

Original Research Article

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***In Vitro* Evaluation of Antimicrobial Activity of *Chaetomorpha antennina* against Biofilm and Beta Lactamase Producing Food Isolates**

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Abstract

This study evaluates the antibacterial activity of methanol and acetone extracts of *Chaetomorpha antennina* collected from Mandapam area, Rameshwaram Dt. The algal extracts were tested for their antibacterial activity against multidrug resistant, biofilm and beta lactamase producing isolates of Gram positive and Gram negative bacteria. The greatest antimicrobial activity was obtained when using methanol extract. Among the 6 bacterial genera, *Enterococcus faecalis* and *Klebsiella pneumoniae* were highly suppressed and *E. coli* was resistant to both extracts. This study was proved *Chaetomorpha antennina* had antimicrobial activity against food pathogens. Further research may help to find the bioactive compounds from algae.

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Introduction

Antibiotic resistance is a global public health problem in 16 the world, especially in developing countries. The infectious disease mortality rate in India today is 416.75 per 100,000 persons. The overuse and inappropriate use of the drug were creating the resistance strain; this phenomenon was a greater threat in poorer nations than in richer ones.

Studies from WHO report have shown very high rates of resistance in bacteria such as *Escherichia coli* against antibiotics as cephalosporin and fluoroquinolones, *Klebsiella pneumoniae* against cephalosporin and carbapenems, *Staphylococcus aureus* against methicillin. These isolates causing infection was untreatable because its causative agent has been found to be resistant to cephalosporin as well as carbapenems due to extended spectrum β -lactamases (ESBL) mediated mechanism

(Bennett et al., 2010). In India and Pakistan, 95% of adults were carrying bacteria that are resistant to β -lactam antibiotics (Reardon, 2014). Treatment of these drug-resistant organisms is a deep scientific worry. These antibiotics were mostly used for common infection and continue to be the prominent cause of resistance to β -lactam antibiotics. The repeated exposure of bacterial strains to a β -lactams has induced production of β -lactamases in these bacteria, this situation, expanding their activity even against the newly developed β -lactam antibiotics. These enzymes are also known as extended-spectrum β -lactamases (ESBLs) (Paterson and Bonomo, 2005). Therefore, alternative antimicrobial strategies are urgently needed.

The natural antimicrobial substances have been recorded in marine environments. Among the marine organisms, the macro algae (seaweeds) occupy a special site as a source of beneficial substances which are active

against bacteria, fungi, virus and cancer (Perez et al., 2016). Among the macroalgae, Chlorophyceae members have a number of beneficial activities such as antioxidant, anticancer, antibacterial and antifungal, especially, *Chaetomorpha antennina* has potential activity (Manchu et al., 2015). According to the literature review, no one studies the antimicrobial activity against ESBL producing isolates. This work aims to evaluate the antimicrobial activity of *C. antennina* extracts against ESBLs isolates and also find alternative drugs and promising source of pharmaceutical agents.

Materials and methods

Seaweed collection and preparation

C. antennina was collected from Mandapam, Rameshwaram district, Tamil Nadu, India. Collected algae were cleaned with seawater to remove impurities. The seaweeds were transported to the laboratory in sterile polythene bags. In the laboratory, samples were rinsed with tap water and were shade dried, cut into small pieces and powdered in a mixer grinder.

Five grams of each powdered sample were soaked in 40 ml of the solvent for three days. Remain extracts were filtered and concentrated in a rotatory evaporator at 35°C. The residual water was removed with a vacuum pump. The weighted crude extracts were suspended in the dimethyl sulfoxide (DMSO) to a final concentration of 50 mg/ml and stored in a refrigerator (El Shafay et al., 2016).

Isolation of food pathogens

Five types of meat samples were collected from the local market. Samples were homogenized using a meat grinder under aseptic conditions and were inoculated on following selective media such as EMB, MSA, SS agar, Cetramide, MacConkey and chromogenic media. After 24 hrs the morphological characters were observed. Bacterial identification was conducted by standard biochemical methods (Collee et al., 1996).

Antibiotic susceptibility testing

The antimicrobial susceptibility of the collected bacteria was assessed using the modified Kirby-Bauer disk diffusion method. Following antibiotics were used for this study. Gentamycin, cefotaxime, ceftriaxone, erythromycin, kanamycin, penicillin, bacitracin, ampicillin, tetracyclin and amoxicillin. The antibiotic

disks were then applied to the prepared plates and incubated at 37°C for 18 hrs then the diameter of the growth inhibition zones was measured.

Isolation of biofilm producing isolates

Brain heart infusion agar supplemented with 5% sucrose and Congo red (0.08 g/l) was prepared and autoclaved at 121°C for 15 minutes. The food isolates were inoculated and incubated aerobically at 35°C for to 48 hrs. Biofilm production was indicated by black colonies with a dry crystalline consistency whereas biofilm non-producers remain pink, though occasional darkening at the center of the colony was observed (Suganya et al., 2014).

Assay for beta-lactamase production

Beta-lactamase production was assayed using the method of Lateef (2004). Broth culture of the test organism was spot inoculated on to Mueller-Hinton agar and 1% starch and then incubated overnight at 37°C. The plates were then flooded with sterile phosphate buffered saline containing potassium iodide, iodine and penicillin. Beta-lactamase production was assessed by the presence of clear colorless zones around the bacterial growth. All the bacterial isolates were tested for the production of beta-lactamases.

Determination antibacterial activity

Twenty ml of sterilized Mueller Hinton agar (MHA) was poured into each Petri plate (90 mm diameter) and allowed to solidify. The plates were incubated with freshly prepared inoculums which were swabbed over the entire surface of the medium, rotating the plate degrees after each application by using a sterile cotton swab, to ensure the spread of the tested microbes on the surface of the plate completely. Inoculums were 10⁸ CFU/ml of bacteria. One well of 6mm diameter was bored in the medium of each plate with the help of sterile cork-borer. Different concentration of extract was filled each well with the help of micropipette. Gentamycin (5µg/ml) was used as positive control.

Results and discussion

The number of mechanisms of resistance has been described; the most common being enzymatic inactivation of the ATB molecule, altered target sites, decreased uptake and/or increased efflux of the antimicrobial agents. β -lactamases are a major resistance

mechanism against the widely used β -lactam antibiotics, which target penicillin-binding proteins (PBPs) involved in bacterial cell wall synthesis. β -lactamase enzymes inactivate β -lactam antibiotics, by hydrolyzing their β -lactam ring essential to antibiotic function (Khan et al., 2014).

In this study, of the total 17 isolates subjected to antimicrobial susceptibility test, 100%, 89%, 83.2% and

75% exhibited resistance to ampicillin, amoxicillin, penicillin, tetracycline and bacitracin in that order. The worrying aspect of the current study is that (29.4%) of the isolates were resistant to antibiotics and single isolate resistance to antibiotics (Fig. 1). Among the 6 types of bacterial isolates, highest resistance was observed in *E. coli* (70%), *Salmonella* species (65%), *E. faecalis* (63.3%) *S. aureus* (60%), *K. pneumoniae* (57%) and *P. aeruginosa* (53.3%) (Fig. 2).

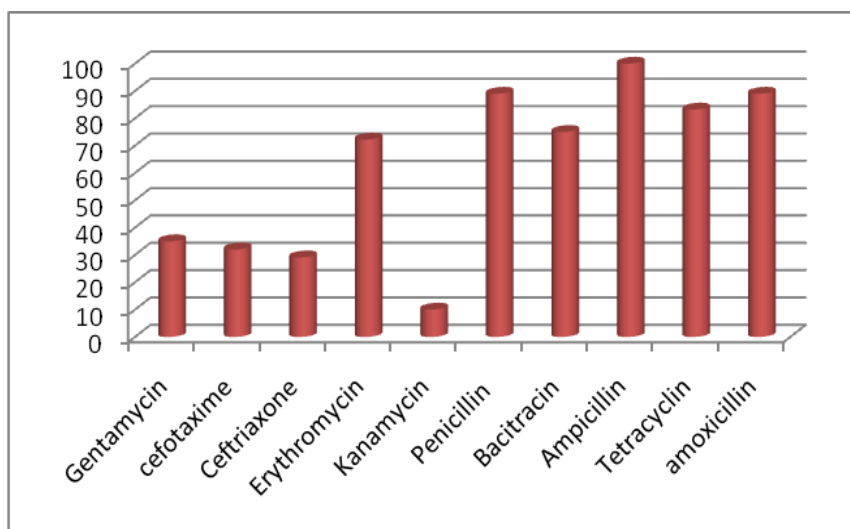


Fig. 1: Antibiotic resistance patterns of food isolates.

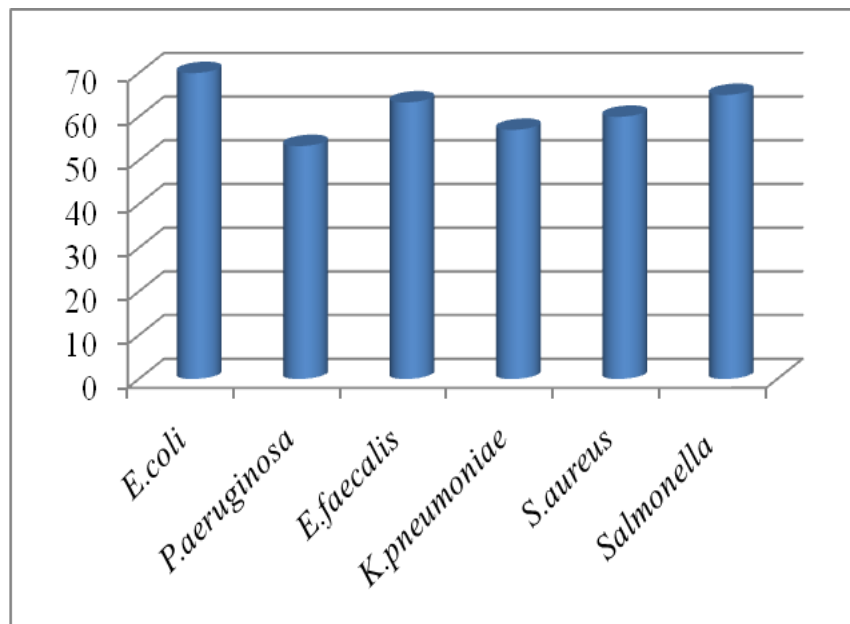


Fig. 2: Percentage of antibiotic resistance in food isolates.

Furthermore, antibiotic resistance is due to the formation of biofilm by microorganisms, this biofilm formation enhanced resistance to antibiotics and other environmental stresses (Branda et al., 2005). Biofilm

promotes bacterial persistence by resisting, antibiotic treatment and host immune responses (Namasivayam et al., 2012). Hence, urgently need new ways to deal with pathogens, especially ESBL and Biofilm producing

isolates. Against a backdrop of this information, our investigation was focused on biological derivatives, because the biological nature of these compounds involves reduced side effects compared to conventional chemical agents. This study was considered to evaluate

the antibacterial potential of *C. antennina* against six clinical bacterial isolates. Table 1 reveals the algal extracts prepared with methanol and acetone had active principles that could inhibit the growth of the pathogenic bacteria tested.

Table 1. Antimicrobial activity of *C. antennina*.

Name of the isolates	Methanol extract (Result in mm)		Acetone extract (Result in mm)		Gentamycin
	50 µg	100µg	50 µg	100µg	
<i>E. coli</i>	-	-	-	-	10
<i>K. pneumoniae</i>	10	13	9	12	-
<i>P. aeruginosa</i>	9	12	-	-	-
<i>E. faecalis</i>	10	16	13	15	-
<i>S. aureus</i>	-	-	9	13	-
<i>Salmonella</i> species	13	15	-	-	-

The methanol and acetone extract of *C. antennina* of 2mg and 4mg concentrations did not provoke a zone of inhibition against *E. coli*. In the case of methanol extract, the zone of inhibition range from 9mm from 16mm among the six types of genera, highly suppressed by *K. pneumoniae* and *E. faecalis*. This extract had less inhibitory effect against *P. aeruginosa* isolate. Among the 6 genera, 4 of were suppressed when using acetone extract and zone of inhibition ranged from 9mm to 15mm and which extract was highly suppressed to *E. faecalis* and lowest inhibition activity against to *S. aureus*. Sivakumar and Safhi (2013) reported that 50 µg of petroleum ether extract of *C. antennina* showed the zone of inhibition ranged between 6 to 25 mm against the tested organisms. Recently, Jolly and Iyer (2015) reported methanol extract of *C. antennina* was showed low activity against *E. coli*. The present study was contrary to Premalatha et al. (2011), who observed highest antimicrobial activity when using methanol extract of *C. antennina*.

From the overall studies, 5 isolates were inhibited by using methanol extract compared with acetone extract. The *C. antennina* showed more or less equal zone of inhibition or slightly greater against some pathogens when compared with positive control gentamycin impregnated positive control discs. Antibacterial activities of algal extracts depend on algal species, the efficiency of the extraction method and concentration of the extract. Previous reports on the most effective solvent for the extraction of antimicrobials have been varied; Kolanjinathan et al. (2009) selected methanol as a solvent for extraction of antimicrobial compounds from seaweeds. Shanmughapriya et al. (2008) found methanol: toluene (3:1) as the best solvent for extracting antimicrobials from fresh algae. In this current study,

superlative activity was observed when using methanol extract. This was agreed with the previous study of Manivannan et al. (2011). They were found highest antibacterial activity when they screened brown algae with methanol. A similar result was found in present study. This antimicrobial activity also depends on the habitat and the season of algal collection. Although a variety of solvents have been employed in screening seaweeds for antimicrobial activity, it is still uncertain what kinds of solvent is the most effective and suitable for extraction of seaweeds.

From these preliminary investigations, the algal extract was activity against to biofilm and beta-lactamase producing isolates. The present study was conducted to develop newer lead for better and safer antimicrobial agents from seaweed. Further studies are needed to identify the pure component and establish the exact mechanism of the seaweed extract.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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