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An *In Vitro* Antibacterial and Cytotoxic Potentials of Bioactive Metabolites Extracted from *Padina tetrastromatica*

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Abstract

Marine brown algae, *Padina tetrastromatica* was extracted using diverse organic solvents of increasing polarity such as diethyl ether, chloroform, acetone, dichloromethane: methanol and methanol. The obtained extracts were evaluated for antibacterial and brine shrimp cytotoxicity assays. It was found that methanolic extract of *P. tetrastromatica* exhibited the broadest and highest range of antibacterial activity. In cytotoxic activity, the methanolic extract of *P. tetrastromatica* showed significant activity ($LD_{50}=354.4 \mu\text{g/mL}$). Based on the present findings, it could be inferred that the bioassay guided purification and fractionation of crude methanolic extract of *P. tetrastromatica* may bring forth potent novel antibiotic and anticancer leads.

Keywords: Algal metabolites; Antimicrobial activity; Cytotoxic activity

Introduction

Naturally occurring antimicrobial agents were reported more than a century ago [1] mainly by Botanists [2]. According to the World Health Organization, plants should be the best source to obtain a variety of drugs [3]. Plant-based antimicrobial compounds continue to play an essential role in primary health care of about 80% of the world's population [4]. Investigation of antibiotic compounds from macroalgae has been determined during several hundred years [5,6]. Algae from varied locales have been evaluated for a range of biological activities such as antibacterial [7], antiviral [8] and antifungal activities [9]. Diverse algal species have shown to have bactericidal or bacteriostatic substances [10]. This paper deals with the details of the findings of antimicrobial and cytotoxic properties of secondary metabolites isolated from a marine algae, *P. tetrastromatica*.

Specimens of *P. tetrastromatica* was garnered from the intertidal and subtidal habitat of Thirumullavaram located in Kollam prefecture (08° 54' N and 76° 38' E). The extraneous matters were removed from the specimen, chopped into tiny pieces were stored at 2°C till its use. The fresh thallus material was extracted with different solvents of increasing polarity as described elsewhere [11]. Extracts were tested against a panel of clinical isolates such as *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Escherichia coli*. Antimicrobial assay was performed as per the methodology described by Manilal et al., [12]. Brine shrimp cytotoxicity of the methanolic extract of various concentrations (100 to 500 $\mu\text{g/ml}$) was evaluated after Manilal et al., [13].

Area of inhibition in millimetre square was used as an indication of antimicrobial activity. The organic extract of algae that produced zone of inhibition $\geq 28 \text{ mm}^2$ was considered as active. In the present study, methanolic extract from *P. tetrastromatica* exhibited the broadest and highest activity, which successfully inhibited all the tested bacterial pathogens in varied range. The bactericidal potential of *P. tetrastromatica* was high against *S. aureus* to the extent of $96.23 \pm 5.5 \text{ mm}^2$ followed by *M. luteus*, ($87.19 \pm 4.8 \text{ mm}^2$) and *E. faecalis* ($73.03 \pm 4.7 \text{ mm}^2$). The microbicidal activity produced against gram negative bacteria was lower and accounted for $41.63 \pm 4 \text{ mm}^2$, $39.63 \pm 4.05 \text{ mm}^2$ and $38.56 \pm 4.7 \text{ mm}^2$ respectively against *E. coli*, *P. aeruginosa* and *K. pneumoniae*. However, the area of inhibition zone varied according to the kinds and concentration of extracts and microbial strains tested. These differences in efficacy at the *in vitro* level can be linked to the level of active substances in the alga and to the purity of the extract. The present findings will have immense potential on the control of clinical pathogens using marine algal bioactives, since the strains used in the study were collected from hospital sources and most of the strains appeared as multidrug resistant. The Gram positive bacteria are more sensitive to algal extracts compared to Gram negative bacteria. This may be due to the permeability barrier provided by the cell wall or to the membrane accumulation mechanism [14]. Numerous

studies have documented the antibacterial activity of *P. tetrastromatica* [15-17]. Antimicrobial assay is a primary screening investigation to evaluate the presence of bioactive metabolites in source organisms. The use of algal extracts and secondary metabolites, both with known antimicrobial trait, can be of great significance in therapeutic treatments.

The bioactive metabolites present in the crude methanolic extract of *P. tetrastromatica* exhibited high toxicity against *Artemia salina*. The crude extract of *P. tetrastromatica* showed the LD₅₀ value of 354.4 µg/ml. The brine shrimp assay is considered as a reliable indicator for the preliminary assessment of toxicity [18] and it can be extrapolated for cell line toxicity and anti-tumour activity. This assay is widely employed in the screening process of botanical for the isolation of bioactive metabolites. Cytotoxic activity of other algae from the Indian coast is already reported [13,19]. Therefore the bioassay guided purification and fractionation of *P. tetrastromatica* may give-forth potent antibiotic and anticancer leads.

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