World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 4.223

EFFECT OF ALGAL EXTRACTS ON HAEMATOLOGICAL AND BIOCHEMICAL CHANGES IN RABBITS

Ms. S. Gokila[1], Dr. B. Bharathi[2]* and Dr.R.Dominic Sahaya Rajan[3]

[1]Lecturer, Dept. of Biochemistry, MMM College of Health Sciences, Chennai 600 037.
[2]Senior Lecturer, Dept. of Microbiology, MMM College of Health Sciences, Chennai 600 037.
[3]Principal, Matha College of Arts and Sciences, Manamadurai.

***Corresponding Author: Dr. B. Bharathi**
Senior Lecturer, Dept. of Microbiology, MMM College of Health Sciences, Chennai 600 037.

Article Received on 21/02/2017 **Article Revised on 15/03/2017** **Article Accepted on 05/04/2017**

ABSTRACT

In the present study, four seaweeds were selected to investigate their bacterial activity againt six bacterial strains and the effect of these seaweed extracts on hematological and biochemical changes in rabbits were studied. Three solvents such as methanol, ethanol and water were used successively for extraction of seaweeds. The bacterial screening wsa done with six bacterial strains containing both gram positive and gram negative strains as test microorganisms. It was concluded that methnol extracts of seaweeds showed highest antibacterial activity than ethanol extracts. Here it reveals that the extracts of *Enteromorpha flexuosa* have high antibacterial activity. The extracts by water have no bacterial activity in all the test plants. The present investigation suggest that the crude extact of *Enteromorpha flexuosa*, *Ulva lactuca, Gracilaria Corticata, Spatogossum asperum* have some significant effect on haematological and biochemical parameters in rabbits.

KEYWORDS: Gram positive, Gram negative, *Enteromorpha flexuosa, Ulva lactuca, Gracilaria Corticata, Spatogossum asperum.*

INTRODUCTION

Modern medicine is dependent on chemotherapeutic agents that are used to treat infectious diseases by way of destroying the pathogenic microorganisms. Most of these chemical agents are antibiotics, microbial products or their derivatives. A large portion of the world population, especially in developing countries depends on the traditional system of medicine for a variety of diseases (Wantanabe, 1967). Nowadays herbal drugs are prescribed widely for their effectiveness, minimal side effects in clinical experiences and relatively low cost.

Drug resistance is a growing public health threat with pathogenic microorganism that learn to cope with challenges posed by therapeutic agents. When antibiotics like pencillin were discovered some fifty years back they were considered as miracle therapeutic drugs. But these scene had suddenly changed and human beings are now confronted with new resistant types of bacteria (Prasad., 1996). Once bacteria have learnt a strategy to circumvent the toxic effects of an antibiotic they exchange the genetic information without any species specificity with other bacteria through episomes that carry the gene for resistance (Mc Cracken, 1980). As a result development of antibiotic resistance in infectious pathogens is observed to be a common phenomenon.

Medicinal and aromatic plants constitute a major source of natural organic compounds, which are widely used as medicine as reported by Caccamese *et al*., 1985. In this regard, Ayurveda being one of the oldest system of medicine which has descriptions of about a thousand plants for the treatment of curable diseases, owns the maximal responsibility as studied by Glombitza in 1996.

The world is now looking towards India for new drugs to manage various challenging diseases became of its rich biodiversity of medicinal plants and abundance of traditional know-how such as Sidda, Ayurveda etc., to cure different diseases. Hence, an ethnobotanical approach is being to search for new drugs. The rationale behind this approach is simple i.e., instead of testing plants at random only those, which are being used by traditional societies to cure their illness, will be tested. In otherwords one should try to take advantage of folk knowledge (Chapman, 1970).

Seaweeds are macroscopic algae which form an important component of the marine living resources. The marine algal resources of India are estimated to be 2,60,876 tonnes. Although all the seaweeds are beneficial to man in oneway or the other, only 49 species are used now as directly edible material or industrial raw

materials. The seaweeds are available largely in shallow waste waters wherever, there is a substratum on which they can grow and flourish (Fables *et al*., 1995).

Protein rich seaweeds such as species of Caulerpa, Porphyra, Gracilaria, Acanthophpra and Laurencia are used for human consumption. There is a practice along some coastal areas in India of utilizing seaweeds used to manure coconut plantations. Because of the severity of the present AIDS epidemic and debilitating effects of Herpes simplex and Epstein –Barr virus, it is becoming more important than ever to reexamine the antiviral immune modulatory effects of red marine algae (Hodgson, 1984).

The seaweed extracts also inhibit the growth of veterinary pathogen, viz. *Bacillus cereus* and *Listeria monocytogenes.* The following sea weeds were used in this study.

Ulva Lactuca
Division: Chlorophyta
Class: Chlorophyceae
Order: Ulvales
Family: Ulvaccae
Genus: Ulva
Species: Lactuca

Gracilaria Corticata
Division: Rhodophyta
Class: Rhodophyceae
Order: Gigartinales
Family: Gracilariaceae
Genus: Gracilaria
Species: Corticata

Enteromorpha Flexuosa
Division: Chlorophyta
Class: Chlorophyceae
Order: Siphonales
Family: Caulerpaceae
Genus: Enteromoropha
Species: Flexuosa

Spatoglossum Asperum
Division: Phaeophyta
Class: Phaephyceae
Order: Dicotyotales
Family: Dictyotaceae
Genus: Spatoglossum
Species: Asperum

Enteromorpha flexuosa occur both as free acids and as a part of mono,di and triglycerides showing antibacterial activity as studied by Mc Cracken *et al*., in 1980.

MATERIALS AND METHODS

Four different marine algae were selected for the study are
1.Ulva lactuca
2.Gracilaria corticata
3.Enteromorpha flexuosa
4.Spatoglossum asperum

Extraction of seaweeds (Arunkumar and Rengasamy, 2000):
1. The collected seaweed samples were thoroughly washed to remove the adherents and air dried for 24 hrs.
2. The different parts were separated, pooled, macerated and extracted repeatedly with the solvent methods, ethanol and water separately.
3. The extracts were cold sleeped overnight at -80^0 Celsius. Filtered with whatmann No.1 filter paperevaporated and concentrated. The crude extracts were used for antibacterial assay against human pathogens.

Collection of Bacterial Strains
The following microorganisms were collected from Majestic lab, Madurai.
1.Escherichia coli
2.Bacillus subtilis.
3.Vibrio species
4.Staphylococcus aureus
5.Salmonella typhimurium
6.Pseudomonas aeruginosa

Identification of organisms based on the microscopic characters
The shape of the bacteria was studied by Gram staining method (Gunasekaran *et al*., 1995). A loop of culture (24 hrs) was transferred to clean microscopic slide and smear was prepared. Then the slide was treated with the solution of basic dye (crystal violet) for 1 minute. The smears were then covered with grams iodine solution for 1 minute and rinsed. After destaining with 95% ethanol for 30 seconds and a preparations were rinsed in the tap water and counterstained for 12 seconds with a saffranin solution. The stained preparations were rinsed with tap water, dried and examined under the light microscope.

Identification of the organism done by using the biochemical tests namely Catalase test, Oxidase test, Indole test, Citrate utilization test, Methyl red test, Voges-Proskaver Test (Kanai Mukherjee, 1984).

Antibacterial Assay
Antibacterial assay was carried out by the standard disk diffusion method Kirby – Bauer method (Baba, 1988).

RESULTS

The microorganisms collected from Majestic lab were identified and characterized by standard methods (Table1 and 2).

Antibacterial activity of seaweeds
The crude extacts prepared individually with methanol, ethanol and water showed various degrees of activity against pathogenic bacteria.

Enteromorpha flexuosa

Extract of *Enteromorpha flexuosa* was active against five bacterial strains. Methanol extract showed highet antibacterial activity against *E.coli* (16mm) and moderate activity against *S.aureus, Vibriosp, S.typhimurium* and *B.subtilis*. Ethanol extracts of *Enteromorpha flexuosa* showed highest antibacterial activity against *Vibrio sp* and *S.aureus* (8mm) and moderate activity against *E.coli, S.typhimurium* and *B.subtilis. P.aeruginosa* was resistant against all three types of extracts (Table 3).

Ulva lactuca

Methanol extract of *Ulva lactuca* showed highest antibacterial activity against *E.coli* and moderate activity against *S.aureus.* Ethanol extract showed highest antibacterial activity against *E.coli* moderate activity againt *S.aureus. B.subtilis, Vibrio sp, S.typhimurium, P.aerunginosa* was resistant against all three type of extracts (Table 4).

Gracilaria corticata

Methonol extract of *Gracilaria corticata* showed highest antibacterial activity against E.coli and moderate activity against *B.subtilis*. Ethanol extract showed highest antibacterial activity against *S.aureus, E.coli, S.aureus. B.subtilis, Vibrio sp, S.typhimurium, P.aerunginosa* were resistant against all three type of extracts (Table 5).

Spatoglossum asperum

Methanol extracts of *Spatoglossum asperum* showed highest antibacterial activity against Vibrio sp., and moderate activity against E.coli and Ethanol extract showed highest antibacterial activity against Vibrio sp and moderate activity against *E.coli., B.subtilis, S,aureus, S,typhimurium, P.aerunginosa* was resistant against all three types of extracts. The test seaweeds were extracted by water have no antibacterial activity (Table 6).

Among four algal species tested, *Enteromorpha flexuosa* showed highest antibacterial activity when compared to all other algae studied.

Table 1: Identification of organisms based on microscopical characters.

Organism	Shape	Gram reaction
Escherichia Coli	Rod	Gram –ve
Bacillus subtilis	Rod	Gram +ve
Vibrio sp	Rod	Gram +ve
Staphylococcus aureus	Coccus	Gram +ve
Salmonella typhimurium	Rod	Gram –ve
Pseudomonas aeruginosa	Rod	Gram –ve

Table 2: Identification of organisms based on biochemical characters.

Organism	Oxidase Test	Catalase Test	Indole Test	Voges Proskauer Test	Methyl red Test	Citrate Test
Escherichia coli	+ve	+ve	+ve	-ve	+ve	-ve
Bacillus subtilis	-ve	+ve	-ve	+ve	-ve	-ve
Vibrio sp	-ve	+ve	-ve	+ve	-ve	+ve
Staphylococcus aureus	-ve	+ve	-ve	+ve	+ve	-ve
Salmonella typhimurium	-ve	-ve	+ve	+ve	-ve	-ve
Pseudomonas aeruginosa	+ve	+ve	-ve	-ve	-ve	+ve

Table 3: Antibacterial activity of *Enteromorpha flexuosa.*

SL no.	Human pathogens	*Enteromorpha flexuosa* Zone of inhibition (mm)		
		Methanol	Ethanol	Water
1	*Escherichia coli*	16	12	-
2	*Bacillus subtilis*	14	17	-
3	*Vibrio sp.,*	13	18	-
4	*Staphylococcus aureus*	12	18	-
5	*Salmonella typhimurium*	13	16	-
6	*Pseudomonas aeruginosa*	-	-	-

Table 4: Antibacterial activity of *Ulva lactuca.*

SL no.	Human pathogens	*Ulva lactuca*		
		Zone of inhibition (mm)		
		Methanol	Ethanol	Water
1	*Escherichia coli*	18	17	-
2	*Bacillus subtilis*	-	-	-
3	*Vibrio sp.,*	-	-	-
4	*Staphylococcus aureus*	16	12	-
5	*Salmonella typhimurium*	-	-	-
6	*Pseudomonas aeruginosa*	-	-	-

Table 5: Antibacterial activity of *Gracilaria corticatrta*.

SI No.	Human pathogens	*Gracilaria corticatrta*		
		Zone of inhibition (mm)		
		Methanol	Ethanol	Water
1	*Escherichia coli*	16	-	-
2	*Bacillus subtilis*	14	-	-
3	*Vibrio sp.,*	-	-	-
4	*Staphylococcus aureus*	-	11	-
5	*Salmonella typhimurium*	-	-	-
6	*Pseudomonas aeruginosa*	-	-	-

Table 6: Antibacterial activity of *Saptoglossum asperum*.

Sl No.	Human pathogens	*Saptoglossum asperum*		
		Zone of inhibition (mm)		
		Methanol	Ethanol	Water
1	*Escherichia coli*	11	12	-
2	*Bacillus subtilis*	-	-	-
3	*Vibrio sp.,*	12	13	-
4	*Staphylococcus aureus*	-	-	-
5	*Salmonella typhimurium*	-	-	-
6	*Pseudomonas aeruginosa*	-	-	-

DISCUSSION

The present study revealed the poor activity of water extract in all the test plant samples. This was in conformity with the earlier reports of Naqvi *et al*., 1981, Reichelt and Borowitzka, 1984. The extracts of Spatoglossum asperum did not show inhibitory effect on *B.subtilis, S.aureus, S.typhimurium* by Dobhal, 1994, Mareau *et al*., 1984 and Rao, 1991. Ballantine and Almodovar, 1977 reported moderate antibacterial activity in chlorophyceae against gram-positive bacteria.

From the studies, the green algae *Enteromorpha flexuosa* showed antimicrobial activity during all the months. This was supported by Rao and Parekh, 1981. Gram-positive bacteria were more effectively controlled by the extracts of the algae used than Gram-negative bacteria (Pereira, 1988) Similar observations indicating that the more susceptibility of Gram-positive bacteria to the algal extracts is due to the differences in their cell wall structure and the composition of the cell wall (Tripathy 1996, Paz *et al*., 1995). The principle strength of the active components depends on the use of a suitable solvent to extract it (Parekh *et al*.,1985), Hornesy and Hide,1985 reported that crude extracts of marine algal species showed inhibitory activity against pathogenic bacteria.

SUMMARY AND CONCLUSION

Marine algae provide a rich source of chemically diverse compounds that can be used to develop various therapeutic agents. Certain marine algae reported for their antibacterial activities. In the present study, methanol extracts of seaweeds showed highest antibacterial activity than ethanol extract reveals that the extracts of *Enteromorpha flexuosa* have high antibacterial activity. The inhibitory effect increased with the level of lipophilic extracts. The extracts by water have no antibacterial activity in all the test plants.

The absence of activity or moderate activity may also be attributed to the varying solubility behaviour if the compounds, variations in phytoconstituents, depending upon the ecological factors prevailing at the time of collection and the growth stage of the plants. Further, this activity seems to be seasonal and has been reported todecrease directly with plant vigour as a function of latitude (Conover and Sieburth, 1964, Naqvi *et al*., (1981). From this study, it is evident that the selected

seaweeds possess bioactive compounds active against human bacterial pathogens.

REFERENCES

1. Arunkumar K and Rengasamy R. Evaluation of bioactive potential of seaweeds occurring along the cost of Tamilnadu, India against plant pathogenic bacterium, *Xanthomonas oryzae* pv.Oryzaer (Ishiyama) *Dye. Bot. Mar*, 2000; 43:409-415.
2. Baba B. Mechanism of inhibitory effect of dextran sulfate and heparin in replication of human immune deficiency virus invitro, proc. *Nati acad. Sci*, 1988; 85: 6132-6136.
3. Ballantine, D.L and L.R Almodovar. Epipytisam and antibiotic activity of tropical marine algae in progress in marine research in the Caribbean and adjacent regions.2nd Symposium caracas, Venezuela, 1977; 23-30.
4. Caccamese, S., R. Azzolina, G. Furnari, M. Cormaci and S. Grasso. Antimicrobial and antiviral activities of some marine algae from Eastern Sicily. *Planta Med.*, 1985; 37: 333-339.
5. Chapman, V.J. Seaweeds and their uses. Methuen and Co. Ltd, London, 1970; 304.
6. Dobhal P. Medicinal and aromatic plants. *Curr. Science*, 1994; 71: 397-398.
7. Fables. C.L., A. Arias, M.C. Gil –Rodriguez, A. Hardission and A. Sierra Lopez. Invitro Study of antimicrobial activity in algae collected from the coast of tenerite. Anuariodel institute de Estudios Canarios, 1995; 34: 181-192.
8. Gunsekaran, S.P., Mc Carthy,P.J and Kelly-Borges,M., Hamacanthin A and B. New antifungal bis-indole alkaloids from the marine sponge Hamacantha sp. *J.Toxicol. Toxin rev*, 1995; 14: 136.
9. Hodgson,L.M. Antimicrobial and antineoplastic activity in some south Florida Seaweeds. *Bot Mar*, 1984; 28: 387-390.
10. Hornesy IS and Hide D. The production of antimicrobial compounds by British Marine algae. IV Variation of antimicrobial activity with algal generation. J. British phycological Society, 1985; 20: 21-25.
11. Kanai L.Mukherjee. Medical Laboratory technology, 1984; 385-415.
12. Mareau.J, Pesando.D,Caram B. Antifungal and Antibacterial screening of Dictyotales from the French Mediterranean coast.*Hydrobiologia*, 1984; 116/117: 521-524.
13. Mc Cracken, M.D., R.E. Middaugh and R.S.Middaugh. A chemical characterization of an algal inhibitor obtained from chlamydomonas. *Hydrobiologia*, 1980; 70: 271-276.
14. Naqvi,S.W.A., Solimbi,S.Y. Kamat,L. Fernandas, C.V.G. Reddy, D.S.Bakuni and B.N.Dhawan. Screening of some marine bplants from the Indian coast for biological activity. *Bot. Mar.*, 1981; 24: 51-55.
15. Paz EA, Lacy RN and Bakhtian M. The β-Lactum antibiotics penicillin and cephalosporin in perspective. Hodder strong, London, 1995; 324.
16. Pereira P. Screening of marine algae from Brazilian Northeastern Coast for antimicrobial activity. *Bot. Mar*, 1988; 31: 375- 377.
17. Prasad P. 1996.Multi drug Resistance. An emerging threat. School of Life Sciences, Jawaharlal Nehru University. New Delhi-110067, India.
18. Rao S. 1991. Bioactivity in Marine Algae. In: Bioactive compounds in marine organisms with Emphasis on the Indian Ocean. An Indo-United states symposium, Thompson, M.F., R.Sarojini and R.Nagabhushanam (Eds) Oxford and IBH pub.co., New Delhi,ISBN-10:8120405749,PP:373-377.
19. Rao P.S.Parekh,K.S. .Antibacterial activity of Indian seaweed extracts. *Bot. Mar*, 1981; 24: 577-582.
20. Riechelt,J.L., and Borowitzka,M.A. Antimicrobial activity from Marine algae: Results of a large scale screening Programme.*Hydrobiologia*, 1984; 116/117: 158-168.
21. Tripathy,Y.B. Research Strategy and Ayurveda. *Current Science*, 1996; 73:418.
22. Wantanabe W. A general description of the mechanism of infectious drug resistance. National institute of Genetics, Misima, Shizuoka-Ken, 411, Japan. Chromosomal Polymorphisms in natural populations of Drosophila Melanogaster. *Mem. Fac.Sci.Kyushu Univ. Ser.E (Biol)*, 1967; 4:159-178.