Chemical Constituent and Determination of Antimicrobial and Antifungal Activities of Ulva Lactuca Species Obtained from Iranian Gheshm Island

Nooredin Goudarzian[1], Zahra Sadeghi[2], Seyyed Mojtaba Mousavi[3], Seyyed Alireza Hashemi[4], Nastaran Banaei[5]

Abstract— In this study, the total flavonoid and invitro antioxidant activity of two seaweeds including Ulva Lactuca (UL) and Sargassum Wightii (SW) were evaluated. The genus UL is a marine group of bacteria belonging to the class Gammaproteo which has attracted attention due to its applications in various fields including natural products and microbial ecology science. Pigmented species of the genus can produce an array of low and high molecular weight compounds with antimicrobial, anti-fouling, algicidal and various pharmaceutically-relevant activities. Moreover, obtained data showed that an essential oil associated with UL species, which was obtained from Gheshm island, presenting fantastic antibacterial and antifungal activities. Besides, examination of the extracted oil containing UL species revealed that this essential oil contains a variety of compounds including hexahydrofarnesyl acetone (20.46 %), hephtane (19.30 %), 3-methyl pentanol (11.30 %), limonene (10.98 %), 8-heptadecene (7.09 %). What is more, further examination via HPLC analysis showed eight more compounds, which carvacerol (0.1736 %) was the predominant constituent. Furthermore, both phenolic extract and the essential oil containing lowest amount of phenolic content, while UL showed the highest antimicrobial activity ranging from 0.390 to 12.5 mL/mL against bacterial activity and all of fungal strains.

Keywords— Ulva Lactuca, Clevenger, Rotary Evaporator, Antifungal Effect, Antimicrobial Effect, Essential Oil, Microorganism

1 INTRODUCTION

In past few years, marine resources have attracted wide range of attention toward themselves due to their rich source of antioxidants which could be used in various fields including drugs and foods development [1, 2]. It is known that seaweeds containing numerous bioactive substances with low cholesterol that could lead to whether reduce in the blood pressure or promote healthy digestion, while they presenting fantastic antioxidant activity. Moreover, algal polysaccharides play an important role as free-radical scavengers vitro and antioxidants for the prevention of oxidative damage in living organisms [3]. Extracted Polysaccharide from ulva pertusa is a group of heteropolysaccharide, mainly composed from rhamnose, xylose, glucose, glucuronic acid, iduronic acid, sulfate with smaller amounts of mannoses, arabinose and galactose. Meanwhile, Cholesterol-enriched diet has been reported to cause severe damages on people health [4, 5]. The prevalence of dyslipidemia resulting from excess energy intake and physical inactivity is accumulating in Egypt. High level of blood cholesterol is a contributory factor of atherosclerosis and many lipid-associated ailments like obesity, heart attack, stroke and kidney failure [6]. Similarly, high level of fat could increase the overall rate of fat-mediated oxidative stress and decrease the antioxidative enzyme activity. Besides, there are various reports that highlighting the beneficial effects of antioxidant supplementation in preventing dyslipidemia and cardiovascular disease. Thus, oxidative damage and its consequences may result in many chronic health problems that are attributed to high fat diet. The green algae *Ulva lactuca (UL)* commonly known as a sea lettuce, which has been using as a food and traditional medical agent to treat helminthic infections, fever, urinary diseases and dropsy [6, 7]. The antimicrobial activity of UL is due to the acrylic acid, which commonly could be found in the algae. Extract of green algae *caulepra prolifera* was reported to exhibit significant activity against strains of marine bacteria [8]. These kinds of seaweeds (Marine algae) belong to a group of eukaryotic known as algae. Seaweeds are classified as rhodophyta (red algae), phaeophyta (brown algae) or chlorophyta (green algae), which depend on their nutrient, pigments and chemical composition. The same as other plants, seaweeds contain various inorganic and organic substances which can benefit human health [9]. Seaweeds are considered as a source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities. Compounds with antioxidant, antiviral, antifungal and antimicrobial activities have been detected in brown, red and green algae [10]. The environment in which seaweeds grow, is harsh as they are exposed to a combination of light and high oxygen concentra-

[1,2]Department of Applied Chemistry, Shiraz Branch, Islamic Azad University, Shiraz, Iran.
Email: NGoudarzian@iaushiraz.ac.ir

[3]Department of Chemical Engineering, School of Chemical and Petroleum Engineering, Shiraz University, Shiraz, Iran.
[4]Department of Mechanical Engineering, Islamic Azad University, Najafabad Branch, Najafabad, Iran.
5Department of Polymer Engineering, Yazd Branch, Islamic Azad University, Yazd, Iran.

International Journal of Scientific & Engineering Research, Volume 8, Issue 8, August-2017
ISSN 2229-5518

tions. These factors can lead to the formation of free radicals and other strong oxidizing agents but seaweeds seldom suffer from serious photodynamic damage during metabolism. This fact implies that seaweed cells have some protective mechanisms and compounds [11]. Marine algae are source of bioactive natural products and it has been studied as potential biocide and pharmaceutical agent. In this case, several scientific reports revealed the significant antibacterial activity of marine plants, while the main focus was on the antibacterial and antifungal activities of the marine algae compared to various kinds of pathogens [12]. The antibacterial activity of seaweeds is generally assayed using extract process in various organic solvent such as acetone, methanol-toluene, ether and chloroformmethanol [13]. Usage of organic solvents provide higher efficiency in matter of compounds extraction for the antimicrobial activity. In recent years, several marine bacterial and protoctist forms have been confirmed as important source of new potentially useful compounds for the development of chemotherapeutic agents. Recent examinations in case of antibiotic substances production by aquatic organisms showed importance of these kinds of materials as rich sources of antibacterial and antifungal agents. In this regard, over 15000 new chemical compounds were determined. By further Focus on bioproducts, recent achievements suggested that algae are a promising group to furnish novel biochemically active substances [14].

The main aim of this study is to evaluate the antimicrobial effectiveness of UL essential oil based on ethanol and methanol on various kinds of microorganism. In fact, results of this study provide a new data base about compositions of UL, which could be beneficial for various industries, including food and drug industries.

2 Materials and Instruments

In this study, used compounds such as anhydrite sodium sulfate (purity 97 %) and normal hexane (purity 95 %) and oleic acid were supplied by Merck & CO. Besides, usual laboratory instruments were used according to the British Pharmacy standards. Gas chromatography (GC) (model HP-6890 manufactured by Hewlett Packard, USA) and mass spectroscopy (MS) (Model HP-5973 manufactured by Hewlett Packard, USA) were used for further evaluation of developed essential oil based on UL.

3 Experimental Section

In order to obtain UL essence oil, hydraulic distillation (HD) method was used. In this case, schema of the procedure can be seen in Figure 1. The essence oil was gathered using clevenger instrument. For this regard, 100 g of dried herb was grinded and the essence was gathered in the instrument for 3:30 h. The operation performed using anhydrite sodium sulfate. The produced oil was conserved in a dim and closed container till analyses.

Moreover, as the present components in essence oils are known as volatile and semi-volatile oils, therefore GC-MS method were used for separation and identification of the components. The result spectrums were compared with standard mass spectrum of Adams (Adams, R.P., 2004). In order to confirm the identified components of the standard mass spectrum, quatz deterrence index was applied. Firstly, the Alkanes of C_8-C_{25} were injected into GC-MS and deterrence time for each Alkane was measured using KI=100n when 'n' is the number of carbons in related Alkane. quatz deterrence index of essence oils were calculated using the following equation:

$$KI=100n+100(t_x-t_n / t_{n+1}-t_n) \quad (1)$$

After dewatering the produced oil, it was diluted using normal hexane with the proportion of 1:10 and then injected into the GC-MS. The essence alga UL gathered by HD method, while nineteen different components were identified which form about 90.35 % of the total product. On the other hand, HD method could gather 7 components form about 99.18% of the total volume of essence. In addition, the percentage of HPLC components were also determined using HD method.

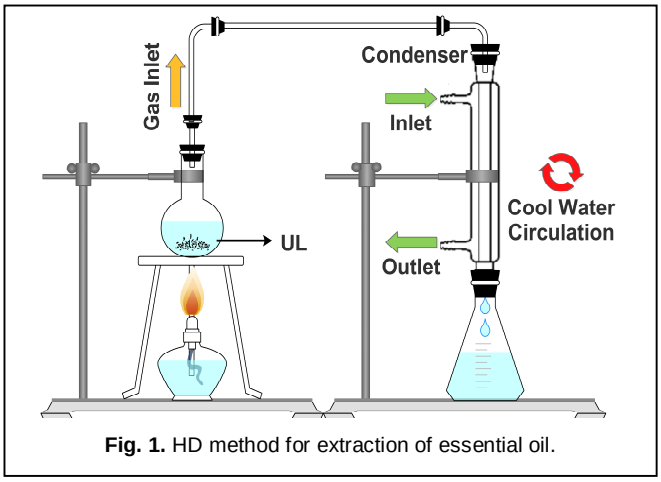

Fig. 1. HD method for extraction of essential oil.

In addition, for evaluation of phenol-based reagent based on folin ciocallatius as reference, a practical method was used. The circulating bath model mLw8 (manufactured by mLwHU) was used in this experiment, which have the ability to control the temperature during the extraction process. In this case, 1 g pre-grinded herb powder poured in 10 ml ethanol or methanol (80 %) and shook for 1 h. Thence, resulting suspension was

International Journal of Scientific & Engineering Research, Volume 8, Issue 8, August-2017
ISSN 2229-5518

placed in a centrifuge for 10 min at 3500 rpm. Then, 7.5 g of sodium carbonate was poured in a 100 mL volumetric flask and thereupon filled with distilled water. Afterward, 100 µL of folin ciocallatius solution poured in a 100 mL volumetric flask

and filled with distilled water. Resulting solution was used to produce other solutions with diverse weight percentages of essential oil. Further process conducted as follow: 50 µL of essential oil with 450 µL distilled water and 2.5 mL folin ciocallatius (1 %) were poured in a volumetric flask and mixed for 3 min. Then, 2 mL sodium carbonate (7.5 %) was added to the resulting solution and placed in a Benn Murray for 15 min at 45 °C. Thence, the resulting solution was cooled to room temperature and absorption level was determined using spectrophotometer at 765 nm wave length.

4 Results and Discussion

Examination of UL essential oil revealed that its containing too many anti- bacterial compounds. In this regard, GC-MS results of UL essential oil can be seen in Figure.2, while HPLC results based on HD method can be seen in Tables 1-3. Afterward, the concentration of phenolic materials was measured using following equation to draw standard curve via determination of diverse amount of gallic acid concentrations:

Concentration of phenolic materials=(garllic acid / 1000)×((20×1) / 1) (2)

In table 4 and 5, different concentration of gallic acid and overall amount of phenol in alga UL can be seen, respectively.

TABLE 1
THE COMPOSITION OF ALGA UL ESSENCE USING HD METHOD.

Number	RIa	Constituent Compounds	Isolated Material (%)	Identificatio Method
1	700	Hephtane	19.30	GC, MS
2	703	2-Ethyl furan	2.31	GC, MS
3	801	Hexanal	2.07	GC, MS
4	841	3-methyl pentanol	11.30	GC, MS
5	975	Methionol	3.67	GC, MS
6	1000	Decane	1.11	GC, MS
7	1029	Limonene	10.98	MS
8	1160	Terpineol	1.25	GC, MS
9	1488	Trans β-Ionone	2.54	MS
10	1500	Pentadecane	1.52	MS
11	1683	8-Heptadecene	7.09	GC, MS
12	1852	Hexahydrofarnesyl acetone	20.46	MS
13	1875	Cetanol	3.20	GC, MS
14	1878	Isobutylphthalate	5.10	GC, MS
15	1943	Isophytol	1.43	MS
16	2100	n-Heneicosane	1.68	GC, MS
17	2300	n-Tericosane	1.56	GC, MS
18	2400	n-Tetracosane	1.30	GC, MS
19	2500	n-Pentacosane	2.13	GC, MS

TABLE 2
OVERALL PERCENTAGE OF EACH COMPOSITION IN THE ALGA UL ESSENCE USING HD METHOD.

Constituent compounds	Overall percentage (%)
Monoterpene hydrocarbons	10.98
Oxygenated Monoterpene	3.79
Oxygenated sesquiterpenes	23.66
Oxygenated Diterpenes	1.43
Organic compound	60.14
Total identified	100

TABLE 3
CONSTITUENT COMPOUNDS OF ALGA UL ESSENCE USING GC-MS METHOD.

Number	Constituent compounds	mg.lit^{-1}	Retention (min)
1	Chloregenic acid	0.01095	10.5
2	Rutin	0.07000	12.6
3	Quercetin	0.00260	21.6
4	Carvacerol	0.17360	28.4
5	Hesperidin	0.00430	18.5
6	Hesperetin	0.01699	22.4
7	Rosmarinic acid	0.01486	19.2

TABLE 4
DIVERSE CONCENTRATIONS OF GALLIC ACID.

Acid Gallic (µl)	0	5	10	-	100
Distilled Water (µL)	500	495	490	-	400

TABLE 5
OVERALL AMOUNT OF PHENOL IN ALGA UL.

Name of plant	Essence	Acid Gallic (mg) / Herb (g)
alga UL	Extraction	0.037

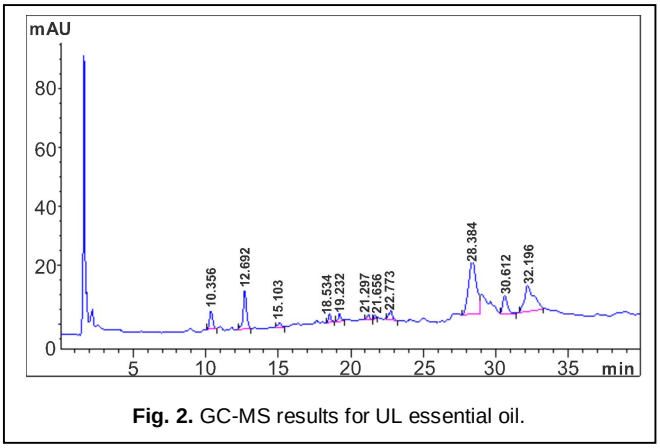

Fig. 2. GC-MS results for UL essential oil.

International Journal of Scientific & Engineering Research, Volume 8, Issue 8, August-2017
ISSN 2229-5518

4.1 Antimicrobial Effect of Alga UL on Microorganisms

In this section, antimicrobial effect of alga UL essential oil on eight different human pathogenic bacteria and five diverse human pathogenic mushrooms were examined. Achieved results revealed that, the higher the concentration of essential oil, the higher the antimicrobial activity. Besides, UL essential oil was produced based on two diverse solvents including ethanol and methanol. Table.6 shows the variance analysis of antimicrobial properties of ethanol-based essence, while Tables 7 shows the effectiveness of ethanol-based essence on inhibition of microorganism growth. Moreover, in Table 8, the minimum rate of bacterial growth (MBC) and microorganism growth inhibition via ethanol-based essence can be seen. Such results for methanol-based essence can be seen in Tables 9-11, respectively. As can be seen in Tables 6-11, both ethanol and methanol based UL essences presenting significant effectiveness against bacterial growth and activity, which justify their role as fantastic sources of antimicrobial materials.

TABLE 6
VARIANCE ANALYSIS OF ANTIMICROBIAL PROPERTIES OF ETHANOL-BASED ESSENCE.

Statistical indicators	Source of changes	Sum of Squares	df	Average Square	F
Diameter of Herb growth inhibition	Outgroup	8316.049	12	693.004	13.111
	Intergroup	9620.031	182	52.857	
	Total	17936.079	194	-	

TABLE 7
EFFECTIVENESS OF ETHANOL-BASED ESSENCE ON INHIBITION OF MICROORGANISM GROWTH.

Classification	Average Diameter of Herb growth inhibition (mm)	Type of Microorganism
bc	13.8267±1.77409	Arizona
cd	8.9267±1.73121	Salmonella
a	21.0000±2.76734	Morganella
ab	18.9667±1.74699	Entrobacter
a	22.8667±1.85643	E.Coli
cd	12.4933±1.25467	Klebsia Pesudomon
ab	19.0067±1.94083	Pseudomonas aeruginosa
de	7.7867±2.27137	Staphylococcus aureus
ab	19.1200±1.65331	Candida albicans
cd	10.2067±2.37818	Cryptoccus neoformans
ef	3.3267±0.95586	Aspergillus flavous
f	1.2333±0.66344	Terichophyton verrucosum
bcd	13.3333±2.29369	Epidermophyton floccodum

TABLE 8
THE MINIMUM RATE OF BACTERIAL GROWTH (MBC) AND MICRO-ORGANISM GROWTH INHIBITION VIA ETHANOL-BASED ESSENCE.

Type of Microorganism	DMSO	MIC	MBC
Arizona	-	1.562	3.125
Salmonella	-	1.562	3.125
Morganella	-	0.781	1.562
Entrobacter	-	3.125	6.25
E.Coli	-	3.125	6.25
Klebsia Pesudomon	-	3.125	6.25
Pseudomonas aeruginosa	-	3.125	6.25
staphylococcus aureus	-	3.125	6.25
Candida albicans	-	0.781	1.562
Cryptoccus neoformans	-	1.562	6.125
Aspergillus flavous	-	3.125	6.25
Terichophyton verrucosum	-	6.125	6.25
Epidermophyton floccodum	-	1.562	3.125

TABLE 9
VARIANCE ANALYSIS OF ANTIMICROBIAL PROPERTIES OF METHANOL-BASED ESSENCE.

Statistical indicators	Source of changes	Sum of Squares	df	Average Square	F
Diameter of Herb growth inhibition	Outgroup	5203.215	12	433.601	9.481
	Intergroup	8323.408	182	45.733	
	Total	13526.623	194	-	

TABLE 10
EFFECTIVENESS OF METHANOL-BASED ESSENCE ON INHIBITION OF MICROORGANISM GROWTH.

Classification	Average Diameter of Herb growth inhibition (mm)	Type of Microorganism
ab	13.9867±1.26404	Arizona
fg	2.4133±0.91516	Salmonella
ab	14.4267±3.37429	Morganella
abc	13.0800±1.26950	Entrobacter
a	16.5067±2.96094	E.Coli
cde	8.2800±1.21953	Klebsia Pesudomon
def	7.0667±1.56767	Pseudomonas aeruginosa
abcd	11.0400±0.99804	Staphylococcus aureus
a	15.7933±1.47517	Candida albicans
efg	3.9133±1.39962	Cryptoccus neoformans
efg	3.2133±1.05103	Aspergillus flavous
g	1.0733±0.40900	Terichophyton verrucosum
bcd	9.5200±2.25552	Epidermophyton floccodum

International Journal of Scientific & Engineering Research, Volume 8, Issue 8, August-2017
ISSN 2229-5518

TABLE 11
THE MINIMUM RATE OF BACTERIAL GROWTH (MBC) AND MICRO-ORGANISM GROWTH INHIBITION VIA METHANOL-BASED ESSENCE.

Type of Microorganism	DMSO	MIC	MBC
Arizona	-	0.781	1.562
Salmonella	-	6.25	12.5
Morganella	-	3.125	6.25
Entrobacter	-	0.781	1.562
E.Coli	-	1.562	3.125
Klebsia Pesudomon	-	0.781	1.562
Pseudomonas aeruginosa	-	1.562	3.125
Staphylococcus aureus	-	0.781	1.562
Candida albicans	-	0.781	1.562
Cryptoccus neoformans	-	6.25	12.5
Aspergillus flavous	-	3.125	6.25
Terichophyton verrucosum	-	6.25	12.5
Epidermophyton floccodum	-	1.562	3.125

4 CONCLUSIONS

Obtained results revealed that these species of seaweeds, which was collected form Iranian Gheshm island, contain a variety of antimicrobial compositions, thereby make them as interesting sources for antimicrobial materials for various applications. Minimum inhibitory concentration (MIC) of investigated sea weeds against bacterial isolates were evaluated in mueller hinton broth by Broth macro dilution method. The seaweed extracts were dissolved in Sodium carbonate solution (80 %) to obtain 128mg/ml stock solutions. Obtained essential oil from UL showed significant effectiveness against growth of various kinds of bacterias, which make it as a fantastic source for extraction of antimicrobial materials such as hexahydrofarnesyl acetone, hephtane, 3-methyl pentanol, limonene (10.98 %) and 8-heptadecene (7.09 %).

REFERENCES

[1] W.S. Aronow, Treatment of high-risk older persons with lipid-lowering drug therapy, American journal of therapeutics 15(2) (2008) 102-107.
[2] A. Belfield, D. Goldberg, Revised assay for serum phenyl phosphatase activity using 4-amino-antipyrine, Enzyme 12(5) (1970) 561-573.
[3] E. Beutler, Improved method for determination of blood glutathione, J Lab Clin Med 61(5) (1963) 882-888.
[4] M.L. Bishop, E.P. Fody, L.E. Schoeff, Clinical chemistry, Principles, procedures, correlation, Quality assurance 3 (2005) 4.
[5] I.H. Kim, D.-G. Lee, S.H. Lee, J.-M. Ha, B.-J. Ha, S.-K. Kim, J.-H. Lee, Antibacterial activity ofUlva lactuca against methicillin-resistantStaphylococcus aureus (MRSA), Biotechnology and bioprocess engineering 12(5) (2007) 579-582.
[6] K. Kranl, K. Schlesier, R. Bitsch, H. Hermann, M. Rohe, V. Böhm, Comparing antioxidative food additives and secondary plant products–use of different assays, Food chemistry 93(1) (2005) 171-175.
[7] T. Kuda, E. Taniguchi, M. Nishizawa, Y. Araki, Fate of water-soluble polysaccharides in dried Chorda filum a brown alga during water washing, Journal of Food Composition and Analysis 15(1) (2002) 3-9.
[8] A. Bansemir, M. Blume, S. Schröder, U. Lindequist, Screening of cultivated seaweeds for antibacterial activity against fish pathogenic bacteria, Aquaculture 252(1) (2006) 79-84.
[9] Y. Chew, Y. Lim, M. Omar, K. Khoo, Antioxidant activity of three edible seaweeds from two areas in South East Asia, LWT-Food Science and Technology 41(6) (2008) 1067-1072.
[10] A. Abdel-Khaliq, H. Hassan, M.E. Rateb, O. Hammouda, Antimicrobial activity of three Ulva species collected from some Egyptian Mediterranean seashores, International Journal of Engineering Research and General Science 2(5) (2014) 648-669.
[11] S.G. Rangaiah, P. Lakshmi, E. Manjula, Antimicrobial activity of seaweeds Gracillaria, Padina and Sargassum sps. on clinical and phytopathogens, Int. J. Chem. Anal. Sci 1(6) (2010) 114-117.
[12] K. Kolanjinathan, D. Stella, Antibacterial activity of marine macro algae against human pathogens, Recent Research in Science and Technology 1(1) (2009).
[13] R.A. Cordeiro, V.M. Gomes, A.F.U. Carvalho, V.M.M. Melo, Effect of proteins from the red seaweed Hypnea musciformis (Wulfen) Lamouroux on the growth of human pathogen yeasts, Brazilian Archives of Biology and Technology 49(6) (2006) 915-921.
[14] İ. TÜney, B.H. Cadirci, D. Ünal, A. Sukatar, Antimicrobial activities of the extracts of marine algae from the coast of Urla (Izmir, Turkey), Turkish Journal of Biology 30(3) (2006) 171-175.