

ASSESSMENT OF THE ANTIMICROBIAL ACTIVITY OF *Chlorella vulgaris* EXTRACTS ON BETALACTAMASE PRODUCING BACTERIAL ISOLATES

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Abstract : In the present study, the solvent extract of *Chlorella vulgaris* have tested with the agar well diffusion method for their antibacterial against betalactamase producing gram negative and positive bacteria (*E.coli*, *E.faecalis*, *K.pneumoniae*, *Salmonella spp*, *Proteus spp*, *Shigella spp*, *P.aeruginosa* and *S.aureus*). The results indicated that both extracts of *Chlorella vulgaris* were efficient against the betalactamase bacterial isolates. The zone of inhibition ranges from 10 to 24 mm and chloroform extract was high active against to *Proteus spp*. whereas these extracts were ineffective against *P. aeruginosa* and *S. aureus*. As a result, it may be concluded that extracts of *C. vulgaris*, have the capabilities to be explored for antibiotic production. Additional, efforts must be made to identify the compounds straight responsible for antibacterial properties.

Index Terms: *Chlorella vulgaris*, *Betalactamase*, *Antibacterial activity*

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I. INTRODUCTION

Antimicrobial resistance is one of the most important public health issues particularly in developing countries wherever comparatively easily availability and over utilization of medicines have cause to a disproportionately higher incidence of inappropriate use of antibiotics and greater levels of resistance compared to developed countries. In India, the infectious disease trouble is among the highest in the world and a recent report showed the inappropriate and unreasonable use of antimicrobial agents against these diseases, which led to increasing in development of antimicrobial resistance (Gansesh *et al.*, 2013). The crude infectious disease mortality rate in India today is 416.75 per 100,000 persons (Laxminarayan and Ranjit, 2016).

Studies from WHO file have proven very high rates of resistance in micro organism such as *Escherichia coli* against antibiotics as cephalosporin and fluoroquinolones, *Klebsiella pneumoniae* in opposition to cephalosporin and carbapenems, *Staphylococcus aureus* against methicillin. These isolates inflicting infection was untreatable due to the fact its causative agent has been observed be resistant to cephalosporin as well as carbapenems due to extended spectrum of β -lactamases (ESBL) mediated mechanism (Bennett *et al.*, 2010).

The repeated exposure of bacterial isolates to a β -lactams has triggered production of β -lactamases in those bacteria, this example, increasing their pastime even in opposition to the newly advanced β -lactam antibiotics, this circumstance, extending their movement even against the recently created β -lactam antibiotics. In this situation urgently we need a choice solution of eradication of bacterial isolates. The natural antimicrobial substances have been recorded in marine environments. Among the marine organisms, the microalgae (seaweeds) occupy a special site as a source of beneficial substances which are active against to bacteria, fungi, virus and cancer (Maria *et al.*, 2016).

Many bioactive and pharmacologically active components have been isolated from algae. Algae based antimicrobials substance has enormous therapeutic potential as they can serve the motive with lesser side effects that are often associated with synthetic antimicrobials (Justella *et al.*, 2011). Among the various microalgae, *Chlorella vulgaris* have been explored for their suitability for commercial potential, chlorella species are the major type that has been used successfully to produced high concentration of valuable compounds. (Priya *et al.*, 2012).

Several authors studied the antimicrobial activities of *Chlorella algae* in different parts of our country. According to previous literature, no one study was the antimicrobial activity of *Chlorella vulgaris* against beta-lactamase producing isolates. Therefore, the present study was to investigate the antimicrobial effect of *Chlorella vulgaris* against betalactamase producing food pathogens.

II. METHODS

a. Food pathogens

For antimicrobial activity of algal extracts against microbial pathogens including Gram positive and Gram negative bacteria were isolates from food samples. Different 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 media such as chromogenic and SS agar. After 24 hrs observed the morphological character. Bacterial identification was conducted by morphological and gram staining method (Collee *et al.*, 1996).

2.2 Isolation of betalactamase producing isolates by Tube Method

Penicillin solution was dispensed in 0.5ml volume in small test tubes. Test bacteria were took with a loop from an overnight culture on solid medium and suspended in the Penicillin solution to give a density of at least 10^4 CFU/ml. After one hour at room temperature, two drops of the starch indicator was added to the suspension, followed by one drop of Iodine reagent. The positive reaction was indicated by the disappearance of blue color immediately. Persistence of blue color for longer than 10 minutes constituted a negative test (Christensen *et al.* 1990).

2.3 Collection of algae

The sample microalgae species *Chlorella vulgaris* was obtained from Royal research centre, Chennai. The algae was cultured on using Bold Basal Medium(BBM).The cultures were grown at $24\pm 1^\circ\text{C}$ in a thermostatically controlled room with cool white in fluorescent lamps at 2-3weeks. After incubation, algal growth was measured by using UV-VIS spectrophotometer at 680nm. Ten ml from cultures were filtered under vacuum using filter membrane (0.45 μm) and washed several times with distilled water. Then, the algae cells were dried at 80°C for 30min and weighed.

2.4 Preparation of algae extracts

Extraction was performed using the solvents of different polarity: chloroform and Methanol. Five grams of the algal dry powder was suspended in the solvents at the ratio of 6:1 (v/w), left at room temperature for 48 h and then, were homogenized for 30 min. Finally, the suspensions were centrifuged at 3000 g for 10 min and the supernatants were filtered and dried. Then dried material was dissolved in DMSO and extracts were kept in dark at 4°C until use (Ahmad *et al.*, 2017).

2.5 Determination antibacterial activity

This test was carried out according to the method of Jahir *et al.*, 2011. The 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 60 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 10^8 CFU/ml of bacteria. One well of 6mm diameter was bored with the medium of each plate with the help of sterile cork-borer. Different concentration of extract was filled in each well with the help of micropipette. Ampicillin (5 μg /ml) was used as positive control.

III. RESULT AND DISCUSSION

In the past few years, ESBL-producing *E. coli* have been increasingly isolated from food-producing animals raising global concerns for veterinary and public health. In the past few years, ESBL-producing *E. coli* have been increasingly isolated from food-producing animals raising global concerns for veterinary and public health (Seiffert *et al.*, 2013). In the past few years, ESBL-producing *E. coli* have been increasingly isolated from food-producing animals raising global concerns for veterinary and public health (Seiffert *et al.*, 2013). According to chromogenic and SS agar media, totally 15 isolates from 8 types of bacterial genera were obtained from meat namely as *E.coli*, *E.faecalis*, *K.pneumoniae*, *Salmonella spp*, *Proteus spp*, *Shigella spp*, *Pseudomonas aeruginosa* and *S.aureus*.

In the past few years, betalactamase producing isolates have been increasingly isolated from food-producing animals raising global concerns for veterinary and public health (Seiffert *et al.*, 2013). The current study reports on the higher occurrence 67% of betalactamase producing isolates. This report concerns more often the occurrence of the mentioned microorganisms in animals at the farm or the slaughter-house. Previous studies showed a high prevalence of betalactamase producing Enterobacteriaceae in poultry production in India (Charles *et al.*, 2017).

For the last ten years, there have been studies on ESBL in India. In 2015, Kar *et al* has conducted the first systematic report on multidrug resistance ESBL producing isolates in meat samples in India. In Hyderabad, India, ESBL producing isolates were observed from various meat samples (Rasheed *et al.* 2014).

ESBL-producing Enterobacteriaceae are highly resistant to a number of drugs, can make a contribution to obtained resistance via horizontal gene transfer throughout a wide range of bacterial species, which are associated with long hospital stay and spend the much money. Recently a number of researches were developed on secondary metabolites based on bioassay-guided screening of prokaryotic and eukaryotic microalgae. However, so far these

potentials haven't been exploited to any great extent. In the prevailing look at, we targeted to overcome the betalactamase producing Gram-negative and positive isolates causing infection. Therefore, algae of *Chlorella vulgaris* was collected and subjected to inhibition of betalactamase producing bacterial isolates.

The antimicrobial activity of extracts of *C. vulgaris* prepared by using methanol, chloroform solvents was recorded in Figure 1 and 2. Data clearly show the great potency of *C. vulgaris* extracts to inhibit the bacterial growth. Both extracts were able to inhibit the growth of one or more pathogens and the zone of inhibition ranges from 10 to 24 mm whereas methanol extract was ineffective against *P.aeruginosa* and *S.aureus* spp. Currently, none of the isolates were suppressed when using standard antibiotic and DMSO.

In the current study, chloroform extract showed better activity but there is no much more different when using the methanol extract. These results were similar to previous studies of Entesar (2016). This result was showed antimicrobial activity of *C. vulgaris* against various bacterial isolates. On the other hand, *C. vulgaris* crude extract showed antibacterial activity against *S. aureus* and *St. pyogenes*, *K. pneumoniae* and *E. coli* with inhibition zones (19.3 ± 2.1 , 21.1 ± 0.63 , 20.1 ± 0.58 and 22.4 ± 0.63 mm respectively). In 2016, Shakeel *et al* determined the antibacterial activity of lake isolates *C. vulgaris*, they also report that chloroform extract was activity against wide range of bacterial isolates.

According to literature reports, this is the first study of antibacterial activity of *C. vulgaris* extracts against betalactamase producing food borne isolates. The present results provide an illustration to the presence of promising the antimicrobial compounds in solvents extract of *C. vulgaris* under study. Further phytochemical studies are needed to elucidate the components responsible for antimicrobial activity of these extracts against bacteria.

IV REFERENCE

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Figure 1
Antibacterial activity of methanol extract of *C. vulgaris*

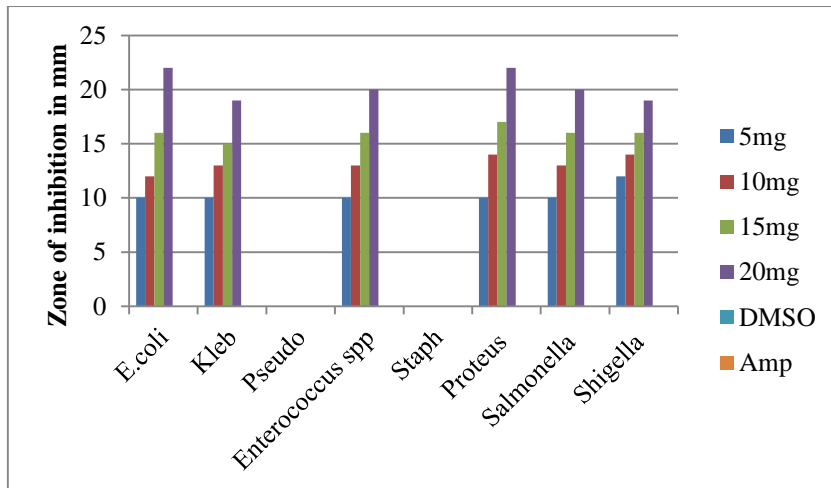


Figure 1
Antibacterial activity of Chloroform extract of *C. vulgaris*

