Evaluation of Antibacterial Activity of Different Solvent Extracts of Marine Macro Algae: Chaetomorpha media (C.Ag.) Kuetzing

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ARTICLE INFO ABSTRACT

Background: Chaetomorpha media (C.Ag.) Kuetzing is marine macro green algae and commonly found in the south east coast of Tamil Nadu, India. Marine macro algae have a number of secondary metabolites which can be used to cure various diseases.

Objectives: The objective of the present study is to evaluate the antibacterial efficiency of Chaetomorpha media (C.Ag.) Kuetzing, a marine macro algae using ethanol, ethyl acetate, chloroform, benzene and petroleum ether as solvents and tested against four human pathogenic bacteria such as Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa and Salmonella typhi.

Methodology: Antibacterial properties of Chaetomorpha media (C.Ag.) Kuetzing were evaluated using agar well diffusion method and Minimum inhibitory concentration.

Results: All the extracts showed significant activity against all pathogens, but the ethanolic extract of Chaetomorpha media (C.Ag.) Kuetzing showed maximum zone of inhibition and minimum inhibitory concentration against all the microorganisms. The minimum zone of inhibition and comparatively greater inhibitory concentration were determined in ethyl acetate extract of Chaetomorpha media (C.Ag.) Kuetzing showing less antibacterial activity against all the experimental strains.

Conclusion: The Spectrum of activity observed in the present study may be indicative that ethanolic extract of Chaetomorpha media (C.Ag.) Kuetzing could be a possible source to obtain new and effective herbal medicines to treat infections against various infectious diseases.

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INTRODUCTION:
Natural bioactive compounds are the compounds which extracted from living organisms such plant and animal sources\(^1\). These compounds are classified into terpenoids, polyketides, amino acids, peptides, proteins, carbohydrates, lipids and nucleic acid bases\(^2\). Many substances obtained from seaweeds have been used for decades in medicine and pharmacotherapy, whereas some of the isolated substances have bacteriostatic and bactericidal properties\(^3\)-\(^5\). Marine organisms are the source materials for structurally unique natural products with pharmacological and biological activities\(^6\).

Among the marine organisms, the macro algae occupy an important place as a source of biomedical compounds\(^7\). Marine macro algae have been found to be rich in secondary metabolites that include alkaloids, glycosides, saponins, tannins, flavonoids, steroids which are of immense medicinal value and useful in broad spectrum of biological activities\(^8\). Among the marine macro algae, the Chlorophyceae commonly known as green algae is a very large group of algal which include approximately 425 genera and 6500 species and they are closely related to human life. It is used in various purposes of food, feed, fertilizer, medicine etc. With this background, the present study was undertaken to evaluate the antibacterial activity of Chetomorpha media (C.Ag) Kuetzing.

MATERIALS AND METHODS

Collection of Plant Sample

Chetomorpha media (C.Ag) Kuetzing, one of the important green marine macro algae shows much attention in the present study as it has potential to supplement native vegetation. Chetomorpha media (C.Ag) Kuetzing was collected from Manapad, Thoothukudi district in the south east coast of Tamil Nadu, India. Samples were rinsed with marine water to remove debris and epiphytes. The entire epiphyte s were removed using soft brush. In the laboratory, the collected plants were again washed in freshwater and stored in refrigerator for further analysis\(^9\).

Preparation of Extracts

For the preparation of various algal extracts, the plant specimens were washed thoroughly and placed on blotting paper and spread out at room temperature in the shade condition for drying. The shade dried samples were grounded to fine powder using a tissue blender. The powdered samples were then stored in the refrigerator for further use. 30g powdered samples were packed in Soxhlet apparatus and extracted with ethanol, ethyl acetate, chloroform, benzene and petroleum ether for 12h separately\(^10\). All the extracts were stored in sterile glass bottles at room temperature until screened.

Culture and Maintenance of microorganisms

Pure cultures of all experimental bacteria were obtained from the Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh. The pure bacterial cultures were maintained on nutrient agar medium. Each bacterial culture was further maintained by subculturing regularly on the same medium and stored at 4°C before use in experiments.

Media Preparation and Its Sterilization

For agar well diffusion method Murray et al.,\(^11\) later modified by Olurinola\(^12\), antimicrobial susceptibility was tested on solid (Agar-agar) media in Petri plates. For bacterial assay nutrient agar (NA) (40g/L) was used for developing surface colony growth. The minimum inhibitory concentration (MIC) was determined by serial micro dilution assay. The suspension culture, for bacterial cells growth was done by preparing 2% Lauria Broth (w/v) was taken for evaluation. All the media prepared was then sterilized by autoclaving the media at (121°C) for 20 min.

Agar well diffusion method

Agar well-diffusion method was followed to determine the antimicrobial activity. Nutrient agar (NA) plates were swabbed (sterile cotton swabs) with 8 hour old - broth culture of respective bacteria. Wells (10mm diameter and about 2 cm a part) were made in each of these plates using sterile cork borer. Stock solution of each algal extract was prepared at a concentration of 1mg/ml in different plant extracts viz. ethanol, ethyl acetate, chloroform, benzene and petroleum ether. About 100µg of different concentrations of plant solvent extracts were added sterile syringe into the wells and allowed to diffuse at room temperature for 2h. Control experiments comprising inoculums without plant extract were set.
up. The plates were incubated at 37°C for 18-24h for bacterial pathogens. The diameter of the inhibition zone (mm) was measured and the activity index was also calculated. Triplicates were maintained and the experiment was repeated thrice, for each replicates the readings were taken in three different fixed directions and the average values were recorded.

**Determination of MIC**

The minimum inhibitory concentration (MIC) was performed by a serial dilution technique using 96-well microtiter plates. The different plant extracts viz. ethanol, ethyl acetate, chloroform, benzene and petroleum ether were taken (1mg/ml) and serial dilution of the extract with luria broth for bacterial culture with respective inoculum were used. The microplates were incubated for 72 hours at 28°C. The lowest concentrations without visible growth (at the binocular microscope) were defined as MICs.

**RESULT AND DISCUSSION**

In the present investigation, the inhibitory effect of different extracts (viz. ethanol, ethyl acetate, chloroform, benzene and petroleum ether) of *Chetomorpha media* (C.Ag.) Kuetzing were evaluated against bacterial strains. The antibacterial activity was determined using agar well diffusion method. The activity was quantitatively assessed on the basis of inhibition zone and their activity index was also calculated along with minimum inhibitory concentration (MIC). The antimicrobial potential of the experimental plant was evaluated according to their zone of inhibition against various pathogens and the results (zone of inhibition) were compared with the activity of the standard, viz., Gentamycin (1.0 mg/disc). The results revealed that all the extracts are potent antibacterial against all the microorganisms studied.

**Antibacterial effects of Chaetomorpha media (C.Ag.) Kuetzing.**

**Effect on *Escherichia coli***

Ethanol extract of *Chaetomorpha media* (C.Ag.) Kuetzing showed significant result against the bacterium *Escherichia coli* which has the inhibition zone of 22 mm (100µg) and it was followed by 18 mm (80µg), 15mm (60µg), 13mm (40µg) and 11mm (20µg). Ethyl acetate extract showed 13mm (100µg) and it was followed by 12mm (80µg), 10mm (60µg), and 9mm (40µg). Chloroform extract showed 14 mm (100µg) and it was followed by 12 mm (80µg) and 9mm (60µg). Benzene extract showed 15mm (100µg) and it was followed by 12mm (80µg), and 10mm (60µg), 9mm (40µg) and 8mm (20µg). Petroleum ether extract showed 13mm (100µg) and it was followed by 10mm (80µg), 9mm (60µg) and 8mm (40µg) as against the standard antibiotic Gentamycin used as a positive control which showed an inhibition of 15mm in the present study (Table-1 and Figure-1).

**Effect on *Klebsiella pneumonia***

Ethanol extract of *Chaetomorpha media* (C.Ag.) Kuetzing showed significant result against *Klebsiella pneumonia* which has the inhibition zone 23 mm (100µg) and it was followed by 18 mm (80µg), 15mm (60µg) 14mm (40µg) and 12mm (20µg). Ethyl acetate extract showed 12mm (100µg) and it was followed by 10mm (80µg), 9mm (60µg), and 8mm (40µg). Chloroform extract showed 13mm (100µg) and it was followed by 12 mm (80µg). Benzene extract showed 15mm (100µg) and it was followed by 11mm (80µg), and 10mm (60µg), 8mm (40µg) and 8mm (20µg). Petroleum ether extract showed 15mm (100µg) and it was followed by 12mm (80µg) as against the standard antibiotic Gentamycin used as a positive control which showed an inhibition of 12mm in the present study (Table-1 and Figure-2).

**Effect on *Pseudomonas aeruginosa***

Ethanol extract of *Chaetomorpha media* (C.Ag.) Kuetzing showed significant result against the bacterium *Pseudomonas aeruginosa* which possessed 13mm (100µg) and it was followed by 11mm (80µg), 10mm (60µg) and 8mm (40µg). Ethyl acetate extract showed 13mm (100µg) and it was followed by 12mm (80µg), and 10mm (60µg). Chloroform extract showed 10mm (100µg) and it was followed by 8mm (80µg). Benzene extract showed 12mm (100µg) and it was followed by 10mm (80µg) and 9mm (60µg). Petroleum ether extract showed 11mm (100µg) and it was followed by 10mm (80µg) and 7mm (60µg) as against the standard antibiotic Gentamycin used as a positive control which showed an inhibition of 11mm in the present study (Table-1 and Figure-3).

**Effect on *Salmonella typhi***

Ethanol extract of *Chaetomorpha media* (C.Ag.) Kuetzing showed significant inhibition zone
against the bacterium *Salmonella typhi* which had 10mm (100µg) and it was followed by 9mm (80µg) and 8mm (60µg). Ethyl acetate extract showed 9mm (100µg) and it was followed by 8mm (80µg). Chloroform extract showed 8mm (100µg). Benzene extract showed 10mm (100µg) and it was followed by 8mm (80µg). Petroleum ether extract showed 8mm (100µg) and it was followed by 7mm (80µg) as against the standard antibiotic Gentamycin used as a positive control which showed an inhibition of 18mm in the present study (Table-1 and Figure-4).

### Table 1: Antibacterial effect of Chaetomorpha media (C.Ag.) Kutzing

<table>
<thead>
<tr>
<th>S. No</th>
<th>Bacteria</th>
<th>Conc. of Extracts (µg)</th>
<th>Inhibition zones (mm)</th>
<th>Ethanol Extract</th>
<th>Ethyl acetate extract</th>
<th>Chloroform extract</th>
<th>Benzene extract</th>
<th>Petroleum ether extract</th>
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<td><em>Escherichia coli</em></td>
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<td>22±0.97</td>
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<td>80</td>
<td>18±0.53</td>
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<td>4.</td>
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<td>10±0.13</td>
<td>9±0.13</td>
<td>9±0.13</td>
<td>10±0.13</td>
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</tbody>
</table>
Figure 1: Antibacterial effect of *Chaetomorpha media* (C. Ag.) Kutzing on *Escherichia coli*

**Ethanol extract**

**Ethyl acetate extract**

**Chloroform extract**

**Benzene**

**Petroleum ether extract**

1. 20µg  
2. 40µg  
3. 60µg  
4. 80µg  
5. 100µg
Figure 2: Antibacterial effect of Chaetomorpha media (C. Ag.) Kutzing on Klebsiella pneumonia

Ethanol extract

Ethyl acetate extract

Chloroform extract

Benzene

Petroleum ether extract

1. 20µg  2. 40µg  3. 60µg  4. 80µg  5. 100µg
Figure 3: Antibacterial effect of *Chaetomorpha media* (C. Ag.) Kutzing on *Pseudomonas aeruginosa*

**Ethanol extract**

**Ethyl acetate extract**

**Chloroform extract**

**Benzene**

**Petroleum ether extract**

1. 20µg  
2. 40µg  
3. 60µg  
4. 80µg  
5. 100µg
Figure 4: Antibacterial effect of *Chaetomorpha media* (C. Ag.) Kützing on *Salmonella typhi*

**Ethanol extract**

**Ethylacetate extract**

**Chloroform extract**

**Benzene**

**Petroleum ether extract**

1. 20μg  2. 40μg  3. 60μg  4. 80μg  5. 100μg
The search for antimicrobials from natural sources has received much attention and efforts have been put in to identify compounds that can act as suitable antimicrobials agent to replace synthetic ones. Previously Kandhasamy and Arunachalam\(^\text{(13)}\) found out the in vitro antibacterial property of marine macroalgae viz. Caulerpa racemosa, Ulva lactuca, Gracilaria folifera, Hypnea musciformis, Sargassum teneerimum, S. myriocystem and Padina tetrastomatica against gram negative and gram positive pathogenic bacteria. Some commonly occurring marine algae Caulerpa scalpelliformis, Ulva lactuca, Pandina tetrastromatica, Stoecchospermum marginatum and Acanthophora spicifera have been collected and evaluated for antibacterial activity by using various solvents such as petroleum ether, chloroform, methanol and benzene\(^\text{(14)}\). Arulsenthil \(\text{et al.}\)\(^\text{(15)}\) found out the antibacterial activity of the methanol and acetone extracts of Padina boergesnii collected from Tuticorin coast against ten human pathogenic bacteria. In the present investigation, different extracts of Chaetomorpha media (C.Ag.) Kuetzing was evaluated for exploration of the antibacterial activity against certain bacteria which was regarded as human pathogenic microorganism. The study revealed that the selected various solvent extracts show the antibacterial effect against various bacteria.

**CONCLUSION**

In conclusion of the present investigation, Chaetomorpha media (C.Ag.) Kuetzing contains potential antibacterial components that may be of great use for the development of pharmaceutical industries as a therapy against various diseases. The ethanol, ethyl acetate, chloroform, benzene and petroleum ether extracts of Chaetomorpha media (C.Ag.) Kuetzing possess significant inhibitory effect against tested pathogens. The results of the study support the traditional medicine claim along with the development of new antibacterial drugs from the selected green algae.

**CONFLICT OF INTEREST**

The author declares that she has no conflict of interest.

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How To Cite This Article:

Source of Support: Nil

Conflict of Interest: None declared

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