Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Asian Journal of Plant Science and Research, 2014, 4(6):36-40

ISSN : 2249-7412
CODEN (USA): AJPSKY

Antimicrobial activities of *Turbinaria conoides* (J. Agardh) Kutzing and *Marsilea quadrifolia* Linn.

Poonam Sethi

Guru Nanak College, Chennai, India

ABSTRACT

Turbinaria conoides (J. Agardh) Kutzing ,(Phaeophyceae) a marine seaweed, and Marsilea quadrifolia Linn. (Marsileaceae) an aquatic fern, were tested for activity against Tobacco necrotic virus (TNV) and bacteria (antimicrobial). Local lesion method was adopted and half leaf comparisons were made in cotyledonary leaves of Cyamopsis tetragonoloba Taub.(Linn.). Extracts of the experimental plants were prepared with water , methanol and toluene: methanol (2:1 v/v). Various concentrations were tested and the toluene : methanol extract of Turbinaria exhibited good activity at a concentration of 1000 µg/mL when compared to Marsilea. These results clearly depict the potential of the algae against plant virus when compared to the aquatic fern. Contradictory to the antiviral activity the extracts of Marsilea showed good antibacterial activity and produced the zone of inhibition of 32mm. The aqueous leaf extract showed maximum zone of inhibition at 300 µg/ml against Pseudomonas fluorescens. The present study clearly indicates that M. quadrifolia had a profound antimicrobial activity and it may be useful in the treatment of various infectious bacterial diseases. Thus both the plants of aquatic habitat have different activities against microbes.

Key words: Antiviral, Cyamopsis ,Gram negative bacteria, Marsilea, Turbinaria, Tobacco necrotic virus**.**

INTRODUCTION

The recent increase in compounds isolated from land plants, has open doors to the poorly exploited marine ecosystem which appears to be a good candidate of natural resource [1].The aquatic ecosystem covers about 70 % of the earth's surface and India has a vast coastline of 6100 km supporting a rich flora of marine plants such as seaweeds, mangroves and sea grasses [2]. As a part of our ongoing research a natural source providing mankind with various secondary metabolites having activity against viruses, which provides a new way for control of plant virus diseases. Marine algae exhibit interesting nutritional properties in addition to their ecological properties. The results of the study suggest that the algae which are abundantly available in this ecosystem also have considerable potential of carbohydrates, amino acids, proteins, phenols and lipids for their use as food and pharmaceutical industry as a source in preparation of nutrient supplements, medicine and fine chemical synthesis. As suggested by [3] the efficacy of lipid extracts was tested hence toluene :methanol extracts were used.

Ferns form a major division of the Plant Kingdom also known as Pteridophyta ,it has been observed that pteridophytes are not infected by microbial pathogens which may be one of the important factors for the evolutionary success and the fact that they survived for more than 350 million years. As per folk medicine, the ayurvedic systems of medicine recommended the medicinal use of the ferns it showed bioactivity properties such as antimicrobial, anti-inflammatory, antitussive, antitumor, etc. But a very little research has been carried out on the

evaluation of bioactive properties of pteridophytes. Hence an attempt has been made to evaluate the antimicrobial properties of Marsilea.

MATERIALS AND METHODS

a)Experimental plant

Turbinaria conoides (J. Agardh) Kutzing a marine brown alga was collected from shores of Rameswaram, India during the month of March. Both the plants were identified, authenticated by Dr. R. Thevanathan, Presidency College, Chennai.[Fig.1] *Marsilea quadrifolia* Linn. is a pteridophyte belonging to Family Marsileaceae commonly known as European water clover, resembles a four leaved clover plant shows differentiation into stem, leaves and roots. *Sushni* in parts of India; "aalaik keerai" in Tamil also enlisted in the Red Data Book of the International Union for Conservation of Nature (IUCN) [4].It is also eaten by various tribal communities such as Kadars, Pulaiyars, Malasars, Malaim. Common in rice fields.

FIGURE 1: Experimental plants

Turbinaria conoides **(J. Agardh) Kutzing**

Marsilea *Quadrifolia* Linn.

b)Microbes

Local strain of the TNV (Tobacco Necrosis Virus) were obtained from Madurai Kamaraj University, Tamil Nadu. Seeds of *Cyamopsis tetragonoloba* Taub.(Linn.) var. Navbahar were purchased from a government certified seed centre and surface sterilized with 0.1% acidified mercuric chloride for 3 to 5 minutes. The seeds were then sown in sterilized soil and grown in earthen pots.

In vitro antimicrobial activity was examined for the methanolic extracts of Marsilea against four bacterial species , the gram negative strains *Salmonella typhi* (ATCC 00215), *Pseudomonas fluorescens* (ATCC 06341), *Pseudomonas aeruginosa* (ATCC 02150) and *Escherichia coli* (ATCC 10263) were obtained from Regional Research Institute of Unani, Chennai.

c)Preparation of extracts

Both the experimental plants were shade dried separately and ten grams of each was extracted with 100 mL of the solvents double distilled water, methanol and toluene : methanol, 2:1 v/v and later kept in a shaker for 48 hours. The three extracts were then filtered through Whatman No.1 filter paper and dried in an oven at 40^{o}C. The residues obtained were then weighed and stored at 4^{o}C and used for the assay.

d)Methodology

The procedures outlined by [5] were followed in the assay. The inoculum was prepared by grinding 1.0 g of virus-

infected cotyledonary leaves , celite was added as an abrasive at the rate of 2 mg/ mL. Inoculation was carried out by rubbing the leaves from the base of the leaf towards the tip with the forefinger previously dipped in the inoculum. The readings were taken on the basis of dark green spots of 1 mm diameter which developed as a result of infection through inoculation were observed at every 24 hour interval. The intensity of the viral infection was scored after 72 hours based on the number of lesions formed.

The extract residues of the experimental algae were weighed and dissolved in 0.25% DMSO (Dimethyl sulphoxide) to obtain 1.0 mg/1.0 mL concentrations. From this solution, 1 : 10 water dilutions were made and the inhibitory effect of these solutions were tested against tobacco necrosis virus (TNV) on the cluster bean plant, *Cyamopsis tetragonoloba.*

Inoculations were performed on 9-day old cotyledonary leaves all the tests were made in replicates of ten, and half-leaf comparisons were made. The experimental half-leaves were inoculated with known concentrations of the extract and virus suspension. The plants were monitored for 72 hours and the number of local lesions induced by TNV was estimated using the following formula

inhibition = A – B / A x 100

where, control-A , experimental-B

Inhibition is based on the reduction of the number of local lesions induced by TNV, in the absence (A) or the presence (B) of the algal extract.

The testing of antibacterial activity of Marsilea extracts was carried out *invitro* by Kirby-Bauer disc diffusion technique [6]. Culture of bacteria was made on Muller Hinton agar plates. Sterile paper discs 5mm diameter (Himedia) were placed over the plate at an equidistant position. The discs were loaded with 10 µl of the drug at the concentration of 100 µl/ml, 150 µl/ml, 200 µl/ml, 250 µl/ml and 300 µl/ml. DMSO was used as solvent. Separate control disc was also included using the solvent. Ciprofloxacin was used as standard for comparison. The plates were incubated at 37°C for 24 hours. The microbial growth was determined by measuring the diameter of Zone of inhibition.

Minimum inhibitory concentration (MIC) is the lowest of concentration of an antimicrobial that will inhibit the visible growth of the microorganism after overnight incubation. The MIC is determined by agar dilution method [7]. The test were performed at four concentration 60 µg/ml, 70 µg/ml, 80 µg/ml, 90 µg/ml and 100µg/ml employing the methanolic extract of the plant.

Results were analysed for statistical significance using SPSS (Statistical Package for Scientific Studies) programme.

RESULTS AND DISCUSSION

Antiviral assay showed extracts of the experimental algae with water, methanol were less effective than toluene: methanol (2:1 v/v) The effect of the extract residues on TNV are given in Table1. At a concentration of 1000 µg/mL, the toluene: methanol extract residue of *Turbinaria* was able to reduce the lesion formation by nearly 64%. At a concentration of 1.0 µg/mL, the active fraction of toluene: methanol extract residue , could reduce the formation of lesions by nearly 44% . Increasing the concentration of the residue from 1.0 µg/mL to 10 µg/mL and 100 µg/mL, the percent reduction in lesion formation by TNV was enhanced by 14% respectively However, at a concentration of 1000 µg/mL, the reduction in lesion formation was 64%.

The antibacterial activity of methanolic extract of Marsilea against the four pathogenic bacteria *Salmonella typhi, Pseudomonas fluorescens, Pseudomonas aeruginosa* and *Escherichia coli* were assessed by zone of inhibition. The results are shown in Table-2. All the microbes used in the present study were sensitive to the methanolic extract of the plant and showed a potential activity. Maximum activity was seen in case of *Pseudomonas fluorescens* where the zone diameter was 32 mm (300µg/ml).

The minimum inhibitory concentration study revealed that the value for the bacteria *Salmonella typhi and Escherichia coli* as 80 µg/ml and 60 µg/ml for *Pseudomonas fluorescens* and *Pseudomonas aeruginosa*.

Table 1: Effect of the Crude Extract Residue of the Experimental Algae on Lesion Formation by TNV on the Leaves of *Cyamopsis tetragonoloba*

Extract	Varying Concentrations of the Crude Extract (µg mL $^{-1}$)	Mean of Number of Lesions ±SE	P Value
Aqueous	Control	22.07± 0.92	0.000
	1	20.60± 0.77	0.070
	10	16.90 ± 0.91	0.000
	100	10.60 ± 0.24	0.000
	1000	10.90 ± 0.45	0.000
Methanol	Control	22.32 ± 0.05	0.000
	1	19.25 ± 0.45	0.003
	10	15.20 ± 0.10	0.000
	100	12.70 ± 0.29	0.000
	1000	11.40± 0.56	0.000
Toluene : Methanol, 2:1 V/V	Control	22.32 ± 0.05	0.003
	1	14.25 ± 0.45	0.000
	10	12.20 ± 0.10	0.000
	100	11.70 ± 0.29	0.000
	1000	0 9.40± 0.56	0.001

TABLE 2: Antibacterial activity of methanolic extract of marsilea

Bacteria	Zone of inhibition (in mm)					Ciprofloxacin (50µg/ml)
	100µg/ml	150µg/ml	200µg/ml	250µg/ml	300µg/ml	
Salmonella typhi	8±0.1	11±0.1	12±0.2	13±0.1	18±0.1	38±0.1
Pseudomonas fluorescens	10±0.2	17±0.1	21±0.1	28±0.2	32±0.1	46±0.1
Pseudomonas aeruginosa	11±0.1	15±0.2	17±0.2	22±0.3	25±0.1	34±0.0
Escherichia coli	7±0.1	13±0.1	18±0.1	20±0.2	22±0.2	33±0.0

This *in vitro* study demonstrated that folk medicine can be as effective as modern medicine to combat pathogenic microorganism. The antibacterial activity of Marsilea would help for development of a new alternative medicine system which has no side effects. The anti-inflamatory activity of this experimental plant also opens doors for further research[8]. This study serves as a baseline in identification of new medicinal plant and further investigation on the same may yield new compounds of medicinal importance for specific microbial disease.

Presently there is an increasing interest worldwide on herbal medicines accompanied by increased laboratory investigation and pharmacological properties of the bioactive ingredients and their ability to treat various diseases . Bacteria cause serious infection in humans as well as other animals. The rapid spread of bacteria expressing multidrug resistance (MDR) has necessitated the discovery of new antibacterials and resistance – modifying agents. There are many studies suggested that the necessity of developing alternative antimicrobial drugs [9]. Antimicrobials from plant source would be an excellent choice due to no side effects. A number of infectious agents are becoming more resistant to commercial antimicrobial compounds. So, there is a needful to develop new drugs, which requires varied strategies among them the secondary metabolites produced by medicinal plants are more important. According to World Health Organization (WHO) medicinal plants would be the best source to obtain a variety of drugs in developed countries.

Compared with Gram-positive bacteria, Gram-negative bacteria are more resistant against antibiotics, because of their impenetrable wall. In the present study M. quadrifolia leaf extracts have showed effective inhibition against gram negative bacteria such as *Klebsiella pneumonia*, *Escherichia coli* and *Pseudomonas fluorescens* when compared to gram positive bacteria like *Bacillus subtilis* and *Streptococcus pyogenes*. These results are in accordance with the previous results of [10]in that M. quadrifolia revealed profound antibacterial, cytotoxic and antioxidant effects. In this study, the antibacterial activity clearly showed that the leaf extract of M. quadrifolia was specific in action against the growth of pathogenic bacteria.

In conclusion, the M. quadrifolia will be used to identify the new potent antimicrobial agents in future and it will also be useful to treat infectious diseases caused by microorganism.

Organic extracts obtained from the brown-alga *Lessonia trabeculata* inhibited bacterial growth and reduced both the number and size of the necrotic lesion in tomato leaves following infection with *Botrytis cinerea* whereas

aqueous and ethanolic extracts from the red-alga *Gracillaria chilensis* prevent the growth of *Phytophthora cinnamomi*, showing a response which depends on doses and collecting-time[11] Similarly, aqueous and ethanolic extracts from the brown-alga *Durvillaea antarctica* were able to diminish the damage caused by tobacco mosaic virus (TMV) in tobacco leaves [12] but not much work has been done on *Turbinaria* a brown alga against microbes hence this report with the world's worst invasive species being highly active against necrotic virus was the first one. Thus it is a promising antiphytoviral agent.

Recently marine sources are receiving much attention mainly because of functional ingredients such as polyunsaturated acids, carotene and their pigment carotenoids, sulphated polysaccharide and sterol. Among different compounds with functional properties, anti oxidants and antibacterials are mostly widely studied.

Hence both the experimental plants are promising antimicrobials and further research on isolation of activated compounds can be further carried, both plants though being from aquatic environment behaved differently invitro studies yet active against bacteria and plant virus.

REFERENCES

[1] Baker, J. *Hydrobiologia* **1984** 116/117: 29-40.
[2] Chapman, V. J. and Chapman, D. J. (eds). Seaweeds and their uses. 2nd ed. Chapman & Hall New York ,**1980**.
[3] Jamieson, G. R. and Reid, E. H. *Phytochemistry* **1972** 11: 1423 – 1432.
[4] Tuba, Z., Overview of the flora and vegetation of the Hungarian Bodrogköz. Tiscia, **1995**, 29: 11-17.
[5] Caccamese, S., Azzolina, R., Furnari, G., Cormaci, M. and Grasso, S. *Bot. Mar*.**1981** 24: 365 – 367.
[6] Bauer, A. W., Kirby, W. M. M., Sherrio. J. O., Turck, M. *Americal Journal of clinical pathology* **1996**; 45: 493-496.
[7] Bennet, J. V., Brodie, J. L., Benner, E. J., Kirby, W. M. M. *Applied Microbiology*. **1996**, 14:170-177.
[8] Lin, J., Opak War, and Geheeb-Keller, M. *Journal of Ethnopharmacology*. **2008**, 68: 267–274.
[9] Evans, C. E., Banso, A., and Samuel, O. A. *Journal of Ethnopharmacology*. **2002**, 80: 21–24.
[10] Ripa, F.A., Nahar, L., Haque, M., Islam, M.M., *European Journal of Scientific Research*, **2009**, 33. (1): 123-129.
[11] Edra Jiménez , Fernando Dorta , Cristian Medina, Alberto Ramírez , Ingrid Ramírez and Hugo Peña-Cortés. *Mar*. *Drugs*. **2011,** 9, 739-756.
[12] Pardee, K. I., Ellis, P., Bouthillier, M .and Towers, G. H.N. C. J . *Canadian Journal of Botany* **2004**,82(3): 304-309.