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In-Vitro Antibacterial Activity of Extract of *Ulva reticulata* Grown Fruit of *Cyamopsis tetragonoloba* (L.) Taub.

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ABSTRACT

The aim of this research was to evaluate the antibacterial activity of the extract of *Ulva reticulata* grown fruit of *Cyamopsis tetragonoloba* against human pathogens. The methanolic extracts of *Ulva reticulata* grown fruit of *Cyamopsis tetragonoloba* against *Escherichia coli*, *Bacillus subtilis*, *Proteus vulgaris*, *Serratia marcescens*, *Streptococcus pyogenes*, *Salmonella typhi* and *Klebsiella pneumoniae*. The *in vitro* antimicrobial activity was performed by Disc diffusion assay. The streptomycin was used as the control. The results showed that fruit extract of plants grown under normal growth condition without seaweed extract (SWE) supplement exhibited comparatively lesser antagonistic effect against all the pathogens studied. From the results, it is concluded that SWE grown *C. tetragonoloba* fruits hold a tremendous potential to serve as a source of newer, effective, safer and antimicrobials rich vegetable.

INTRODUCTION

Over the years, the cases of infectious disease had been elevating in an alarming rate, despite the rapid advancement in the medical field. Infections caused by bacteria can be prevented, managed and treated through anti-bacterial group of compounds known as antibiotics [1]. Antibiotics are natural, semi-synthetic or synthetic compounds that kill or inhibit the growth of bacteria. The natural products are found to be more effective with least side effects as compared to commercial antibiotics. So they are used as an alternative remedy for treatment of various infections [2]. 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 [3]. 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 seagrasses [4]. Seaweeds provide a rich source of structurally diverse secondary metabolites. Some secondary metabolites derived from seaweeds have the potency to be the new material for pharmacy [5]. Chemical compounds contained are the groups of polysaccharides, lipids, proteins, alkaloids and phenolic components [6]. Several recent studies have been revealed that seaweeds are potential sources that can be used as antimicrobial products [7, 8]. Seaweeds are exploited mainly for the industrial production of phycocolloids such as agar-agar, alginate and carrageenan, not for health aspects. Biostimulant properties of seaweeds are explored for use in agriculture and the antimicrobial activities for the development of novel antibiotics [9]. The present study is aimed to find out the antibacterial activity of extract of *U. reticulata* grown fruit of *C. tetragonoloba* against human pathogens.

MATERIALS AND METHODS

Collection of seaweed

Green alga (*U. reticulata* Forsskal) was collected during low tide, at Hare Island, Thoothukudi from November 2014 to February 2015. The sample was washed thoroughly with seawater followed by fresh water to remove sand particles and macroscopic epiphytes. After draining, the seaweed was shade-dried, powdered, sieved and used for the preparation of seaweed concentrate.

Preparation of seaweed liquid fertilizer for foliar application

Seaweed extracts (SWEs) were prepared by adopting the method of [10] with certain modifications. About 20g dried seaweed powder with 200ml distilled water was heated to 60ºC and maintained at the temperature for 24 hr in a hot air oven. The extract was filtered and then centrifuged at 10000 rpm to remove suspended impurities. The filtrate was stored in airtight bottles at 4ºC (100% seaweed concentrate) for further use.

Experimental design

A pot culture experiment was conducted during February to April 2015 at Plant Research Centre, St. Mary's College Campus, Thoothukudi, Tamil Nadu, India. The pots were filled with 3kg of garden soil. 20 seeds of *C. tetragonoloba* were sown in each pot. After the emergence of seedlings, they were thinned to ten plants per pot and allowed to grow up to fruiting stage. Weeding and watering were done at regular intervals. 1% SWE was applied as foliar spray (along with 100ml of distilled water in the ratio of 1: 100) after expansion of first leaf and was continued till fruiting stage. Enough replicates were maintained.

Antibacterial activity-Disc Diffusion Assay

Antibacterial activity of fruit extract of *C. tetragonoloba* was analysed [11] using human pathogens viz., *Escherichia coli* (MTCC 448), *Bacillus subtilis* (MTCC 2394), *Proteus vulgaris* (MTCC 426), *Serratia marcescens* (MTCC 2387), *Streptococcus pyogenes* (MTCC 7405), *Salmonella typhi* (MTCC 733) and *Klebsiella pneumoniae* (MTCC 109) obtained from the Department of Microbiology; St. Mary's College (Autonomous), Thoothukudi. Each bacterial pathogen was sub-cultured in agar medium and maintained. The spread plates were prepared by proper concentration of inocula. Sterile filter paper (Whatman No. l) discs (6 mm) were impregnated with fruit extracts (10 mg/ml) to give final concentration of 0.1 mg/disc and dried aseptically. The impregnated discs were placed on pre inoculated agar medium and incubated at 37°C. Streptomycin disc (100 µg/ml) was used as positive control, whereas methanol was used as negative control. After 24 - 48 hrs of incubation, the diameter of the inhibition zone was measured. All the assays were carried out in triplicates.

Plate 1. *In vitro* antibacterial activity of *C. tetragonoloba* fruit extract against human pathogens

Table 1. Antibacterial activity of *C. tetragonoloba* fruit extract

Name of the pathogens	Inhibition zone (mm)				
	a	b	c	d	e
B. subtilis	3.5±0.07	8.5±0.25	9.5±0.47	9.0±0.34	16.0±0.20
S. pyogenes	6.0±0.17	7.0±0.12	12.0±0.10	8.0±0.27	12.0±0.57
E. coli	4.0±0.09	11±0.41	10.0±0.17	9.5±0.12	11.0±0.45
P. vulgaris	-	9.0±0.25	10.0±0.23	8.0±0.42	14.0±0.62
K. pneumoniae	-	10.5±0.10	11.5±0.15	8.0±0.21	12.0±0.71
S. typhi	4.0±0.11	9.0±0.15	9.5±0.21	10.0±0.14	10.0±0.35
S. marcescens	7.0±0.21	11.0±0.29	7.5±0.32	9.5±0.25	10.0±0.77

Values are the mean of three replicates ±standard deviation. a, b, c and d represent antibacterial activity of methanolic fruit extract (10 mg/ml) of water (control), *U. reticulata*, *S. marginatum* and *K. alvaerezii* grown (treated) plants respectively. e = Streptomycin (100µg/ml)-positive control.

RESULTS AND DISCUSSION

Life threatening diseases and high mortality occur in animal and human population due to bacterial infection. Many bacteria both Gram positive and Gram negative contaminate food, water, air, soil, *etc*., and cause microbial pollution. *B. subtilis* is responsible for causing food borne gastroenteritis, *E. coli* causes diarrhoea and fever, *S. pyogenes* causes tonsillitis, rheumatic fever, *P. vulgaris* and *S. marcescens* cause wound infection and urinary tract infection, *S. typhi* causes typhoid fever and food poisoning and *K. pneumoniae* causes pneumonia and blood stream infection [12],[13],[14]. Now a days, use of antibiotics has increased significantly due to severe infection and resistance acquired by different pathogenic bacterial strains as a consequence of indiscriminate use of drugs, synthetic chemicals and antibiotics. Moreover the cost of drug is becoming high and also they cause adverse side effects on hosts including hypersensitivity and depletion of beneficial microbes. Food as medicine is regaining its popularity among people from all strata of society to get rid of from soaring price of life protecting drugs. Therefore empirical research on the antimicrobial property of *C. tetragonoloba* fruit extract would attain for a new perspective. The bacterial strains were sub-cultural before a day of use to maintain log phase. Agar disc diffusion assay was adopted on pre-seeded nutrient agar spread plates to determine the *in vitro* bactericidal activity. The antibacterial activity of fruit extracts were visualized as clear zone (inhibition zone) around the disc on agar plates. All the extracts were serially diluted and standardized with above said pathogens and found 10 mg/ml to be the effective concentration.

Antibacterial activity of *C. tetragonoloba* fruit extracts is represented in Table (1) and Plate (1). The data revealed that all the seaweed grown fruit extracts were active against all the pathogens tested. It was remarkable to note that SWE grown fruit extract inhibited the growth of *E. coli* and *S. typhi* apparently similar to Streptomycin at the concentration used in the study. Extract of *S. marginatum* treated *C. tetragonoloba* fruit extract interacted with *S. pyogenes* and resulted in growth inhibition (12 mm) as equal as streptomycin. Similarly, *K. pneumoniae* was arrested by *U. reticulata* and *S. marginatum* treated plant's-fruit extract. Bactericidal potential of seaweed (*U. reticulata*) grown fruit extract was greater than streptomycin. Fruit extract of plants grown under normal growth condition without seaweed extract supplement exhibited comparatively lesser antagonistic effect against all the pathogens studied. The results of the present study, is in agreement with [15],[16]. They found that fresh pod extract of *Cyamopsis tetragonoloba*, *Phaseolus vulgaris*, *Vicia faba* retarded the growth of *Escherichia coli* and *Bacillus subtilis*. Extract of *Spinacea oleracea*, *Cucurbita pepo* and *Brassica oleracea* were inhibitory against *Escherichia coli* and *Klebsiella pneumoniae* [17]. The strong antagonistic activities of *S. marginatum*, *U. reticulata* and *K. alvaerezii* grown *C. tetragonoloba* fruit extract could be related to their phenolic compounds and antioxidant activity.

CONCLUSION

From the results, it is concluded that SWE grown *C. tetragonoloba* fruits hold a tremendous potential to serve as a source of newer, effective, safer and antimicrobials rich vegetable.

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