

ANTIMICROBIAL ACTIVITY OF *ULVA LACTUCA* EXTRACTS AND ITS FRACTIONS

G. Alang^{1*}, R. Kaur¹, A. Singh², P. Budlakoti², A. Singh¹ and P. Singla¹

¹G.H.G Khalsa College of Pharmacy, Gurusar Sadhar-141104, Ludhiana, Punjab, India.

²I.T.S Paramedical College, Muradnagar-201206, Ghaziabad, Uttar Pradesh, India

Summary

In the present study, Green alga *Ulva lactuca Linnaeus* has been taken and evaluated for its antimicrobial activity against Gram positive bacteria- *Staphylococcus aureus subsp. aureus* (MTCC 737) (*S.aureus*) and *Staphylococcus epidermidis* (MTCC 3615) (*S.epidermidis*) and Gram negative bacteria- *Pseudomonas aeruginosa* (MTCC 424) (*P.aeruginosa*) and *Escherichia coli* (MTCC 1687) (*E.Coli*) and Fungi- *Candida albicans* (MTCC 183) (*C.A.*) and *Aspergillus niger* (MTCC 228) (*A.N.*). Green alga *Ulva lactuca Linnaeus* was successively extracted with n-hexane, chloroform and ethanol: water (1:1) by maceration. The phytochemical analysis revealed the presence of carbohydrates, steroids and glycosides. The extracts were subjected for study of antimicrobial activity. The antimicrobial activity was determined by disc-diffusion method at 1mg/ml concentration using Dimethyl formamide as the solvent control. Ampicillin and Clotrimazole were used as reference agents. The chloroform extract was chromatographed over silica gel and the elutes were monitored by thin layer chromatography. The fractions were analyzed by UV, IR spectral methods and tested for their antimicrobial activity. Of the eight fractions tested, fraction 3 exhibited more antibacterial activity than chloroform extract against all the tested bacteria, whereas fractions 1, 5 and 6 showed more remarkable antifungal activity than the extract against *Aspergillus niger* and *Candida albicans*.

Key Words: Antimicrobial; *Ulva lactuca*; phytochemical screening

***Corresponding author:** Gaurav Alang; Email: gavrup2000@gmail.com

Introduction

During the latter part of this century the practice of herbalism has become main stream throughout the world because the screening of algae, plant extract and natural products has shown that green algae represent a potential; source as well as serving as lead compounds for discovery. Traditional compounds are facing crisis due to rapidly increasing resistance e.g. *S. aureus*. In contrast to chemical drugs herbs are considered to be non-toxic because of natural origin and long term use as folk medicine. Green algae have been extensively under research for the last two decades. Since number of active constituents have been extracted from different species of green algae (e.g. biologically active steroids [1]). The green algae *Ulva lactuca* Linnaeus have been found to have antimicrobial[2], antibacterial[3,4], preservative[4,5], anticoagulant[5], antiperoxidative[4,6], antihyperlipidemic[6], hepatoprotective[7], anti-inflammatory[8], antiprotozoal[9], antiviral[5,10,11], leishmanicidal activities[14] and also employed as dietary fibers[6,12,13,15,16] in areas of Scotland.

Materials and Methods

Algal material

Ulva lactuca was collected from Thonithurai near Pamban bridge, Mandapam post Ramananthapuram district, Tamilnadu, India in January 2007. It was authenticated by M. Govindraj, Field Man, Central Marine Fisheries research institute, Ramanathapuram, Tamilnadu, India. Green alga, *Ulva lactuca* was air dried for two weeks at room temperature. The dried material was coarsely powdered and stored in polyethylene bag until it was used for screening.

Extraction procedure

The powdered material (500 g) was successively extracted with n-Hexane, Chloroform and Ethanol: Water (1:1) each by maceration with occasional shaking at room temperature for 72 hrs. The n-Hexane extract **H**, chloroform extract **C**, and Ethanol: Water (1:1) extract **E** were concentrated after combining the filtrate and kept in desiccator for further investigation.

Table: 1 Percentage yield of extracts

S. No.	Extract	% Yield
1	H	0.01%
2	C	0.17%
3	E	2.8%

Preliminary Phytochemical Screening

The extracts **H**, **C** and **E** of green alga *Ulva lactuca* obtained by maceration were analyzed by qualitative methods for the presence of alkaloids, carbohydrates, flavonoids, phenol, steroids, proteins, amino acids, glycosides and tannins.

Table: 2 Preliminary Phytochemical Screening of Extracts

S. No.	Chemical class	Test	Extracts		
			Extract H	Extract C	Extract E
1	Carbohydrates	Molisch's test	+	+	+
		Fehling's test	-	+	-
2	Proteins	Biuret test	-	-	-
3	Amino acids	Ninhydrin test	-	-	-
4	Steroids	Salkowski test	+	+	-
		Liebermann-Burchard test	+	+	-
5	Alkaloids	Dragendorff's test	-	-	-
		Wagner's test	-	-	-

6	Glycosides	Legal's test	-	+	+
		Keller-Killiani test	-	+	+
7	Flavonoids	NaOH test	-	-	-

Column Chromatography

Silica gel (60-120 mesh) was used as adsorbent for column chromatography. The column was taken and packed with cotton at the bottom of the column. The slurry was prepared using silica gel and n-hexane solvent to a suspension of free flowing consistency. It was poured slowly from the top of the column apparatus in little quantities slowly allowing for the even and uniform packing. 2/3rd of the column was packed by using the above procedure. The extract C was dissolved in minimum quantity of n-hexane and chromatographed over silica gel. It was then eluted with the solvents of increasing polarity in the order of cyclohexane:ethyl acetate (30:1) **1**; cyclohexane:ethyl acetate (25:1) **2**; cyclohexane:ethyl acetate (20:1) **3**; cyclohexane:ethyl acetate (15:1) **4**; cyclohexane:ethyl acetate (10:1) **5**; cyclohexane:ethyl acetate (5:1) **6**; cyclohexane:ethyl acetate (3:1) **7**; and cyclohexane:ethyl acetate (2:1) **8**. These elutes were monitored by TLC.

Table: 3 Percentage yields of fractions

S. No.	Fraction	% Yield
1	1	5.6
2	2	3.2
3	3	3.6
4	4	3.4
5	5	4
6	6	2.8
7	7	2.4
8	8	2.6

Pharmacological Evaluation

Antibacterial and Antifungal activity [17]

Gram positive bacteria- *Staphylococcus aureus subsp. aureus* (MTCC 737) (S.A.) and *Staphylococcus epidermidis* (MTCC 3615) (S.E.). **Gram negative bacteria-** *Escherichia coli* (MTCC 1687) (E.C.) and *Pseudomonas aeruginosa* (MTCC 424) (P.A.). **Fungi-** *Candida albicans* (MTCC 183) (C.A.) and *Aspergillus niger* (MTCC 228) (A.N.).

Table: 4 Antibacterial activity of extracts

S.No.	Extract	Zone of inhibition (in mm)			
		S. A.	S. E.	P. A.	E. C.
1	H	10	11	11	12
2	C	11	12	10	11
3	E	8	9	7	7
4	Ampicillin	10	11	11	11

Table: 5 Antifungal activity of extracts

S.No.	Extract	Zone of inhibition (in mm)	
		A. N.	C. A.
1	H	12	11
2	C	12	12
3	E	8	9
4	Clotrimazole	20	18

Table: 6 Antibacterial activity of fractions

S. No.	Fraction	Zone of inhibition (in mm)			
		S. A.	S. E.	P. A.	E. C.
1	1	13	14	7	7
2	2	7	8	7	6
3	3	13	14	13	13
4	4	8	6	13	14
5	5	7	8	13	13
6	6	7	6	13	14
7	7	8	7	6	7
8	8	12	13	7	6
9	Ampicillin	10	11	11	11

Table: 7 Antifungal activity of fractions

S. No.	Fraction	Zone of inhibition (in mm)	
		A. N.	C. A.
1	1	17	13

2	2	7	7
3	3	8	7
4	4	9	8
5	5	17	12
6	6	18	13
7	7	7	8
8	8	7	6
9	Clotrimazole	20	18

Results and Discussion

Because of the biological activities exhibited by *Ulva Lactuca Linn.*, present study was undertaken wherein different compounds have been extracted, isolated from the green algae and subjected to antimicrobial activity.

Review of literature has shown that the green algae *Ulva Lactuca Linn.*, possess antimicrobial activity. In the view of this, an effort was made to check the isolated compounds from the algae for their anti-microbial activity. The inhibition of microorganisms under standardized conditions was utilized to demonstrate microbial action of the compounds.

For present work efficacy of eight compounds were detected against *S.aureus*, *S.epidermidis*, *P.aeruginosa*, and *E.coli*. The concentration of the test compound used was 1mg/ml. and Ampicillin was taken as the standard drug. From the tests carried out on the different extracts and fractions. n-hexane and chloroform extract have been found to have comparable activity to that of the standard drugs. The results are presented graphically in Chart 1 and Chart 2.

Chart1: Antibacterial activity of *Ulva lactuca* extract

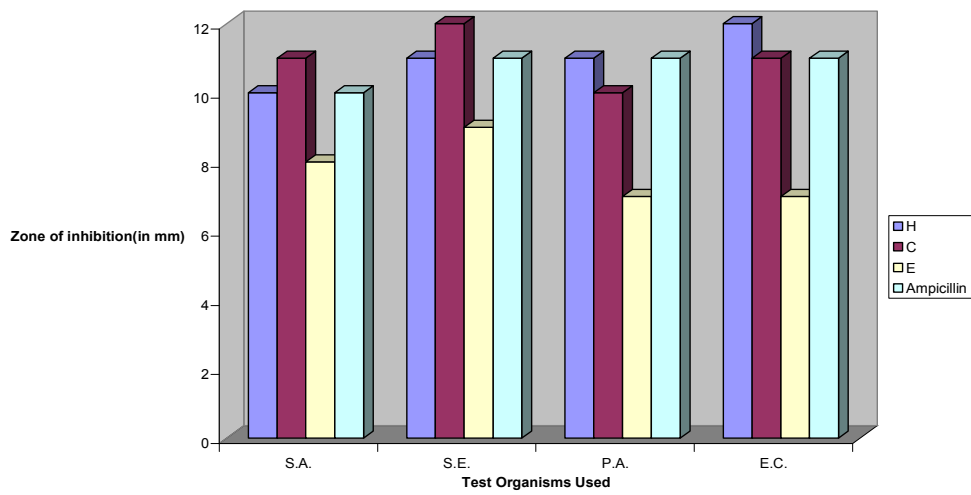
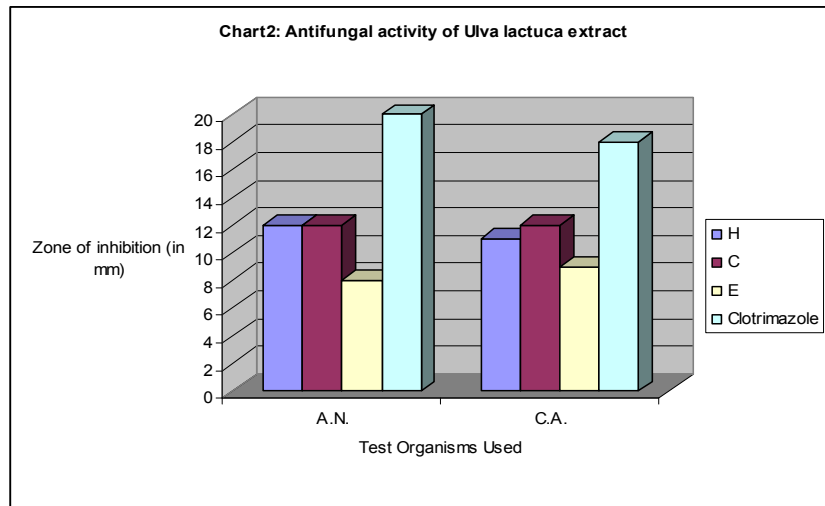


Chart2: Antifungal activity of *Ulva lactuca* extract



Similarly fractions 1, 3 and 8 have significant activity against *S. aureus*. And Fractions 1, 3, 8 have remarkable against *S. epidermidis* while Fractions 3, 4, 5, 6 have been found to be more potent against *P. Aeruginosa* and *E. Coli* when compared to Ampicillin. And fractions 1, 5, 6 have comparable activity against *Aspergillus Niger* and *Candida Albicans* when compared to the standard drug, Clotrimazole. The results are presented graphically in Chart 3 and Chart 4.

Chart3: Antibacterial activity of *Ulva lactuca* fractions

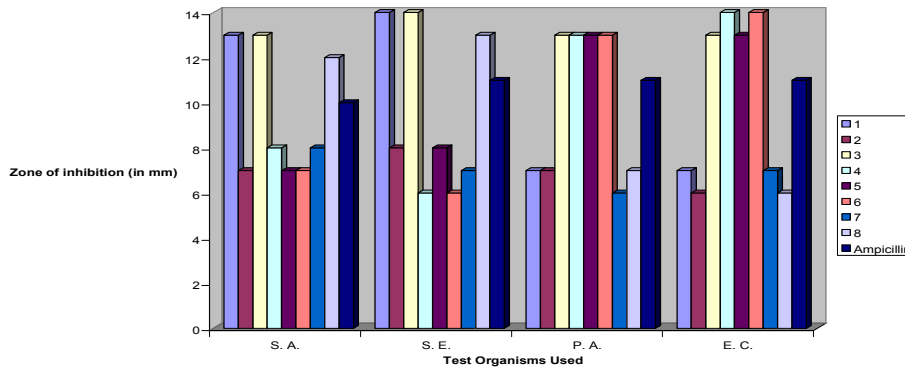
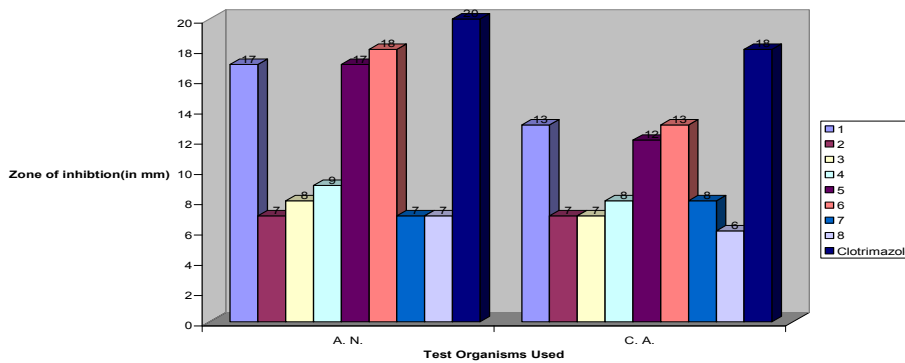


Chart4: Antifungal activity of *Ulva lactuca* fractions



Conclusion

It can be concluded that the different extracts and fractions show moderate to significant antimicrobial activity against the strains used. Active constituents were carbohydrates, steroids and glycosides found in the extracts of n-hexane, chloroform, and water: ethanol may be responsible for antimicrobial activity.

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References

1. Awad, N. E. (2000). Biologically active steroid from the green alga *Ulva lactuca*. *Phytother. Res.* 14: 641-643.
2. Oranday MA, MJ Verde, SJ Martínez-Lozano, NH Waksman, Active fractions from four species of marine algae, *International Journal of Experimental Botany*,2004, 165-70
3. In Hae kim, Dong-Gun Lee, Sang Hyun Lee, Jong-myung Ha, Bae-Jin Ha, Sung-Koo Kim, Jae-Hwa Lee, Antibacterial activity of *Ulva lactuca* against Methicillin-resistant *Staphylococcus aureus* (MRSA), *Biotechnology and Bioprocess Engineering* 2007, 12: 579-582
4. Hanaa H. Abd El-Baky, Farouk K. El Baz and Gamal S. El-Baroty, Potential biological properties of sulphated polysaccharides extracted from the macroalgae *Ulva lactuca* L., *Academic Journal of Cancer Research*,2009.01-11
5. Hanaa H. Abd El-Baky,Farouk K. El Baz and Gamal S. El-Baroty, Evaluation of marine alga *Ulva lactuca* L. as a source of natural preservative ingredient, *Electronic journal of Environmental, Agriculture and Food Chemistry*
6. Arumugam Sathivel, Hanumantha Rao Balaji Raghavendran , Periasamy Srinivasan, Thiruvengadam Devaki, Anti-peroxidative and anti-hyperlipidemic nature of *Ulva lactuca* crude polysaccharide on D-Galactosamine induced hepatitis in rats, *Food and Chemical Toxicology*,2008,46,3262-3267

7. Sathivel, A., BalajiRaghavendran, H.R., Devaki, T., 2003. Hepatoprotective nature of sea weeds (*Ulva lactuca*/*Gracilaria edulis*) against liver injury induced by DGalactosamine/endotoxin in rats. *Seaweed. Res.* 25 (1–2), 109–111.
8. Rajeev Kumar Jha and Xu Zi-rong, *Biomedical Compounds from Marine organisms*, *Marine Drugs*, 2004, 123-146
9. Orhan, B. Sener, T. Atici, R. Brun, R. Perozzo, D. Tasdemir, Turkish freshwater and marine macrophyte extracts show in vitro antiprotozoal activity and inhibit FabI, a key enzyme of *Plasmodium falciparum* fatty acid biosynthesis, *Phytomedicine*, 2006, 13, 388-393
10. Marcia de Padua, Paulo Sergio Growoski Fontoura and Alvaro Luiz Mathias, Chemical Composition of *Ulvaria oxysperma* (Kützinger) Bliding, *Ulva lactuca* (Linnaeus) and *Ulva fasciata* (Delile), *Brazilian Archives of Biology and Technology*, Vol.47, March 2004, n. 1 : pp. 49-55,
11. Ivanova V, Rouseva R, Kolarova M, Serkedjieva J, Rache R & Monolova N, Isolation of a polysaccharide with antiviral effect from *Ulva lactuca*, *Prep. Biochem*, 24(1994) 83-97.
12. Lahaye M, Jegou D. Chemical and Physical–Chemical characteristics of dietary fibers from *Ulva lactuca* (L.) Thuret and *Enteromorpha compressa* (L.) Grév. *J Appl Phycol* 1993; 5:195–200.
13. Arieli, A; Sklan, D. and Kissil, G. (1993), A note on the nutritive value of *Ulva lactuca* for ruminants. *Animal Production*, 57: (2), 329-331.
14. R.Moo-Puc, D.Robledo, Y.Freile-Pelegrin, Evaluation of selected tropical seaweeds for in-vitro anti-trichomonal activity, *Journal of Ethnopharmacology*, 120(2008)92–97
15. J.Ortiz, N.Romero, P.Robert, et al., Dietary fiber, amino acid, fatty acid and tocopherol contents of the edible seaweeds *Ulva lactuca* and *Durvillaea antarctica*, *Food Chemistry* 99(2006)98–104
16. M.R.Ventura, J.I.R.Castanon), The nutritive value of seaweed *Ulva lactuca* for goats, *Small Ruminant Research* 29, 1998, 325–327s
17. Jr., E.C.S. Chan, Noel R. Krieg. *Antibiotics and Other Chemotherapeutic agents* In: Michael J. Pelczar, eds. *Microbiology*, 5th ed. Tata McGraw-Hill Publishing Company Limited, 2003: 510-539.