Plant Archives Vol. 19, Supplement 1, 2019 pp. 1394-1399 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

ANTIBACTERIAL AND WOUND HEALING ACTIVITIES OF ACETONE *CLADOPHORA GLOMERATA* EXTRACT

Ahmed S. Dwaish
Department of Biology, College of Science, Mustansiriyah University, Baghdad, Iraq
Emails : ahmedsahi33@gmail.com and ahmedsahi@uomustansiriyah.edu.iq

Abstract

The green filamentous alga (*Cladophora glomerata*) extract were prepared in acetone and mad four different concentrations (w/vol.) 12.5, 25, 50 and 100 mg/ml and tested against bacterial species were: *Staphylococcus aureus, Staphylococcus epidermidis, Bacillus substilis, Escherichia coli*, *Klebseilla* sp. and *pseudomonas aeruginosa.* The results show that the hot acetone extract was able to make good zone of inhibition in bacterial species between 8-20mm value. Chemical analyses of for hot acetone extract alga (*C. glomerata*) show different active chemical compounds such as alkaloids, phenols, Tannins, Flavones, Resins and tannins. To identify the compounds that responsible for activities, GC–MS technique was use in order to tentative identify these antimicrobial activity compounds. The main compositions were including indoles, terpenes, acetogenins, phenols, fatty acids and volatile halogenated hydrocarbons compounds which had antimicrobial activity. The results of the current research showed that the acetoneextract of *C. glomerata*had the ability to speed healing of wounds as compared to the control and shortened the period of epithelialization nearly by a week.

Key Words **:** *Cladophora glomerata*, antibacterial, active compounds.

Introduction

Macroalgae consider one of the most important of natural bioactive compounds, due to their ability to produce large different types of secondary metabolites through a wide range of biological activities. Many algae types of Chlorophyta and Phaeophyta showed there incredible ability antifungal, antiviral, antioxidant and antimicrobial activities (Bansemir *et al.*, 2006; Chew *et al.,* 2008; Dina and Ahmed, 2016). Since the middle of the last century, they began to employ algae extracts in the medical industry (Christobe *et al.,* 2011; Yousif *et al*., 2014). Previous studies and research have demonstrated the ability of macroalgae to produce antimicrobial activity against different types of bacteria (Kandhasamy and Arunachalam, 2008; Christobel *et al*., 2011; Dina *et al*., 2018). In addition to create various antibiotics that are effective against pathogenic bacteria fish and humans (Das *et al*., 2005; Dina *et al*., 2013). Many management have been used to control different pathogenic bacteria. Unluckily, most these ways has side effect and the problems becoming increasingly difficult (Sieradzki *et al*., 1999). That use over doses to inhibition growth of pathogens, make it resistance to antibiotic, and search for new sources to control disease infection. The main aim of this search is to detect the chemical composition of extracts from fresh water green algae *C. glomerata*, and to study their antibacterial activity in order to find a potential natural source of bioactive compounds and biomedical uses.

Materials and Methods

Collection and Preparation of Sample

From Baghdad University–Iraq, at located on longitude (33°16'09.5"N) and latitude (44°20'19.41" E). Start collected the samples of *C. glomerata* by using hand and spread from the rock and transported in plastic bags to the laboratory immediately during autumn, 2017.

According to (Bellis and McLarty, 1967 ; Burrows, 1991), *C. glomerata was* identify and stored in 5% formalin. The *C. glomerata* specimen gently wash with tap water to remove other materials such as sand, shells, etc. Then, dried at 40°C in an oven and then by using electric blender convert to powder form.

Preparation *C. glomerata* for hot acetone extraction

Soxhlet apparatus was used to prepper hot acetone extraction, through add) 5g of dried algae powder and extracted for 4-5 hrs. with (150 ml) organic solvent (acetone) and concentrated the solvent by using rotary evaporator. To prepper stock solution add 0.5 g of *C. glomerata* to 2.5 ml of DMSO and then the volume was made up to 5 ml that equal 100mg /ml and then make different concentration (Snehlata, *et al*., 2014) .

Antibacterial Assay

In vitro, by using well diffusion assay for antibacterial tests (Siham and Ahmed,2012) through measuring the diameters in millimeter of the bacterial inhibition zones. the bacteria that use in this test were: *S. aureus*, *S. epidermdis*, *E. coli* , *Klebseilla* sp*., P. euroginosa* and *Bacillus substilis*. With and without acetone as positive and negative control. The clear halos greater than 10 mm were considered as positive results, experimental in comparison data represent mean ± SD of each sample. Every tests were performed in triplicate.

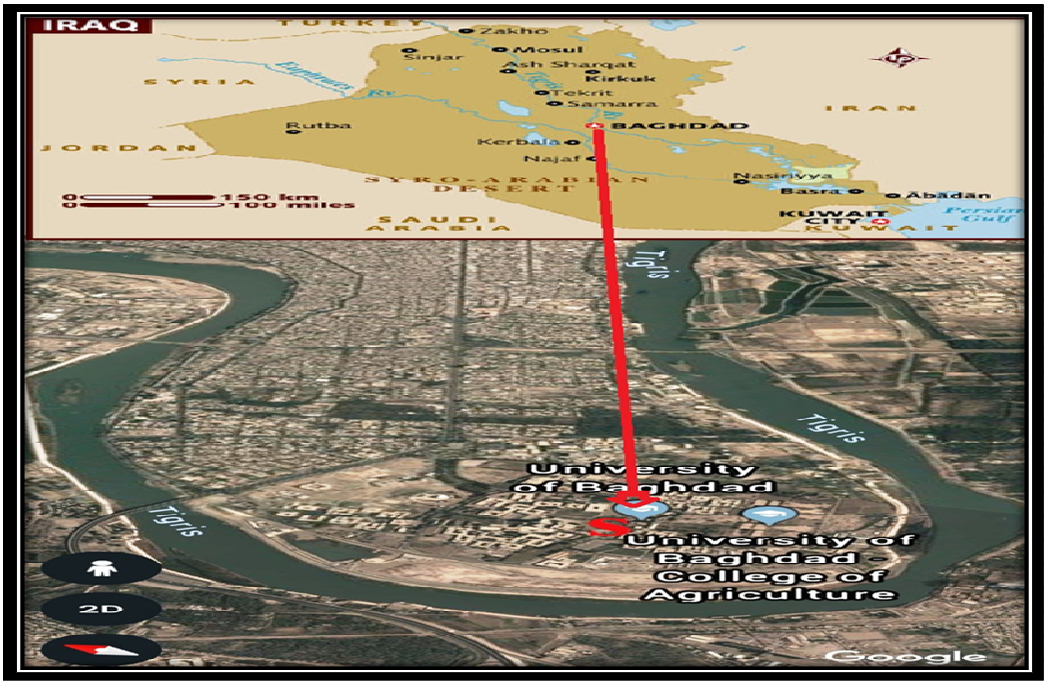

Fig. 1: Site collection of *C. glomerata* at located on longitude (33°16'09.5"N) and latitude (44°20'19.41" E).

Indicators of Active Compound in Extracts

According to standard protocols (Harbone,. 1973; Harbone, 1984) to deterging the presence or absent of active compounds.

Gas Chromatography-Mass Spectrometry

According to (Praveen *et al*., 2010), the compounds were identified by comparison of their mass with NIST library search and authentic standards for GC-MS analysis

Antibacterial test (Open Wound)

In vivo, the most sensitive bacterial strains to acetone *C. glomerata* extract was pick out to antimicrobial studies. Groups of adult Albino mice containing nine mice with weigh between 20-29 grams, were split into tripartite groups. It was anesthetized by ether and then sterilized the surface of each mouse back after shaving. A longitudinal incision was performed by a scalpel with three incisions on the back of the mice (Morton and Malone, 1972). Each mouse was then keeping it in different cages to avoid any nuisance. The groups were treated as follows:

Group 1: (Negative control) the wound treatment with distilled water only for two weeks

Group 2: (Positive control), the wound treatment with 1 ml of *S. aureus* suspension containing (5.5×10^5 cell/ml) and after (24) hours of infection, mice treated with azithromycin antibiotic (concentration 2% per 15g w/w) for two weeks.

Group 3: As algal extract treatment ,the wound treatment with 1 ml of *S. aureus* suspension containing (5.5×10^5 cell/ml) and after (24) hours of infection, mice treated with (acetone) extracts of *C. glomerata* (concentration 100 mg/ml) for two weeks.

Results and Discussion

Morphological Structure of *C. glomerata*

Macrolage *C. glomerata* consider one of the most abundant algae in the river, is green to light green, branching, filamentous in form, attached on rock or cobble in the bed of sallow rivers. Under the microscope, the following points can be observed: thalli are composed of joined cylindrical cells, with lengths of 6-20 µm and widths of 4-10 µm and with dichotomously branching filaments figure (2). Branches are tufted, arising singly, and in older algae, the branches becoming irregular. Branches are narrowed towards tips, cell walls are thick and usually lamellate. The chloroplast is in a parietal network with numerous pyrenoids. Usually tends to assemble in one place, making it easy to remove, this findings agreed with(Fish and Fish, 1989; Gibson *et al.*, 2001).

Antibacterial activity of algae extracts

The crude hot acetoneextracts of algae (*C. glomerata*) that determined by well diffusion assay.

Aqueous forms of the extracts of the algae exhibited no action of antibacterial activities against the test organisms. While the results of the use hot acetone of algae extracts showed heterogeneous degrees of antibacterial activity against organisms in different concentrations testing and the results are summarized in Table 1 and figure 3.

Table 1 : Antibacterial activity of *C. glomerata* hot acetone extract. (inhibition zone was measured to the nearest millimeter).

Bacteria	Concentrations mg/ml				LSD value
	100	50	25	12.5	
S. aureus	20±1	14±2	14±0.5	10±1	3.266*
S. epidermdis	18±2	14±1	13±1	8.5±1	3.089*
E. coli	14±1	11.5±0.5	11.5±0.5	8 ±1	2.341*
Klebseilla sp.	12±1	10±1	8±0.5	7.5±1	2.056*
P. euroginosa	11.5±1	10±1	8.5±1	8 ±1	2.226*
Bacillus substilis	13.5±0.5	11±2	10.5±1	9±1	2.762*
LSD value	2.975 *	2.355 *	3.026 *	1.94 NS	---
* (P<0.05), NS: Non-Significant.					

Due to antibiotics are synthetic chemicals with many side effects, other natural sources must be sought (Ajaib *et al*., 2015). Since bacteria can possess the ability to resist antibiotics, it is necessary to prepare natural extracts that have the effectiveness of antibiotics and medically safe.

As seen in table (1), it was clear that the maximum zone of inhibition was in bacteria *S. aureus* with 20 mm of zone of inhibition at concentration 100mg/ml. while, the minimum zone of inhibition was in bacteria *Klebseilla sp.* with 7.5 mm of zone of inhibition at concentration 12.5mg/ml.

Previous studies, confirmed the susceptibility of algal extracts to the resistance of many bacteria (Dina *et al*., 2013; Dina *et al*., 2016) proved that methanolic extracts of *Spirulina platensis*, *Chlorella pyrenoidosa* and *Nostoc muscorum* were good against the human pathogenic bacteria and fungi (Arun *et al*., 2012).

Phytochemical Evaluation

Biochemical analysis were being undertaken to determine the structure and nature of compounds responsible of the bio activity of the extract with high antibacterial potency.

For determine the structure and nature of compounds of the bio active responsibility in extract by biochemical analysis. Different active compounds in acetone extract of *C. glomerata* has been found. The results showed presence of alkaloids, phenols, Tannins, Flavones, Resins and tannins. While Coumarines and Saponines, were absent as shown in table (2). This results agreed with (Siham and Ahmed, 2012) that screened most active compounds in macroalgae.

Table 2: Presence or absence of active compounds in *C. glomerata* extract.

Chemicals Compound	Hot Acetone Extract
Glycosides	+
Phenols	+
Alkaloids	+
Resins	+
Saponines	-
Tannins	+
Flavones	+
Coumarines	-

Ass seen in table (3) analysis by Gas chromatography–mass spectrometry (GC-MS) Gas chromatography–mass spectrometry (GC-MS) consider essential for the identification of natural organic compounds, in order to determine the compounds responsible for the biological activity. Ass seen in table (3) variousnatural antimicrobial compounds present in *C. glomerata* extract that belong to different range of chemical classes, including indoles, terpenes, acetogenins, phenols, fatty acids and volatile halogenated hydrocarbons (Rodríguez-Meizoso *et al*., 2010). GC–MS methods were used to analyze both, fatty acids and volatile compounds, in separated fraction of acetone extracts that showed antibacterial activity. The result presumes that the existence long chain hydrocarbons may act as potential bioactive substance and able to using in pharmaceutical preparations. Moreover, the cultivable nature of seaweeds is an added advantage of wholesale production of potential antibacterial products. Further study is in progress to find out the mechanism of inhibition of pathogens by the purified compounds and to study the antioxidant in addition to anti-inflammatory properties of *C. glomerata.* Our results are in accordance with the reported investigations (Blumer *et al*., 1971; Youngsblood *et al*., 1971).

Table 3 : GC-MS Analysis of Major Compounds in acetone extract of *C. glomerata.*

Number	Rt.	Area%	Compounds
1.	26.374	1.36	hexadecamethyl
2.	26.597	1.56	hexadecamethyl
3.	30.474	1.57	octadecamethyl
4.	30.541	1.80	octadecamethyl
5.	30.812	4.40	heptane
6.	33.210	2.09	Tetradecanoic acid
7.	33.954	0.83	Dibutyl phthalate
8.	34.612	3.17	n-Hexadecanoic acid
9.	39.388	3.76	octadecamethyl-
10.	39.649	0.95	Oleic Acid
11.	39.727	1.45	Heptadecane
12.	41.477	7.34	Hexanedioic acid
13.	41.776	3.70	octadecamethyl-
14.	42.985	1.42	Pentacosane
15.	44.010	4.28	hexadecamethyl-
16.	49.946	1.58	octadecamethyl

Open wound experiment *(in vivo)*

As shown in the figure (4 and 5), it was clear that the last group treated with acetone extract was more efficient after week of treatment and the wounds were completely curedwithout any pus, while the second group treated with antibiotics lasted longer than two week of treatment. The first group, negative control, did not detect wounds infected with *S. aureus*. The principle of tissue repair involves renewal or replacement and that leading to the healing of a wound. Often, wound healing aims to reduce the time of infection and reducing to minim the undesired consequences (Morton and Malone, 1972; Mrityunjoy *et al*., 2007). In this study, our data showed that the acetone extract of *C. glomerata* increased wound contraction in excision wound as compared to the control group and shortened the period of epithelialization nearly by seven days.

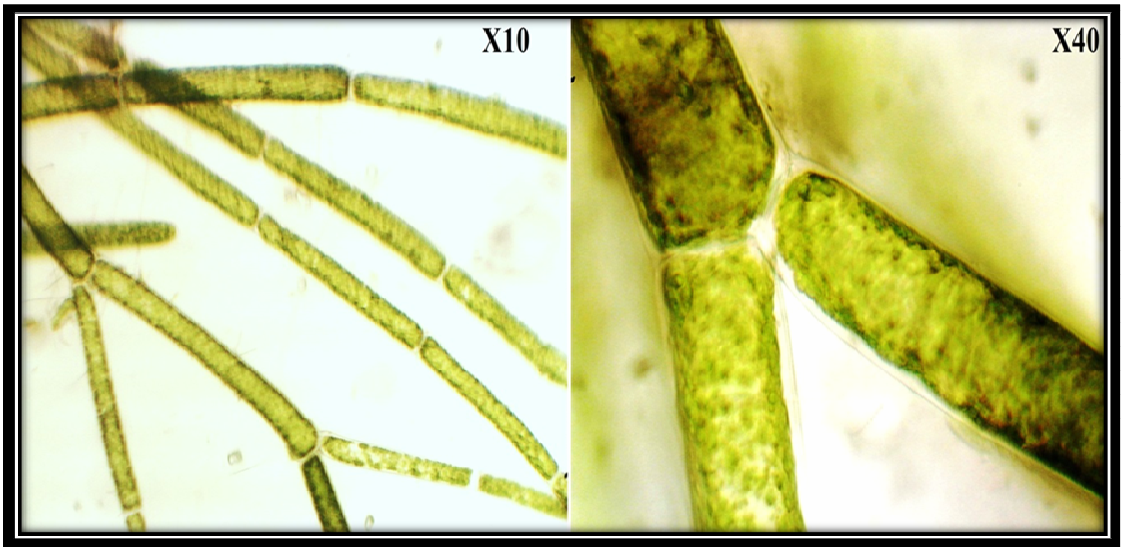

Fig. 2 : Filaments of *Cladophora glomerata* showing the branching.

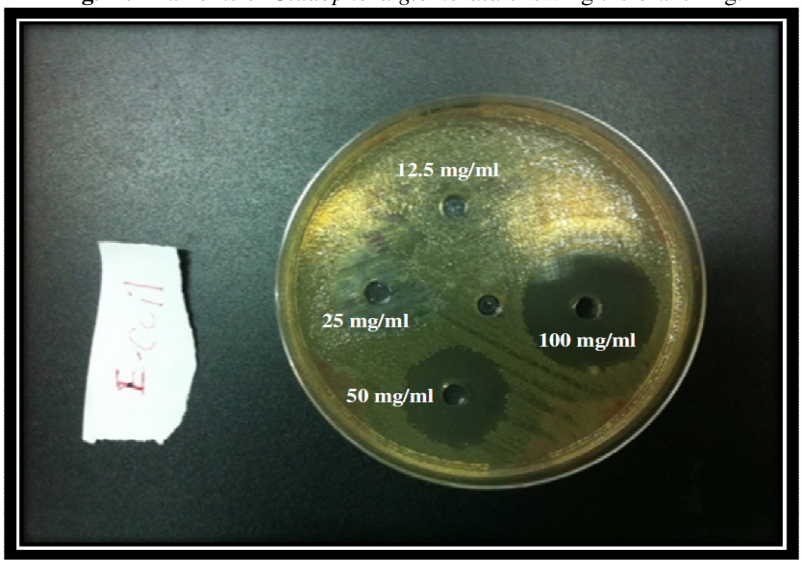

Fig. 3 : Antibacterial activity of Crude hot acetone extract of *C. glomerata* against *E. coli* at different concentrations.

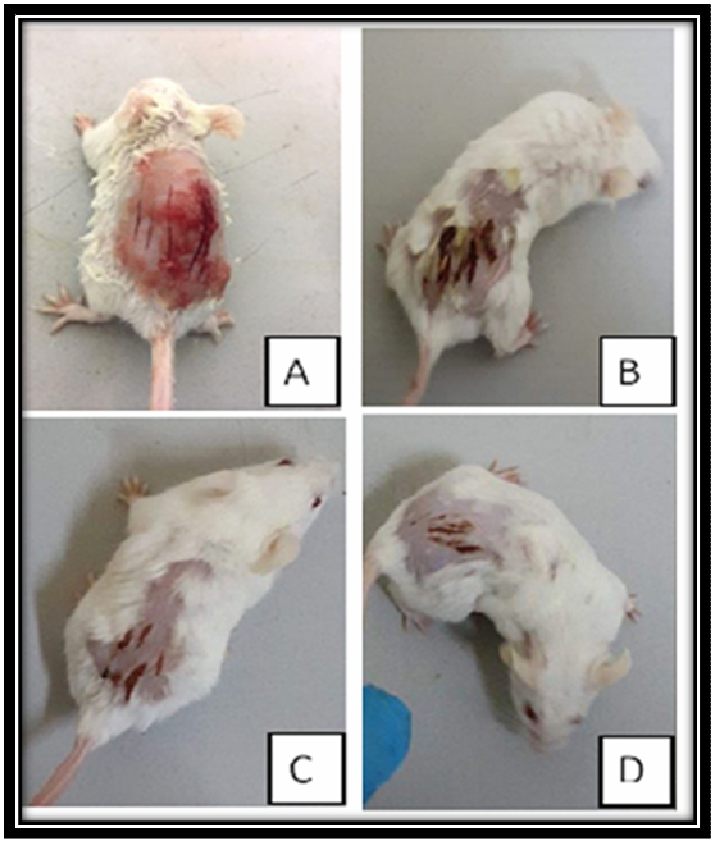

Fig. 4: Treatment of mice skin infection (A) start of infection (B) negative control after 4 days (C) positive control after 4 days (D) Acetone extract of *C. glomerata* after 4 days

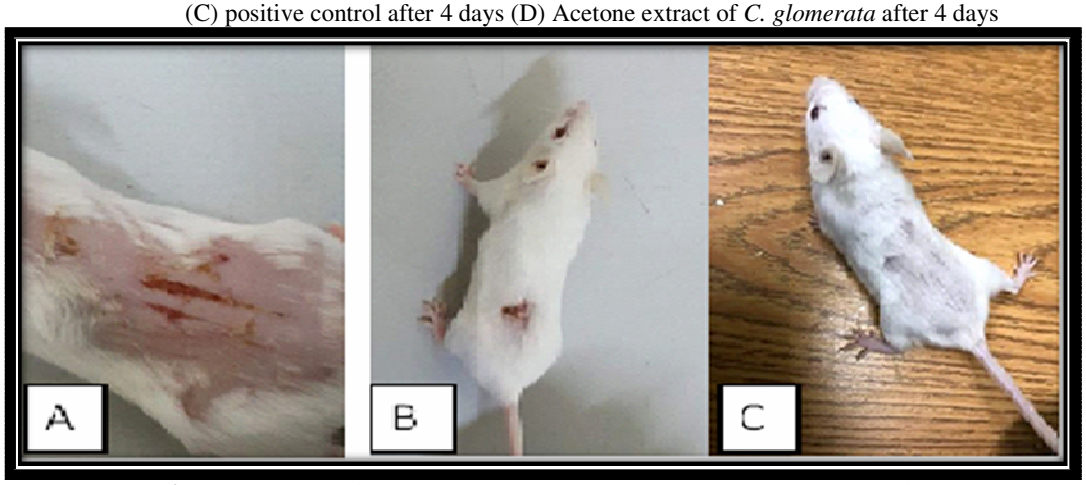

Fig. 5: Treatment of mice wound after 7 days (A) negative control (B) Positive control (C) Acetone extract of *C. glomerata*.

Acknowledgements

Special thanks to the collage of science Biology Department Mustansiriyah University for their kind support for using its labrotaries.

References

Ahmed, S.D.; Dina, Y.M. and Siham, N.L. (2016). "Use of spirogyra sp. extract against multidrug resistant bacterial pathogens.*"International Journal of Advanced Research*, 4(7): 575-579.

Ajaib, M.; Mati-ur-Rehman, A.; Khan, K.M.; Perveen, S. and Shah, S. (2015). *Pulicaria undulata*: A Potential Phytochemical, Antimicrobial and Antioxidant Source. *J. Chem. Soc. Pak.,* 37(3): 559-566.

Arun, N.; Gupta, S. and Singh, D.P. (2012). Antimicrobial and antioxidant property of commonly found microalgae *Spirulin platensis*, *Nostoc muscorum* and *Chlorella pyrenoidosa* against some pathogenic bacteria and fungi. *Int. J. Pharma. Sci. Res.,* 3(12): 4866-4875.

Bansemir, A.; Blume, M.; Schroder, S. and Lindequist, U. (2006). Screening of cultivated seaweeds for antibacterial activity against fish pathogenic bacteria. Aquaculture, 252: 79-84.

Bellis, V.J. and McLarty, D.A. (1967). Ecology of *Cladophora glomerata* (L.) Kütz. in southern Ontario, J. Phycol.; 3(2): 57-63.

Blumer, M.; Guillard, R.R.L. and Chase, T. (1971). Hydrocarbons of marine phytoplankton. Mar. Biol., 8: 183-189.

Burrows, E.M. (1991). Seaweeds of the British Isles volume 2 Chlorophyta, Natural history museum publications London.

Chew, Y.L.; Lim, Y.; Omar, M. and Khoo, K.S. (2008). Antioxidant activity of three edible seaweeds from two areas in South East Asia. LWT 41: 1067-1072.

Christobel, G.J.; Lipton, A.P.; Aishwarya, M.S., Sarika, A.R. and Udayakumar, A. (2011). Antibacterial activity of aqueous extract from selected Macroalgae of Southwest coast of India. *Seaw. Res.,* 33(2): 67-75.

Das, B.K.; Pradhan, J.; Pattnaik, P.; Samantaray, B.R. and Samal, S.K. (2005). Production of antibacterials from the freshwater alga *Euglena viridis* (Ehren). *W.J. Microbiol. Biotech.,* 21: 45–50.

Dina, Y.M.; Ahmed, S.D.; Abd-Ali, G. and Jawad, A.L.M. (2013). Use of *Cladophora glomerata* Extract Against Multidrug Resistant Bacterial Pathogens. J. Env. Sci. and Engin (USA) in No. 8A, Vol 2.

Dina, Y.M.; Sarah, I.M. and Ahmed, S.D. (2018). The effect of hot ethanolic algae extract of *Cladophora glumerata* on some biological aspect of *Callosobruchus maculates* (Fab). J. Pharm. Sci. & Res. 10(3): 532-536.

Dina, Y.; Yousif, M. and Dwaish, A.S. (2016). Activity of two green algae (*Zygnema stellinum* and *hydrodictyon reticulatum*) extracts against some phytopathogenic fungi. *EJBPS*, 3(10) : 45-48.

Dina, Y.M.; Dwaish, A.S. and Jawad, A.L.M. (2013). Anti–Phytopathogenic Fungi Activities of cladophora Glomerata extract. W.J.P.R 2(6): 1868-1877.

Fish, J.D. and Fish, S. (1989). A student's guide to the Seashore. Unwin Hyman Ltd, London.

Gibson, R.; Hextall, B. and Rogers, A. (2001). Photographic Guide to the Sea and Shore Life of Britain and North-west Europe. Oxford University Press, Oxford.

Harbone, J.B. (1984). Phytochemical Methods. A guide to Modern Techniques of Plant Analysis. 2nd. ed., Chapman and Hall. London. 288.

Harbone, J.B. (1973). phytochemical methods. Chapman and Hall. London.

Kandhasamy, M. and Arunachalam, K.D. (2008). Evaluation of *in vitro* antibacterial property of seaweeds of southest coast of India. Afr. J. Biotechnol., 7: 1958-1961.

Morton, J.J. and Malone, M.H. (1972). Evaluation of Vulneray activity by an open wound procedures in rats. Arch Int. Pharmacodyn. Ther., 196: 117-126.

Mrityunjoy, M.; Naira, M.; Jagadish, V.K. and Mohammad, A. (2007). Evaluation of *Tectona grandis* leaves for wound healing activity. Pak. J. Pharm. Sci., 2(2): 120-124.

Praveen, K.P.; Kumaravel, S. and Lalitha, C. (2010). Screening of antioxidant activity, total phenolics and GC-MS study of Vitexnegundo. Afr. J. Biochem. Res., 4:191-195.

Rodríguez-Meizoso, I.; Jaime, L.; Santoyo, S.; Señoráns, F.J.; Cifuentes, A. and Ibáñez, E. (2010). Subcritical water extraction and characterization of bioactive compounds from *Haematococcus pluvialis* microalga. J. Pharm. Biomed. Anal., 51: 456-463.

Sieradzki, K.; Robert, R.B.; Haber, S.W. and Tomasz, A. (1999). The development of vanomycin resistance in patient with methicillin resistant *Staphylococcus aureus*. New Eng. J. Med., 340: 517-23.

Siham, N. and Ahmed, S.D. (2014). Use of *Cladophora glomerata* extract against multidrug resistant bacterial pathogens. W.J. of Pharm. Research., Vol 3, Issue10.

Snehlata, P.; Rajshree, P. and Rita, S. (2014). Phytochemical Screening of Selected Medicinal Plant Cinnamon Zeylanicum bark extract, Area of research; Uttarakhand, India. Int. J. of Sci. and Res. Publications, Volume 4, Issue 6, June.

Youngsblood, W.W.; Blumer, M.; Guillard, R.R.L. and Flore, F. (1971). Saturated and unsaturated hydrocarbons in marine benthic algae. Mar. Biol., 8: 190-201.