2$	
		6	4,5-Octanediol	$C_8H_{18}O_2$	
3	16.267	7	Acetic acid, 2-propenyl ester	$C_5H_8O_2$	19.79
		8	N,N,O-Triacetylhydroxylamine	$C_6H_9NO_4$	
		9	1,1-Dipropoxyacetone	$C_9H_{18}O_3$	
		10	Propanoic acid, 2-oxo-, ethyl ester	$C_5H_8O_3$	
		11	1H-Tetrazole-1,5-diamine	CH_4N_6	
4	17.025	12	Acetamidoacetaldehyde	$C_4H_7NO_2$	15.31
		13	2,3-Hexanediol	$C_6H_{14}O_2$	
		14	4,5-Octanediol	$C_8H_{18}O_2$	

Table 6. GC-MS screening of various substances in hexane extract of *Scenedesmus quadricauda*

Peak	Retention Period	No.	Compounds	Chemical formula	%
1	10.803	1	Acetamidoacetaldehyde	$C_4H_7NO_2$	50.45
		2	2,3-Hexanediol	$C_6H_{14}O_2$	
		3	4,5-Octanediol	$C_8H_{18}O_2$	
2	13.155	4	Acetamidoacetaldehyde	$C_4H_7NO_2$	29.19
		5	2,3-Hexanediol	$C_6H_{14}O_2$	
		6	4,5-Octanediol	$C_8H_{18}O_2$	
3	15.230	7	2,3-Hexanediol	$C_6H_{14}O_2$	12.54
		8	4,5-Octanediol	$C_8H_{18}O_2$	
		9	Acetamidoacetaldehyde	$C_4H_7NO_2$	
4	17.018	10	1H-Tetrazole-1,5-diamine	CH4N6	8.22
		11	Propane, 2-bromo-	C3H7Br	
		12	Propane, 2-nitro-	C3H7NO2	
		13	Diisopropyl 2-oxomalonate	C9H14O5	
		14	Propanesulfonylacetonitrile	C5H9NO2S	

Table 7. GC-MS screening of various substances in ethanol extract of *Scenedesmus quadricauda*

Peak	Retention Period	No.	Compounds	Chemical formula	%
1	6.633	1	1H-Tetrazole, 5-vinyl-	$C_3H_4N_4$	82.12
		2	Divinylene oxide	C_4H_4O	
		3	Tetrahydrocyclopenta[1,3]dioxin-4-one	$C_7H_{10}O_3$	
		4	Cyclobutane, 1,2-dipropenyl-	$C_{10}H_{16}$	
		5	7-Oxabicyclo[2.2.1]hept-5-ene-2,3-dicarboxylic anhydride	$C_8H_6O_4$	
2	11.28	6	Acetamidoacetaldehyde	$C_4H_7NO_2$	17.88
		7	2,3-Hexanediol	$C_6H_{14}O_2$	
		8	4,5-Octanediol	$C_8H_{18}O_2$	

Table 8. GC-MS screening of various substances in ethanol extract of *Coelastrella* sp

Peak	Retention Period	No.	Compounds	Chemical formula	%
1	10.809	1	Acetamidoacetaldehyde	$C_4H_7NO_2$	37.07
		2	2,3-Hexanediol	$C_6H_{14}O_2$	
		3	4,5-Octanediol	$C_8H_{18}O_2$	
2	13.155	4	Acetamidoacetaldehyde	$C_4H_7NO_2$	42.16
		5	2,3-Hexanediol	$C_6H_{14}O_2$	
		6	4,5-Octanediol	$C_8H_{18}O_2$	
3	15.237	7	Acetamidoacetaldehyde	$C_4H_7NO_2$	20.76
		8	2,3-Hexanediol	$C_6H_{14}O_2$	
		9	4,5-Octanediol	$C_8H_{18}O_2$	

Table 9. GC-MS screening of various substances in hexane extract of *Coelastrella* sp

Peak	Retention Period	No.	Compounds	Chemical formula	%
1	10.808	1	Acetamidoacetaldehyde	$C_4H_7NO_2$	7.69
		2	2,3-Hexanediol	$C_6H_{14}O_2$	
		3	4,5-Octanediol	$C_8H_{18}O_2$	
2	13.151	4	Acetamidoacetaldehyde	$C_4H_7NO_2$	27.64
		5	2,3-Hexanediol	$C_6H_{14}O_2$	
		6	4,5-Octanediol	$C_8H_{18}O_2$	
3	20.724	7	Propanoic acid, 2-methyl-, anhydride	$C_8H_{14}O_3$	64.67
		8	Furan-2-carbonyl chloride, tetrahydro-	$C_5H_7ClO_2$	
		9	Butane, 2-iodo-3-methyl-	$C_5H_{11}I$	
		10	Butanoic acid, 2-propenyl ester	$C_7H_{12}O_2$	
		11	Butanoic acid, anhydride	$C_8H_{14}O_3$	

Table 10. Gene Bank Accession Number for algal genera

Algal genera	Gene Bank Accession No.
Tetradesmus nygaardi	MZ801740
Scenedesmus quadricauda	MZ801741
Coelastrella sp	MZ801742

Conclusion:

Hexan extraction of *Tetradesmus nygaardi* and *Coelastrella* sp is more effective than ethanol against all studies pathogenic bacteria such *Acintobacter baumanii*, *Klebsiella pneumonia Enterococcus faecalis* and *Staphylococcus aureus*. On the other hand, ethanol extraction is more effective than hexane in *Scenedesmus quadricauda* against all pathogenic bacteria. This study shows different chemical compounds extracted from three algal genera recorded active against all pathogenic gram^{+ve} and gram^{-ve} bacteria and cause to kill or inhibit their growth. Some of these compounds were found in all or most of the extract by ethanol or hexane of three algal genera, such as Acetamide, Aldehyde, Alcohol, Dimethyl, Benzene, Aceter, Ketone, Heterocycle, Alkane, Furan, Propanoic acid, Tetrazole, Acetic acid, Allyl acid, Butanic acid. Acetamidoacetaldehyde; 2, 3-Hexanediol and 4, 5-Octanediol appear potent antibiotic activity against pathogenic gram^{+ve} and gram^{-ve} bacteria and fungi through screening by ethanol and hexane for algal genera in this study

Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.

- Ethical Clearance: The project was approved by the local ethical committee in Salahaddin University**.**

Authors' contributions statement:

Both researchers F. H. A. and J. J. T. Participated in the development of the idea or concept of the search. F. H. A. developed the theory and performance the computation. J. J. T. has done all the practical part of antibiotic activities of some algae against some human pathogen but with supervision and support by F. H. A. Both authors participated to debate the data to contribute to the final paper to become in better form.

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النشاط المضاد البكتيري لثلاثة أجناس من الطحالب ضد بعض البكتيريا المسببة للأمراض

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الخلاصة:

في الدراسة الحالية تم استخلاص ثلاثة أنواع من الطحالب مثل تيتراديسمس نيكاردي (م ز 801741) ، وسينيديسمس كوادريكودا (م ز 801740)، وكولستريلا اسبي(م ز 801742) بنسبة 95٪ إيثانول و هكسان مقابل نوعين بكتريا موجبة الجرام ونوعين من البكتيريا سالبة الجرام بطريقة الانتشار في الابار. تم استخدام أحد عشر تركيزًا من مستخلص الطحالب (2 ، 5 ، 10 ، 15 ، 20 ، 25 ، 30 ، 35 ، 40 ، 45، 50 ملغم لكل مل). لوحظ ان الاستخلاص بواسطة ايثانول اكثر فاعلية من الهكسان في سينيدسمس كوادريكودا من النوعين الاخرين من الطحالب المذكورة ضد جميع انواع البكتريا المسببة للامراض وهي كليبسلا نيمونيا (أ ت س س:19606)، أكتينوبكتر بوماني (أ ت س س:29212)، اينتيروكوكس فيكاليس (أ ت س س : 13883)، ستافيلوكوكس اوريس (أ ت س س : 13883) . بالاضافة الى ذلك ، كان استخلاص طحلب تيتراديسمس نيكاردي بواسطة هكسان أكثر فعالية من الايثانول ضد جميع البكتريا المسببة للامراض. أظهر مسخلص طحلب كولستريلا بواسطة الايثانول تأثيرا ضعيفا ضد جميع انواع البكتريا المسببة للامراض مقارنة بالانواع الاخرى من الطحالب. العديد من المركبات الكيميائية التي تمتلك أنشطة مضادة للبكتيريا تم الحصول عليها عن طريق تحليل مستخلصات الطحالب بواسطة جهاز كروماتوجرافيا الغاز – مطياف الكتلة.

الكلمات المفتاحية: نشاط، طحالب، مضاد للبكتيريا، أجناس، ممرض.