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Research Article

In vitro antibacterial effects of red alga *Champia parvula* (C. Agardh) of various solvents against human pathogenic bacteria

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Abstract

The aim of the present study is to evaluate the antimicrobial inhibitory effect of *Champia parvula* (red alga) of various solvents at the concentration 100 µg/mL, on pathogenic bacteria like *Klebsiella pneumoniae, Proteus vulgaris, Bacillus cerus, Bacillus subtilius, Staphylococcus aureus*, and *Salmonella typhii* were studied by the disc diffusion method. The present study reveals that a higher zone of inhibition against *Salmonella typhii* (15.4 ± 0.2), *Bacillus subtilius* (13.8 ± 0.1), *Staphylococcus aureus* (10.7 ± 0.2) and *Proteus vulgaris* (10.6 ± 0.1) in the methanol extract alone, followed by acetone, benzene, chloroform, and ethyl acetate extracts showed moderate activity against most of the pathogens, whereas chloroform extract is inactive only against *Bacillus cerus*. The positive control streptomycin shows inhibitory action against all the pathogens studied. This study shows the potential of marine active compounds from *Champia parvula* as an antimicrobial agent for a disease free environment.

1. Introduction

Bacteria are the causative agents for many diseases among human population. Multi-drug resistant bacteria have evolved during recent times and hence, the searches for new drugs from natural resources have been in search for combating the diseases. Marine algae are the major resource available from the marine ecosystem and contain various biologically active compounds which serve as a component for nutraceutical and pharmaceutical industry [1]. Now, more than 2400 marine natural products have been isolated from seaweeds of subtropical and tropical populations [2]. Marine macroalgae are considered to be an excellent source of bioactive compounds with broad range of biological activities including antibacterial [3][4], antifungal [5], antiviral [4][6], antitumor [7], antioxidant [8][9][10], and anti-inflammatory activities [11][12][13]. Marine macroalgae and its active constituents such as carotenoids, terpenoids, steroids, amino acids, phlorotannins, phenolic compounds, halogenated ketones, alkanes and cyclic polysulphide find their applications in pharmaceutical, food, cosmetic industries and in traditional medicine [14][15][16]. Many bioactive compounds of marine algae with antimicrobial activity have been isolated and some of them are under investigation to protect lifestyle related diseases, [6][14][17][18][19]. Seaweeds are considered to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities. The compounds with cytostatic, antiviral, antihelminthic, antifungal and antibacterial activities have been detected in green, brown and red algae [20][21]. Extracts of marine algae were reported to exhibit antibacterial activity [22][23][24]. Several workers have reported that the seaweed extracts exhibit inhibitory activity against a number of gram positive and gram negative bacterial pathogens. A number of seaweeds have been studied for their antibacterial activity both in India and abroad [25][26][27][28][29][30]. India has a long coastline and abundant natural resources of marine algae with very high species diversity. The aim of this study was to analyse the antibacterial efficacy using acetone, benzene, chloroform, methanol, and ethyl acetate crude extracts of marine red alga *Champia parvula* (Rhodophyta) from the Mandapam coast of Tamil Nadu, against bacterial pathogens.

2. Materials and Methods

2.1 Collection of seaweed

The marine red alga *Champia parvula* (C. Agardh) was collected from Mandapam coast, Ramanathapuram District, and Tamil Nadu, India during low tides. The healthy epiphytic free submerged thalli were collected and the debris such as sand particles, pebbles and shells were removed. The seaweed samples were cleaned with sea water and then with fresh sea water and shade dried at room temperature. The dried samples were thoroughly washed with sterile sea water, followed by a rapid rinse with distilled water to remove the salt on the surface of the thalli. Excess water was removed with blotting paper, dried at room temperature until constant weight obtained and ground in an electric mixer and were used for the experimental procedures. The sample was identified by Emeritus Professor, Dr. M. Baluswamy, Associate Professor in Plant Biology and Plant Biotechnology, Madras Christian College, University of Madras, Chennai.

2.2 Preparation of extracts

The powdered sample (100g) was extracted in soxhlet apparatus using five solvents acetone, benzene, chloroform, methanol and ethyl acetate (1000 m L) as solvents for 24hrs at a room temperature maintained not more than the boiling point of the solvent. The resultant crude extracts were filtered with Whatman No.1 filter paper. The filtrates obtained were concentrated under vacuum with a rotary evaporator to obtain the various crude extracts. The crude extracts were collected in an air tight container and stored at 4°C until use.

2.3 Test Microorganisms

The strains of *Klebsiella pneumoniae, Proteus vulgaris, Bacillus cereus, Bacillus subtilis, Staphylococcus aureus, and Salmonella typhi* were obtained from the University of Madras, Taramani campus, Chennai, Tamil Nadu, India, were used as the test organisms for the study.

2.4 Preparation of Inoculum

The Nutrient agar slant slopes stored 4°C were used for inoculum preparation and antibacterial assay activity. The virulent bacterial cultures used for the assay were prepared by transferring a loopful of cells from the stock cultures. The bacteria were sub cultured and routinely maintained on both nutrient agar and Muller-Hinton agar. The antibacterial activity was carried out by was used. Antimicrobial activity was evaluated using Muller-Hinton agar by Kirby-bauer method. The various extracts 100 µg/mL was loaded on sterile filter paper discs and air dried. The bacterial pathogens were streaked on Muller-Hinton agar plates with sterile loops, the discs were then placed on seeded medium plates and incubated for 24 hours at 37°C for clear zone of inhibition. Sterile commercial disc were used as control. [12].

2.5 Antibacterial assay

The antibacterial activity of the algal extract was evaluated by Disc diffusion method[31]. A sterile cotton swab was dipped into the bacterial suspension and evenly streaked over the entire surface of a sterile Muller Hinton agar plate to obtain uniform inoculums. Sterile disc (6mm in diameter) loaded with various crude extract (100 µg/ml) were impregnated on to the plates and incubated overnight at 37^{o}C for 24 hrs. The antibacterial activity was determined by measuring the diameter of the zone of inhibition (mm).

3. Results and Discussion

The antibacterial activity of various solvent extracts of *Champia parvula* (C. Agardh) on bacterial pathogens were studied by the Kirby-Bauer method and the results were measured in Plate-1 and tabulated in Table-1. The maximum inhibitory activity was observed with methanolic extract followed by other solvent extracts at the concentration 100 µg/ml. Streptomycin was used as the positive control in the study. The antibacterial activity of *Champia parvula* methanolic extract against bacterial pathogens are: *Salmonella typhi* (15.4 ± 0.2 mm), *Bacillus subtilis* (13.8 ± 0.1 mm), *Staphylococcus aureus* showed (10.7 ± 0.2 mm), and *Proteus vulgaris* (10.6 ± 0.1 mm) followed by other solvents such as acetone, benzene, chloroform and ethyl acetate which showed minimal antibacterial activity. No activity was observed with chloroform extract for the *Bacillus cereus*. The positive control streptomycin showed resistance to all the bacterial pathogens used in the study.

The marine red algae constituted active compounds which showed antibacterial activity [32][33]. Infections have become the leading cause of death worldwide which has led to an increase in antibacterial resistance, making it a global growing problem nowadays. Thus, there is a need to discover new antimicrobial compounds from natural resources with diverse bioactive compounds and novel mechanisms of action for new and emerging infectious diseases [34]. Reports have

suggested that the samples of powdered fresh or dried seaweeds showed less antibacterial activity than the extracts of powdered samples.

Bioactive and pharmacologically important compounds such as alginate, carrageen and agar as phycocolloids have been obtained from seaweeds and used in medicine and pharmacy have been reported many researchers [22]. Antibacterial activity of the extracts of marine red algae have been reported by other authors [35][36][37]. The results of the present study were direct evidence of Champia parvula possessing antibacterial activity which may be due to the presence secondary metabolites and phytochemicals present in the extracts.

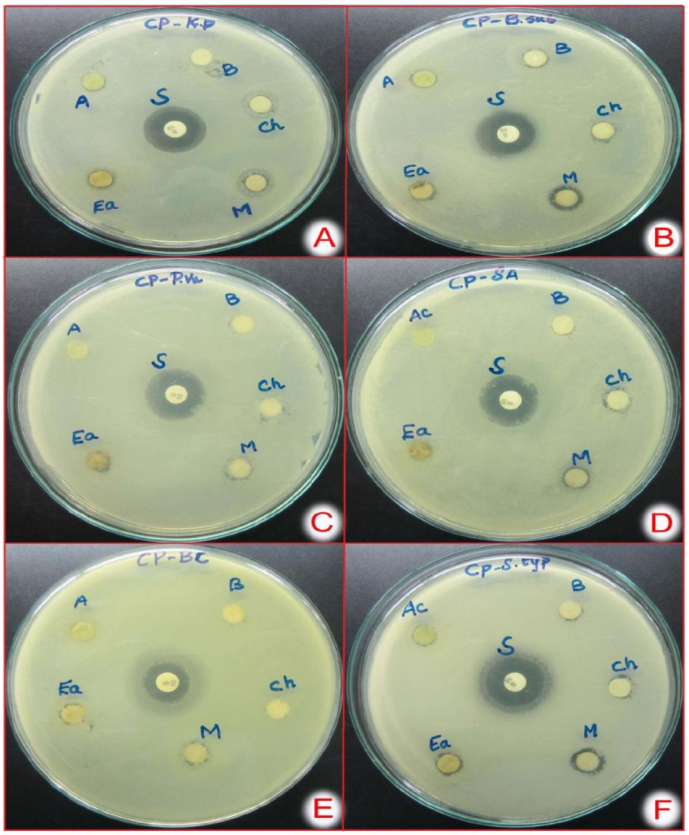


Plate.1 Antibacterial activity of the different solvents extracts of *Champia parvula*

= A- Acetone, B- Benzene, Ch-Chloroform, M-Methanol, Ea- Ethyl acetate, S-Streptomycin

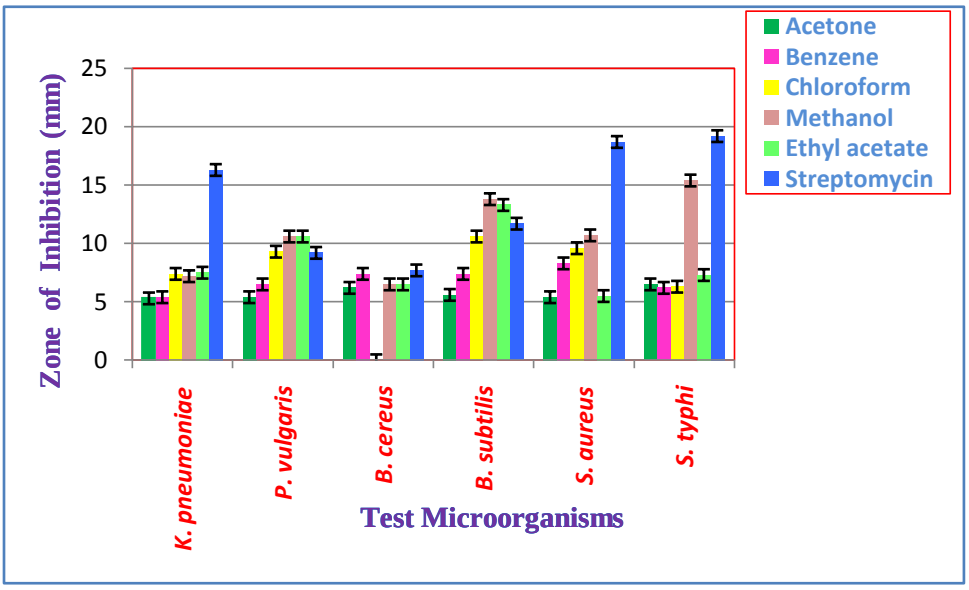
= *Kp - Klebsiella pneumoniae; Pv- Proteus vulgaris; Bc- Bacillus cereus; Bs- Bacillus subtilis; Sa- Staphylococcus aureus; St- Salmonella typhi*

= *Cp-Champia parvula*

Table 1: Antibacterial activity of various solvent extracts of *Champia parvula* against bacterial pathogens

Solvents	Bacterial pathogens showing zone of inhibition (mm in diameter)					
	Klebsiella pneumoniae	***Proteus vulgaris***	***Bacillus cereus***	***Bacillus subtilis***	***Staphylococcus aureus***	***Salmonella typhi***
Acetone	5.3 ± 0.0	5.4 ± 0.2	6.2 ± 0.1	5.6 ± 0.2	5.4 ± 0.4	6.5 ± 0.2
Benzene	5.4 ± 0.2	6.5 ± 0.2	7.4 ± 0.2	7.4 ± 0.2	8.3 ± 0.3	6.2 ± 0.0
Chloroform	7.4 ± 0.1	9.3 ± 0.1	0.0 ± 0.0	10.6 ± 0.1	9.6 ± 0.2	6.3 ± 0.2
Methanol	7.2 ± 0.0	10.6 ± 0.1	6.5 ± 0.2	13.8 ± 0.1	10.7 ± 0.2	15.4 ± 0.2
Ethyl acetate	7.5 ± 0.2	10.6 ± 0.1	6.5 ± 0.2	13.3 ± 0.2	5.5 ± 0.3	7.3 ± 0.2
Positive control Streptomycin	16.3 ± 0.1	9.2 ± 0.1	7.7 ± 0.1	11.7 ± 0.5	18.7 ± 0.2	19.2 ± 0.8

- = No activity, mm = millimeter, values, are expressed as mean ± standard error (n=3)



4. Conclusion

From the above results, it can be concluded that the marine red alga Champia parvula (C. Agardh) shows maximum activity to the bacterial pathogens with methanolic extract compared to other solvents which shows its potential as a good alternative source as antibacterial agents among red seaweeds from the natural source.

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