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ORIGINAL RESEARCH ARTICLE

In vitro Activity of Seaweed Extracts Collected from Gulf of Mannar Coast Islands, Tamilnadu on Clinical Isolates

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ABSTRACT

In the present work we used ten seaweeds such as *Ulva lactuca, Ulva reticulate, Halimeda gracilis,Caulerpa peltata, Padina gymnospora, Stoechospermum marginatum, Spatoglossum asperum, Lobophora variegata, Gracillaria grassa and Acanthophora spicifera* were collected from Mandapam,Nallathanni theevu,Muyal theevu and Sayalkudi in the Gulf of Mannar Coast, Rameswaram ,Tamil Nadu. Methanol:Chloroform (1:1v/v) was taken as solvent for extraction. The extraction of ten seaweeds were prepared for antibacterial activity against selected human pathogens such as *Eschericha coli, Salmonella typhi, Klebsiella pneumonia and Vibrio cholera* using agar disc diffusion method with positive control as Tetracycline . All the seaweeds extract were shown moderate antibacterial activity (22mm to12mm) out of which maximum zone of inhibition (24mm) was exhibited by *Gracilloria grassa* against *Vibrio cholera.* Minimum zone of inhibition (11mm) was exhibited by *Caulerpa peltata* against *Klebsilla pneumonia* and (13mm) zone of inhibition by *Caulerpa peltata* against *E.coli* .Phytochemical analysis of seaweeds may be useful for further antimicrobial activities of seaweeds and to confirm them as a better source for antimicrobial properties in the medical field.

Key words: Seaweeds, Clinical isolates and antibacterial activity.

INTRODUCTION

Seaweeds are considered a rich resource of bioactive activity compounds. Compounds with antiviral, antifungal, antimalarial, antifilarial, hypoglycaemic, antifertility and antibacterial activity have been detected in green, brown and red algae.They produce primary or secondary metabolites which are potentially bioactive compounds of interest in pharmaceutical industries such as carotenoids, dietary fiber, protein, essential fatty acids, vitamins and minerals. Important polysaccharides such as agar, alginates and carrageenans obtained from seaweeds are used in food Industries. The infectious disease is a major cause of morbidity and mortality worldwide (WHO, 2004). Pharmaceutical industries are interested in marine plants because of their rich and active molecules. This is a major concern and an urgent need for searching for new and safe antibacterial agents. Several studies have been investigated about the biological activities of algae extracts. Different active molecules from seaweeds showed the antimicrobial activities against the pathogens e.g.,*S.aureus* or *P. aeruginosa* that commonly cause infection in the human [1]. Enteric pathogens are the most frequent cause of diarrhea illnesses that account for an annual mortality rate of five million people worldwide, the second most common cause of death after cardiovascular disorders. Prominent pathogenic enteric include *salmonella, shigella* and strains of E.*coli* are responsible for diarrhea. Many novel molecules have been isolated from marine ecosystem and some of them are under investigation and are being used to developed new pharmaceuticals.

The usage of organic solvents provides a higher efficacy in extracting compounds always for antimicrobial activity assay [5]. Several extractable compounds such as cyclic-polysulfides and halogenated compounds are toxic to microorganism and they are responsible for antibiotic activity of seaweeds Seasonal and geographical variation also contributes in the antimicrobial activity levels of marine algae. However information is

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lacking on the seasonal and geographical variation in the specific metabolites of marine algae of South India. The extraction of major compounds from the different species of seaweeds was solvent dependent. There are a lot of reports from around the world related to that seaweed species were extracted using organic solvents. The crude extracts of Indian seaweeds are active only against gram positive bacteria [8]. To inhibit their activity new drug is required for this purpose sea source is screened for isolation of their novel compound. Phytochemical analysis of seaweeds may be useful for further antimicrobial activities of seaweeds and to confirm them as a better source for antimicrobial properties in the medical field. Further work was needed to identify the principle compound which were responsible for antibacterial activity against pathogenic bacteria especially those causing the human diseases.

MATERIALS AND METHODS

Sample collection:

Seaweeds were collected by hands picking in period of November – December 2013 from the Islands for the first time from Nallathanni theevu, Muyal theevu and Sayalkudi along the Gulf of Mannar Coast. [8.47°N 79.02°E] Rameswaram,Tamilnadu,India.

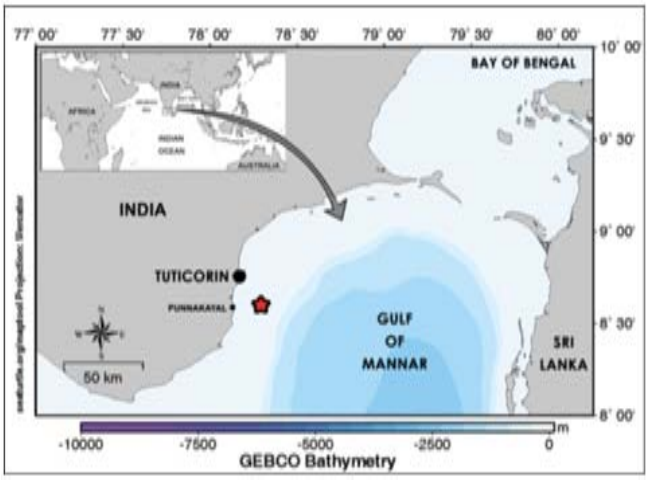

Fig 1: Localization of the collection site of Gulf of Mannar Coast, Tamilnadu, India

Preparation of seaweed extract:

The collected seaweeds were cleaned with sea water and fresh water to remove all epiphytes and sand particle and dried in shade at room temperature. The crude extracts from the seaweeds were prepared by solvent extraction method using Chloroform: methanol (1:1v/v) (Iverson et al., 2001) and kept for 28 days, vigorously shaken at intervals. After the extraction the extracted samples were filtered through Waterman No.1 filter paper.

Preparation of media and cultures:

Media preparation: Peptone – 3g,Nacl-3g,Beef extract -0.18g,Agar- 12grams,Distilled water 600ml,$_P^H$ adjusted to neutral (7.0) .After the sterilization the bacterial culture is inoculate the nutrient broth. The inoculated broth has been incubated for 24 hours 37^{o}c in incubator. The prepared nutrient medium was poured on to the sterilized Petri plates. After the solidification of the medium overnight bacterial cultured were prepared and inoculated by swap method.

Disc diffusion method:

The crude of extracts of seaweeds such as Green:*Ulva lactuca, Halimeda gracilis, Ulva reticulate, Caulerpa peltata,Brown: Padina gymnospora, Stoechospermum marginatum, Spatoglossum asperum Red: Lobophora variegata, Gracillaria grassa and Acanthophora spicifera were tested* against five human pathogenic bacteria namely ,*Escherichia coli ,Klebsiella pneumonia , Vibrio cholera,and Salmonella typhi* by disc diffusion method. The zone of inhibition was measured (mm in diameter). Chloroform as the negative control, antibiotic Tetracycline as positive control.

RESULT AND DISCUSSION

Marine algae are considered as source of bioactive compounds to produce great variety of secondary metabolites characterized by a broad spectrum of biological activities. In the present study antibacterial activities of different types of seaweeds collected from the Rameshwaram, Mandapam, Nallathanni theevu, Uppu Thanni Theevu, Muyal theevu and Sayalkudi is Gulf of Mannar coast Tamil Nadu. Chloroform : methanol (1:1) extracts of 10 Green,brown and red seaweeds were tested at a concentration of 7.5 ug/disc diffusion method against 4 clinical isolate of gram negative bacteria namely, *Eschericha coli, Salmonella typhi, Klebsiella pneumonia, and Vibrio cholera.*

Ulva lactuca extract obtained using methanol: chloroform (1:1) pointed out maximum zone of inhibition against pathogens like *Klebsilla pneumoneae (15mm), Vibrio cholera (14mm), Salmonella typhi (14mm),* and minimum zone of inhibition against *E.coli (12mm).* **(Fig 1).**

Ulva reticulate: The extract obtained using methanol : chloroform (1:1) showed a maximum zone of inhibition against pathogen like *Klebsilla pneumonia(18mm), E.coli(17mm),* and minimum zone of inhibition against *salmonella typhi(13mm), Vibrio cholera(13mm).* (**Fig 2).**

Caulerpa peltata: The methanol : chloroform (1:1) observed the maximum zone of inhibition against *Vibrio cholera(20mm),* and minimum zone of inhibition against *E.coli(13mm) and Klebsilla pneumonia(11mm).*Where no activity against *Salmonella typhi.* (**Fig 4).**

Halimeda gracilis: The observation made from methanol: chloroform (1:1) showed a maximum zone of inhibition against *Klebsilla pneumoneae (18mm), Salmonella typhi (15mm), Vibrio cholera (14mm),* and minimum zone of inhibition against *E.coli (11mm).* (**Fig 3).**

Stoechospermum marginatum: The extract obtained using methanol: chloroform (1:1) showed a maximum zone of inhibition against pathogens like *Vibrio cholera (22mm*) and minimum activity shown against *E.coli (20mm)*. Whereas no activity against pathogens like *Salmonella typhi and Klebsilla pneumonia***.. (Fig 6)**.

Spatoglossum asperum: The methanol : chloroform (1:1) extract obtained showed maximum zone of inhibition against *Vibrio cholera(16mm)* and *Klebsilla pneumonia(16mm)* and minimum zone of inhibition against *Salmonella typhi (15mm),* and *E.coli(14mm).*(**Fig 7).**

Padina gymnospora: The methanol: chloroform (1:1) extract showed the maximum zone of inhibition against pathogen like *Vibrio cholera (22mm)* and minimum zone of inhibition showed against *E.coli (20mm*).Where as no activity against pathogens like *Salmonella typhi and Klebsilla pneumonia.* **(Fig 5).**

Lobophora variegata: The methanol: chloroform (1:1) posses maximum zone of inhibition against *Salmonella typhi(22mm), Vibrio cholera (21mm)*and minimum zone of inhibition against *Klebsilla pneumonia(18mm), E.coli(13mm).* (**Fig 8).**

Gracillaria grassa: The extraction of methanol: chloroform (1:1) observed maximum activity against *Vibrio cholera (24mm),* and moderate activity against *Salmonella typhi (16mm)* and *Klebsilla pneumonia(16mm)*and less activity obtained against *E.coli(12mm).* (**Fig 9).**

Acanthophora spicifera: The extract of methanol: chloroform (1:1) showed maximum zone of inhibition against *Vibrio cholera* (*17mm)* and moderate activity against *Salmonella typhi (14mm)* and *Klebsilla pneumonia (13mm)* and low activity obtained against *E.coli (11mm).* (**Fig 10).**

In our study, ten different marine algae collected from the Coastal area of Gulf of Mannar,Rameswaram,Tamil Nadu were screened for their antibacterial activity using Methanol: Chloroform extract against four different gram negative human pathogens (*E.coli, V.cholera. S.typhi, and K.pneumonia*) these organisms are considered as a danger pathogens because of their fatal causes. According to the past collected data, the different zone of inhibition observed for the same selected test organism with different solvent extract of algae. First time this algae is tested for antibacterial activity using methanol: chloroform extract of *Lobophora variegate.* These algae show antibacterial compound for tested gram negative bacteria showed (18mm) zone of inhibition against the *K. pneumonia* bacteria, (22mm) zone of inhibition against the *S.typhi* bacteria, (21mm) zone of inhibition against the *V.cholera, (13mm)* zone of inhibition against the *E.coli* bacteria.

The overall result showed that 1:1 proportion of seaweed extract: antibiotic Tetracycline against Gracillaria grassa of red algae and Lobophora variegata Stoechospermum marginatum, Padina gymnospora of brown algae, *Caulerpa peltata* of green algae were more active compared to other groups of algae tested. Similar result were also obtained [2,3]. The tested solvent was determined to be the best solvent for isolation of antimicrobial compounds from the tested marine algae. These results were in close agreement with those obtain by [6]. It was revealed that the chloroform: methanol extract G*racillaria grassa, Padina gymnospora, Stoechospermum marginatum, Lobophora variegata and Ulva lactuca* were active against *Vibrio cholera, E.coli, Klebsiella pneumonia, Salmonella typhi.* This may indicate that the extraction method had definite effect on the isolation of bioactive principles by using Chloroform :Methanol (1:1 v/v) ,When compared with other solvents. Some author showed that methanol extraction yield higher antimicrobial activity than n-hexane and ethyl acetate [4, 9]. This difference in results may be firstly due to difference in species used, time and place of sample collection, secondly; there may also be difference in the capability of the extraction protocols to recover the active metabolite and finally, differences in the assay methods that would result in different susceptibilities of the target strains. But, further studies may be made to identify and evaluate the actual substances which are responsible for the antibacterial property.

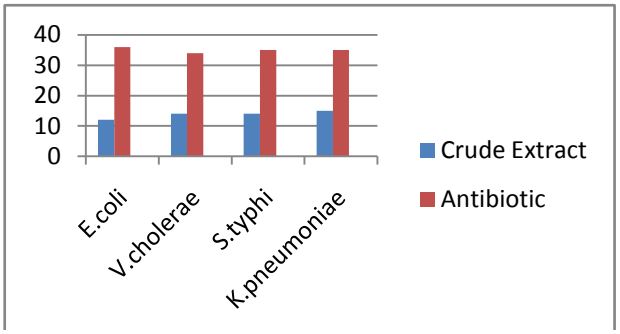

Figure 1: Zone of Inhibition of crude extract of *Ulva lactuca* against clinical isolates.

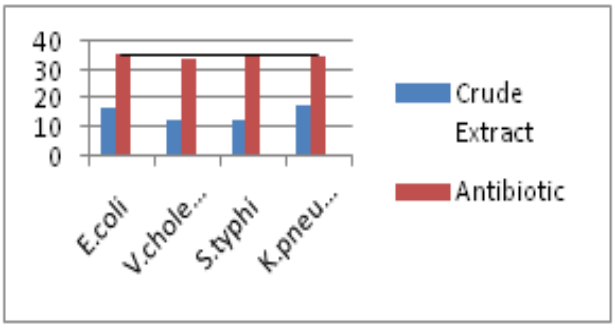

Figure 2: Zone of Inhibition of crude extract of *Ulva reticulata* against clinical isolates.

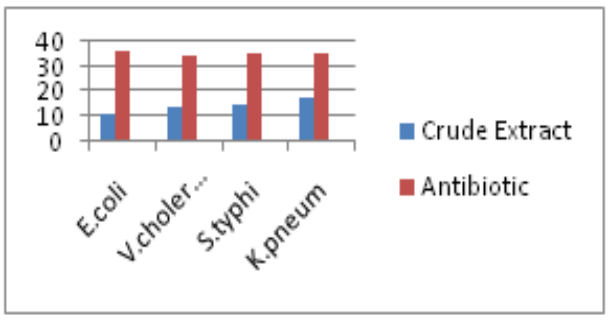

Figure 3: Zone of Inhibition of crude extract of *Helimedia gracils* against clinical isolates.

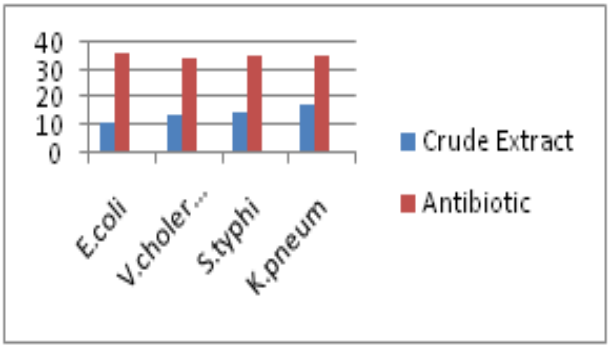

Figure 4: Zone of Inhibition of crude extract of *Caulerpa* peltata against clinical isolates.

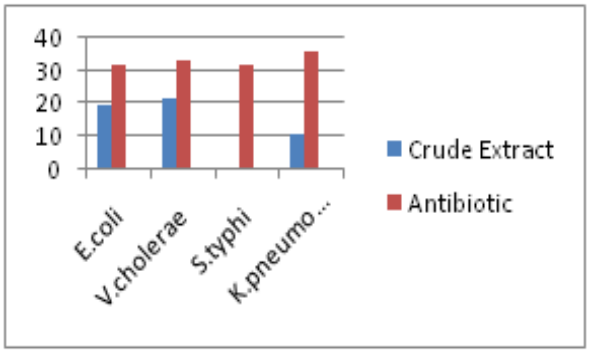

Figure 5: Zone of Inhibition of crude extract of *Padina gymnospora* against clinical isolates.

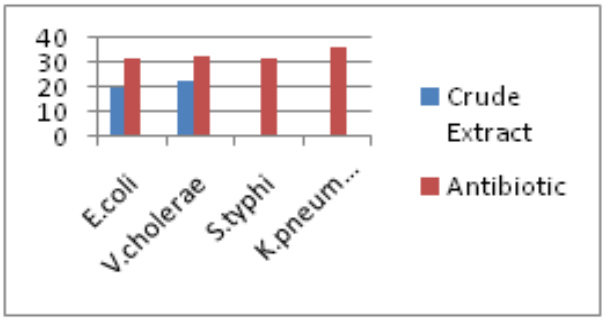

Figure 6: Zone of Inhibition of crude extract of *Stoechospermum marginatum* against clinical isolates.

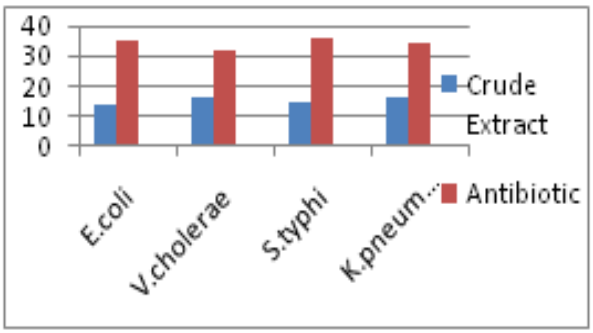

Figure 7: Zone of Inhibition of crude extract of *Spatoglossum asperum* against clinical isolates.

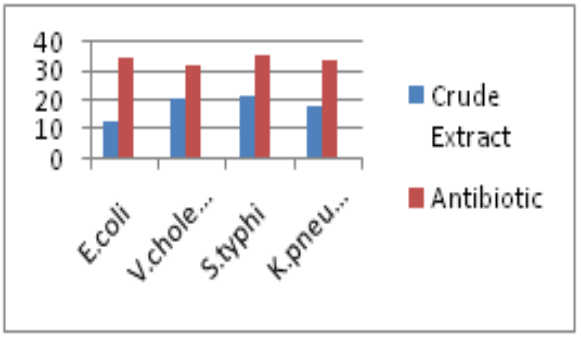

Figure 8: Zone of Inhibition of crude extract of *Lophophora variegata* against clinical isolates.

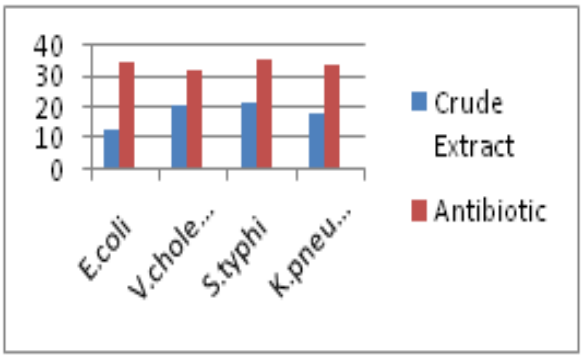

Figure 9: Zone of Inhibition of crude extract of *Gracilaria grassa* against clinical isolates

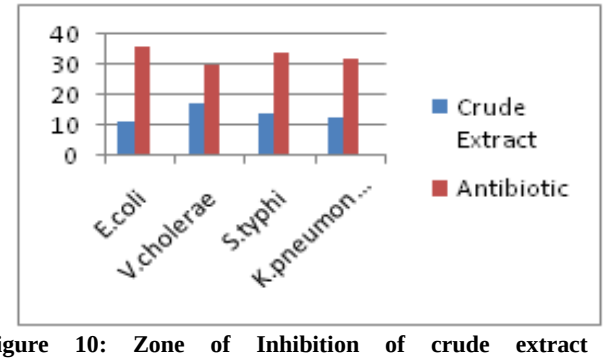

Figure 10: Zone of Inhibition of crude extract of *Achanthophora spicifera* against clinical isolates.

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