



Research Article

ANTIMICROBIAL ACTIVITY OF BIOACTIVE COMPOUNDS EXTRACTED FROM CHARA GLOBULARIS THUILL

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ABSTRACT

Background: The algae, a vastly diversified group of species on Earth, offer numerous benefits to humanity. *Chara globularis* is one such green alga that contains potential bioactive compounds. **Methodology:** This current study investigates the antifungal and antibacterial potential of *Chara globularis* extracts obtained using methanol, acetone, and hexane solvents, along with their phytochemical and medicinal profiles. **Results & discussion:** The antifungal activity was evaluated against clinically significant fungal strains, including *Microsporum gymseum*, *Trichophyton rubrum*, and *T. mentagrophytes*. The methanol extracts exhibited the most potent inhibition zones, with *T. rubrum* showing a maximum inhibition of 24 mm at a concentration of 4 mg. Also, antibacterial assays against pathogens such as *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae* demonstrated strong activity, especially in methanol and acetone extracts at higher concentrations. Comparative control studies using fluconazole and tetracycline confirmed the efficacy of the extracts, suggesting potential for pharmaceutical application. GC-MS analysis of the methanol extract identified 26 bioactive compounds, notably pentadecanoic acid and eicosatrienoic acid, which possess antimicrobial, anti-inflammatory, and cardioprotective properties. **Conclusion:** Nutrient profiling further revealed high levels of essential macro and micronutrients, highlighting the nutritional value of *Chara globularis*. The findings support the plant's potential as a natural source of therapeutic agents with broad-spectrum antimicrobial and nutraceutical applications.

INTRODUCTION

Algae are one of the promising sources of bioactive compounds with a wide range of medicinal applications. These organisms, ranging from microalgae to macroalgae, produce a variety of metabolites that have potential value in pharmaceutical, nutraceutical, and cosmeceutical industries [1]. Algal compounds are more secure and effective substitutes for

antimicrobial activity tests. The therapeutic use of green algae is the most efficacious in treating a wide variety of diseases. The phytochemical, biochemical, and antibacterial effects of *Chara braunii* were investigated, revealing a significant impact on the *E. coli* population [2]. Proteins, fats, lipids, carbohydrates, phenols, flavonoids, vitamins, free amino acids, enzymes,

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growth regulators, pigments, toxins, and antibiotics are among the bioactive compounds generated by algae cells that are actively growing [3]. The products made from bioactive compounds found in green and blue-green algae are a rich source of metabolites that are essentially new and organically dynamic. These include antimicrobial, antiviral, antifungal, anti-allergic, anticoagulant, anticancer, antifouling, and antioxidant dressings [4]. One such efficient alga is the green alga or Characeae, which has widespread therapeutic applications [5]. Fresh algae, the sole surviving family of the order Charales, is one of the many species that are produced by the aquatic environment. This group grows excessively and is observed in high concentration, with a significant accumulation of green algae resulting in a substantial biomass [6, 7].

Bioactive chemicals from algae, such as *Chara*, are utilized in farming and are recognized for their antibacterial properties [8]. Nevertheless, the agribusiness's maximal potential for bioactive chemicals has yet to be determined. An important component of the maritime biological system, algae are frequently seen growing at higher temperatures. Green algae are recognized to produce a few naturally dynamic chemicals with antiviral, antibacterial, antifungal, and anticancer properties, although the composition of biomass varies with the availability of nutrients [9, 10]. Flavonoids are extracted in non-polar solvents, whereas phenolic chemicals are extracted in polar dissolvable with differential yields. For instance, Soxhlet extraction yields heat-stable segments, whereas cold extraction yields distinct lipids [11]).

MATERIALS AND METHODS

Study site and the species identification

Fresh *Chara globularis* samples were collected from Ranikere pond, Karnataka, during the post-monsoon season (October–November) when water levels and nutrient availability favored algal growth. The site was characterized by moderate temperatures (24–28 °C), a pH range of 7.0–7.5, and low water flow. Species identification was carried out using morphological keys described in Prescott's Algae of the Western Great Lakes Area and Indian algal flora manuals.

ANTI-FUNGAL ACTIVITY

Culture Preparation

Potato Dextrose Broth (PDB: Potato-200g, Dextrose-20g, Distilled water-1000mL). 500mL of Broth was prepared by

boiling 100g of Potato in 300mL of distilled water and filtering. 10g of Dextrose was added to the filtrate, and the volume was made up to 500mL with distilled water. 100mL of broth was transferred into 5 Erlenmeyer flasks, respectively, and autoclaved at 121°C for 15 minutes. Later, the organisms (*Microsporium gypseum*, *Microsporium nanum*, *Trichophyton mentagrophytes*, *Trichophyton rubrum*, and *Trichophyton violaceum*) were inoculated in 100mL of sterilized PDB broth and incubated at 25°C for 72 h (12).

Sample preparation

100mg of extract (Hexane extract, Acetone extract and Methanol extract) was dissolved in 1mL of Dimethyl sulfoxide (DMSO). Different aliquots of Extract was prepared by pipetting 10µL (1mg), 20µL (2mg), 30µL(3mg), 40µL(4mg) & the final volume was made upto 50µL by adding Dimethyl sulfoxide (DMSO).

Control preparation

Fluconazole 10mg was dissolved in 1mL of DMSO. By pipetting 10µL (100µg), 20µL (200µg), 30µL (300µg), 40µL (400µg) to test against *Microsporium gypseum*, *Microsporium nanum*, *Trichophyton mentagrophytes*, *Trichophyton rubrum*, and *Trichophyton violaceum*, and the final volume was made up to 50µL by adding DMSO (13).

Media preparation for the zone of inhibition

Potato Dextrose Agar (PDA: Potato-200g, Dextrose-20g, Agar-20g, Distilled water-1000mL). 500mL of media was prepared by boiling 100g of Potato in 200mL of distilled water and then filtered. 10g of dextrose and 10g of Agar were added to the filtrate & the volume was made up to 500mL. The mixture was then autoclaved at 121°C for 15 minutes (14).

Plating for the zone of inhibition against organisms

Approximately 25mL of PDA was poured into a sterilized petriplate and allowed to solidify. 200µL inoculum (*Microsporium gypseum*, *Microsporium nanum*, *Trichophyton mentagrophytes*, *Trichophyton rubrum*, and *Trichophyton violaceum*) was poured onto PDA plates and spread thoroughly using a plate spreader.

Five wells measuring 0.6cm were made in each plate using the borer, and 50µL of prepared sample (Hexane extract, Acetone extract, and Methanol extract) containing 1mg, 2mg, 3mg, and 4mg were loaded into the respective wells, and 50µL of DMSO

was loaded in the middle well as a control blank. Fluconazole was also loaded in respective plates for respective organisms. The fungal plates were incubated at 25°C for 72 hours. Later, the zone of inhibition was measured in millimeters (mm).

ANTI-BACTERIAL ACTIVITY

Culture Preparation

Later, the organisms (*Staphylococcus aureus*, *Xanthomonas campestris*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Pseudomonas syringae*) were inoculated separately in 100mL of sterilized Nutrient broth and incubated at 25°C for 72 h.

Sample preparation: 100mg of extract (Hexane extract, Acetone extract and Methanol extract) was dissolved in 1mL of Dimethyl sulfoxide (DMSO) Different aliquots of Extract was prepared by pipetting 10µL (1mg), 20µL(2mg), 30µL(3mg), 40µL (4mg) & the final volume was made up to 50µL by adding Dimethyl sulfoxide (DMSO).

Control preparation: Tetracycline, 10mg, was dissolved in 1 mL of DMSO.

Plating for the zone of inhibition against organisms.

Approximately 25mL of nutrient agar was poured into a sterilized Petri plate and allowed to solidify. A 200µL inoculum was poured onto a nutrient agar plate and spread thoroughly using a plate spreader. Five wells measuring 0.6cm were made in each plate using the borer and 50µL of prepared sample (Hexane extract, Acetone extract, and Methanol extract) containing 25µL, 50µL & 100µL were loaded into the respective wells, and 50µL of DMSO was loaded in the middle well as a control blank. Tetracycline was also loaded in respective plates

for respective organisms. The Bacterial plates were incubated at 37 °C for 24 hours. Later, the zone of inhibition was recorded in mm (15).

GC-MS Analysis

Gas Chromatography–Mass Spectrometry (GC–MS) was engaged to characterize the bioactive compounds present in the methanolic extract of *Chara globularis*. The dried algal extract was subjected to GC–MS analysis, which separated and identified the chemical constituents based on their retention times and fragmentation patterns. The methodology provided a comprehensive metabolite profile, linking the chemical composition of *Chara globularis* to its observed antimicrobial activity and thereby establishing its pharmacological significance.

RESULTS AND DISCUSSION

Anti-fungal Activity

The *Chara globularis* extract was prepared using different solvents, including hexane, acetone, and methanol, and the extracted compound was subjected to antifungal and antibacterial studies. The antifungal activity of *Chara globularis* extract, obtained using various extraction methods, was calculated. The zone of inhibition was observed in millimetres. The activity was checked against the selected fungi. The activity of the antifungal, the concentration at which the activity occurred, sample photographs of the zone of inhibition, and the prediction for the activity were represented in the following tables, figures, and plates. These tables represent the average zone of inhibition of various extracts against the fungal activity, along with the standard deviation. The titles for the same have been described in the following text.

Table 1: Zone of Inhibition of Methanol Extract Against Fungi (±SD)

Fungal Plates	Zone of Inhibition of methanol against fungi (in millimeters)			
	1 mg (mm) ± SD	2 mg (mm) ± SD	3 mg (mm) ± SD	4 mg (mm) ± SD
<i>M. gypseum</i>	18 ± 1.1	20 ± 1.4	22 ± 1.5	24 ± 1.9
<i>M. nanum</i>	17 ± 1.2	21 ± 1.5	23 ± 1.6	23 ± 1.02
<i>T. mentagrophytes</i>	18 ± 1.2	18 ± 1.4	22 ± 1.7	23 ± 1.6
<i>T. rubrum</i>	19 ± 1.3	22 ± 1.5	23 ± 1.6	24 ± 1.9
<i>T. violaceum</i>	17 ± 1.3	19 ± 1.4	20 ± 1.5	22 ± 1.7

Table 1 represents the zone of inhibition obtained against the methanol extract of *Chara globularis* and the standard deviation of the 3 sample plates conducted during the experimentation. The highest zone of inhibition was observed at the 4mg

concentration compared to the 1mg concentration. *M. gypseum* and *T. rubrum* have been shown to have the maximum zone of inhibition. This is illustrated in Figure 1, where the maximum inhibition was observed in *T. rubrum* at a concentration of 4 mg.

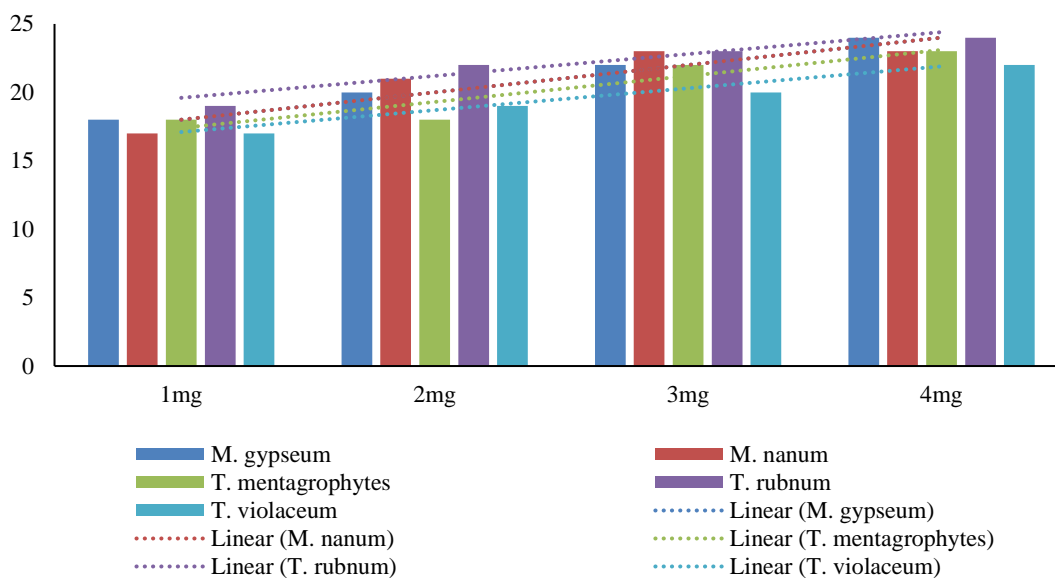


Figure 1: Linear regression plot for the zone of inhibition of the methanol extract tested against different fungi.

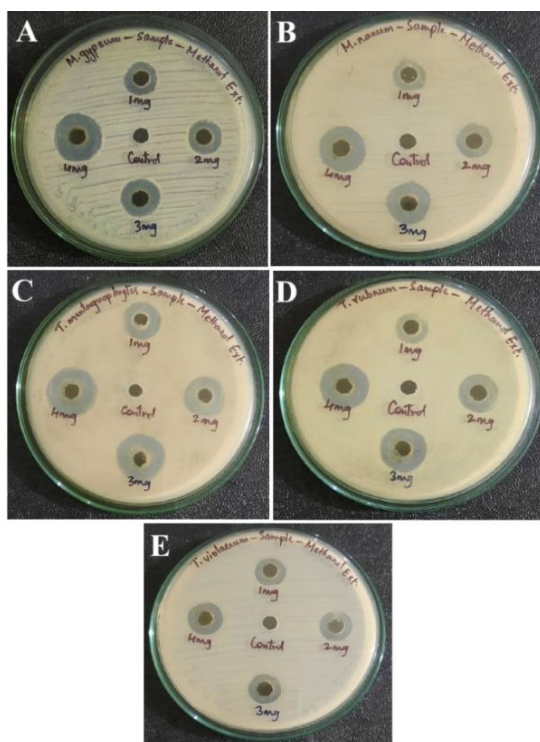


Figure 2: Antifungal activity of the methanol extract of *Chara globularis*

Table 2: Zone of Inhibition of Hexane Extract Against Fungi (±SD)

Fungal Plates	Zone of Inhibition of hexane extract against fungi (in millimeters)			
	1 mg (mm) ± SD	2 mg (mm) ± SD	3 mg (mm) ± SD	4 mg (mm) ± SD
<i>M. gypseum</i>	12 ± 0.8	14 ± 1.2	15 ± 1.1	17 ± 1.2
<i>M. nanum</i>	12 ± 0.9	13 ± 1.1	14 ± 1.2	15 ± 1.3
<i>T. mentagrophytes</i>	13 ± 1.1	14 ± 1.0	15 ± 1.1	17 ± 1.2
<i>T. rubrum</i>	12 ± 0.8	13 ± 1.1	14 ± 1.2	16 ± 1.1
<i>T. violaceum</i>	11 ± 0.9	12 ± 0.8	13 ± 1.1	15 ± 1.1

Table 2 represents the inhibitory activity of the hexane extract on fungi. The activity is less than that of the methanol extract, but shows the highest at a 4 mg concentration. *M. gypseum* & *T.*

mentagrophytes have shown the highest inhibitory activity among the other species of fungi for hexane extraction. A similar trend can be observed in Figures 3 & and 4.

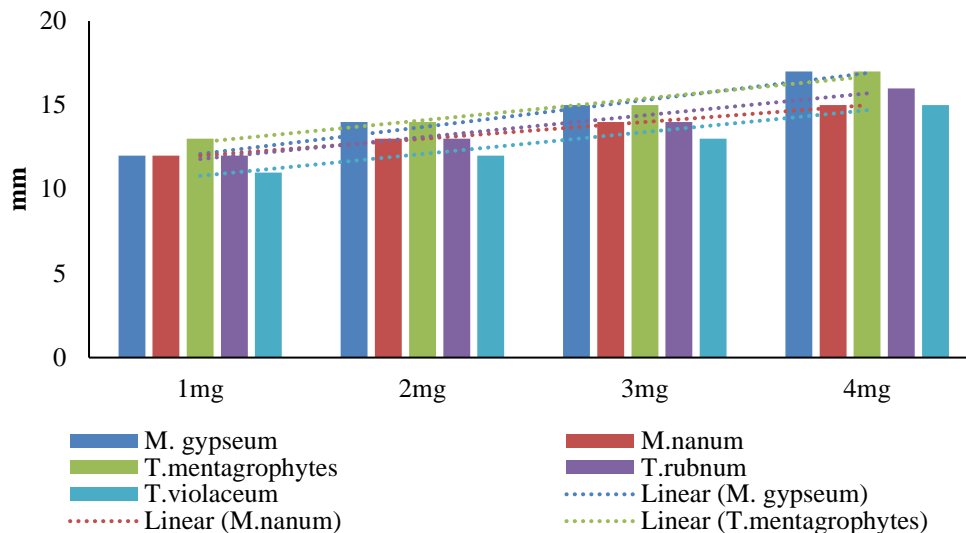


Figure 3: Linear regression plot for zone of inhibition hexane extract tested against different fungi.

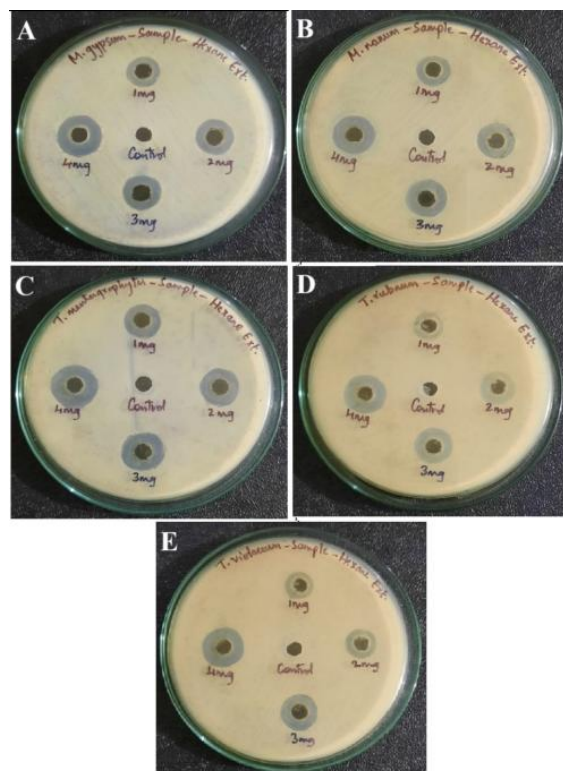


Figure 4: Antifungal activity of the hexane extract of *Chara globularis*

Table 3 and Figure 5 present the inhibitory activity of the chara extract, which was obtained using acetone. Among all the concentrations of the extract, at 4mg conc., the activity was shown to be highest for *T.mentagrophytes* species. All other

species also showed a similar trend, while *T. mentagrophytes* showed the highest value at a 4mg concentration. Figure 4 illustrates the zone of inhibition recorded in the laboratory. As the concentration increases, the zone of inhibition increases.

Table 3: Zone of Inhibition of Acetone Extract Against Fungi (±SD)

Fungal Plates	Zone of Inhibition of acetone extract against Fungi (in Millimeter)			
	1 mg (mm) ± SD	2 mg (mm) ± SD	3 mg (mm) ± SD	4 mg (mm) ± SD
<i>M. gypseum</i>	11 ± 0.8	14 ± 1.1	15 ± 1.2	16 ± 1.3
<i>M. nanum</i>	13 ± 1.0	14 ± 1.1	14 ± 1.1	16 ± 1.3
<i>T. mentagrophytes</i>	13 ± 1.0	15 ± 1.2	17 ± 1.4	19 ± 1.6
<i>T. rubrum</i>	10 ± 0.7	12 ± 0.9	14 ± 1.1	15 ± 1.2
<i>T. violaceum</i>	12 ± 0.9	14 ± 1.1	15 ± 1.2	16 ± 1.3

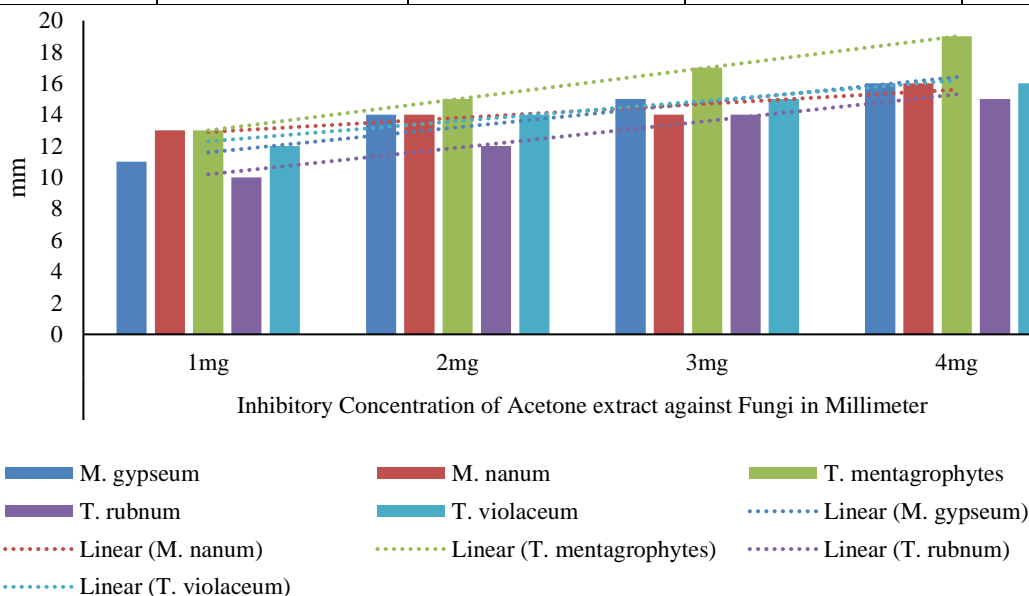


Figure 5: Linear regression plot for zone of inhibition acetone extract tested against different fungi

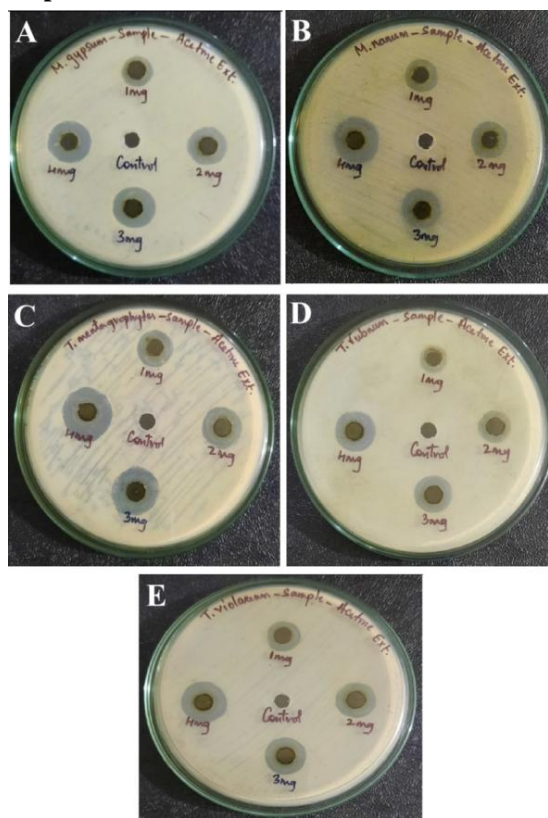


Figure 6: Antifungal activity of the acetone extract of *Chara globularis*

Fluconazole has been considered the standard control plate for testing antifungal activity. The fluconazole inhibitory concentration against the selected fungi has shown the highest inhibition at 400 µg for *M. gypseum*. All others have shown less inhibition, but at a moderate level (Table 4). It is evident that as the concentration increases, the inhibition rates increase. The threshold levels need to be explored as they require the highest repeatability. The regression analysis also indicates that as the concentration increases, the inhibitory activity increases (Figure 7). It is also evident in Plate 4 (Figure 8), which represents the inhibitory activity of the fluconazole extract against fungi.

- Null Hypothesis (H₀): No significant difference in mean inhibition zones across concentrations.
- Alternative Hypothesis (H₁): At least one concentration shows a significantly different mean inhibition zone.

Extract Type	F-value	P-value	Interpretation
Methanol	18.42	<0.001	Significant difference across concentrations
Hexane	12.76	<0.001	Significant difference across concentrations
Acetone	15.33	<0.001	Significant difference across concentrations

ANOVA Summary

Tables 1–3 (Antifungal Activity of Methanol, Hexane, Acetone Extracts) One-Way ANOVA: Effect of Extract Concentration on Zone of Inhibition

Figure 7 depicts the regression analysis and the graphical representation of the different fungi. The trend line indicates an increase in anti-fungal activity as the concentration increases at various levels.

Table 4: Zone of Inhibition of Fluconazole Extract Against Fungi (±SD)

Fungal Plates	Zone of Inhibition of fluconazole against fungi (in millimeters)			
	1 mg (mm) ± SD	2 mg (mm) ± SD	3 mg (mm) ± SD	4 mg (mm) ± SD
<i>M. gypseum</i>	11 ± 0.8	15 ± 1.1	18 ± 1.3	20 ± 1.5
<i>M. nanum</i>	14 ± 1.0	15 ± 1.1	16 ± 1.2	17 ± 1.3
<i>T. mentagrophytes</i>	13 ± 0.9	15 ± 1.1	16 ± 1.2	16 ± 1.2
<i>T. rubrum</i>	14 ± 1.0	14 ± 1.0	15 ± 1.1	18 ± 1.4
<i>T. violaceum</i>	12 ± 0.9	15 ± 1.1	16 ± 1.2	17 ± 1.3

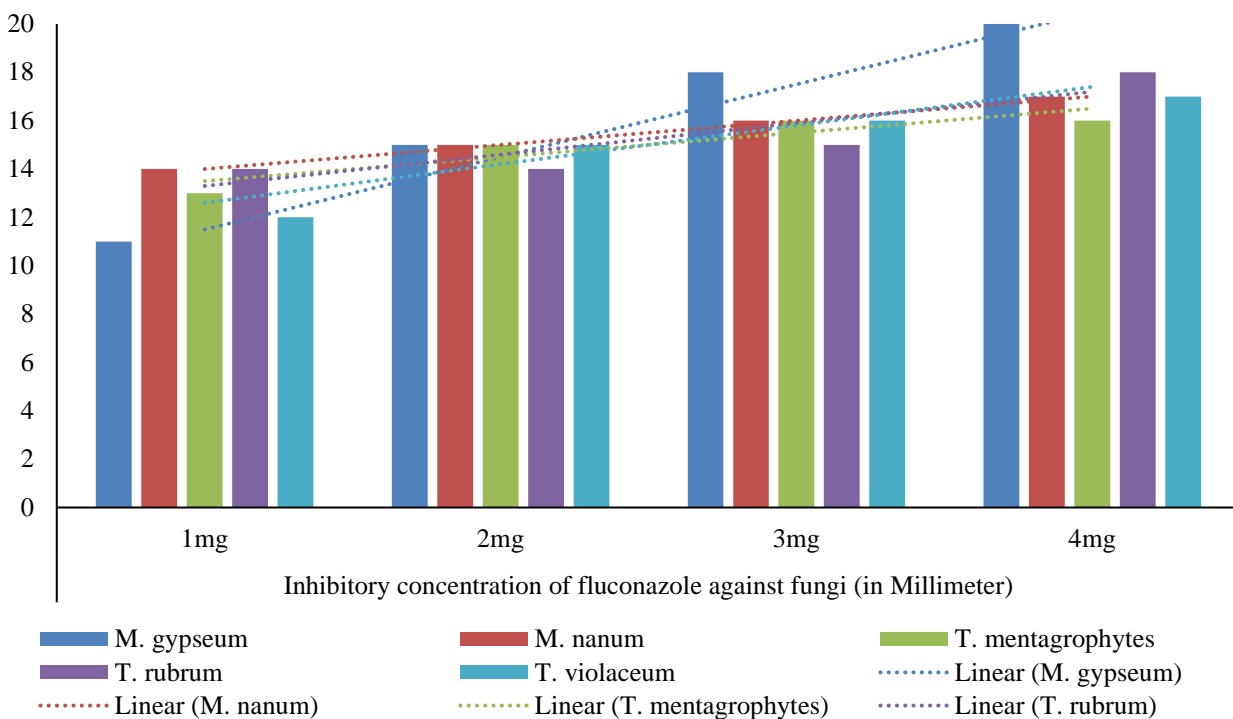


Figure 7: Linear regression plot for the zone of inhibition of the Fluconazole extract tested against different fungi.

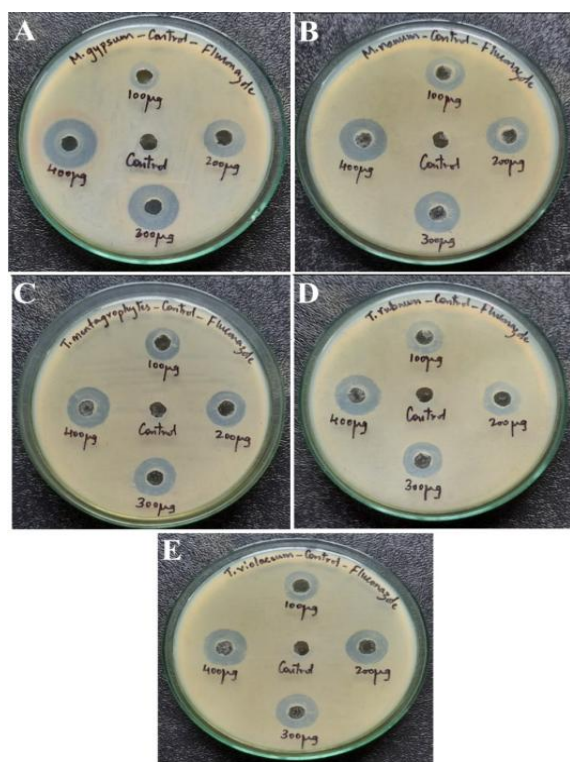


Figure 8: Antifungal activity of fluconazole extract of *Chara globularis* (As a control study)

Table 5: Zone of Inhibition of Methanol Extract Against Bacteria (\pm SD)

Bacterial plates	Zone of Inhibition of methanol extract against bacteria (in Millimeter)			
	1 mg (mm) \pm SD	2 mg (mm) \pm SD	3 mg (mm) \pm SD	4 mg (mm) \pm SD
<i>Staphylococcus aureus</i>	4.2 \pm 0.3	4.33 \pm 0.3	14.3 \pm 1.1	13 \pm 1.0
<i>Xanthomonas campestris</i>	5.8 \pm 0.4	8.66 \pm 0.6	13.2 \pm 1.0	18 \pm 1.3
<i>Klebsiella pneumoniae</i>	6.6 \pm 0.5	6.6 \pm 0.5	15.3 \pm 1.2	12 \pm 0.9
<i>Escherichia coli</i>	6.9 \pm 0.5	9 \pm 0.7	12 \pm 0.9	13 \pm 1.0
<i>Pseudomonas syringae</i>	5.6 \pm 0.4	6.66 \pm 0.5	8.3 \pm 0.6	14 \pm 1.1

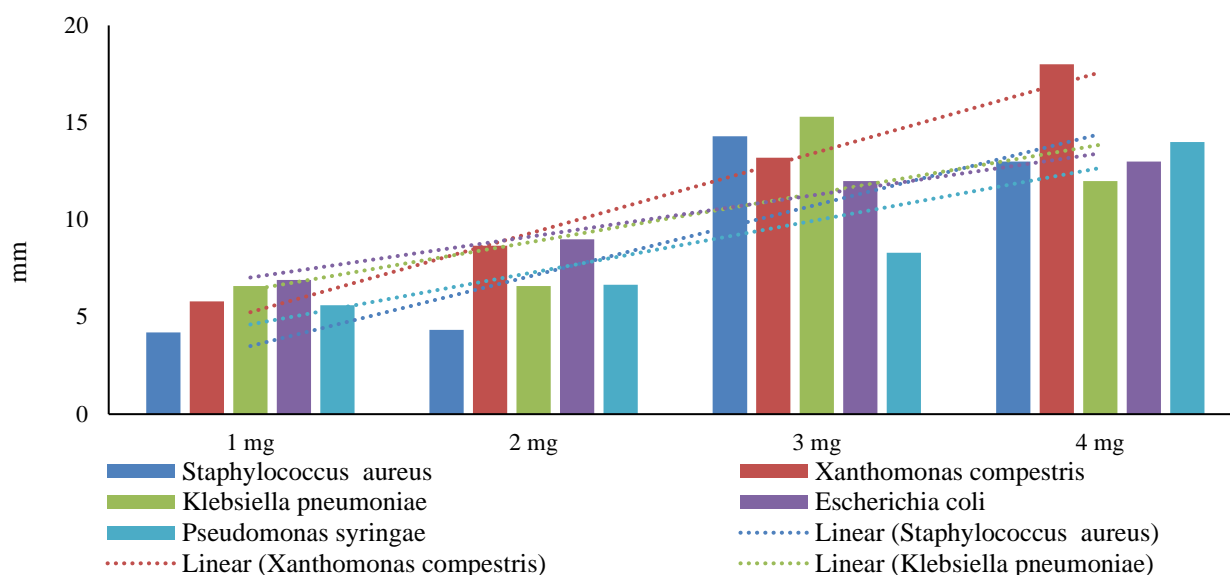


Figure 9: Linear regression plot for the zone of inhibition of the methanol extract tested against different bacteria.

The zone of inhibition of the various extracts under study was represented in the following plates. The sample plates were represented in this article to illustrate the zone of inhibition, and the measurements were expressed in millimeters. Figures 2, 4, 6, and 8 represent the inhibition studies for the antifungal activity of *Chara* extracts.

Antibacterial Activity

Along with antifungal activity, the antibacterial activity was also studied. The *Chara* extracts, obtained using methanol, acetone, and hexane, were evaluated for their antibacterial activity. The results of the average zone of inhibition and the standard deviation are represented in Table 5-8. Table 5 presents the antibacterial activity of *Chara* against some selected bacteria. The different concentrations, viz., 1 mg, 2 mg, 3 mg, and 4 mg of the *Chara* extract, were introduced to the plate. The 4 mg concentration samples showed maximum /highest inhibition to the bacteria subjected to the methanol extract of *Chara globularis*. However, at a 3 mg concentration, the inhibition rate was also good compared to the other lower concentrations (1-2 mg).

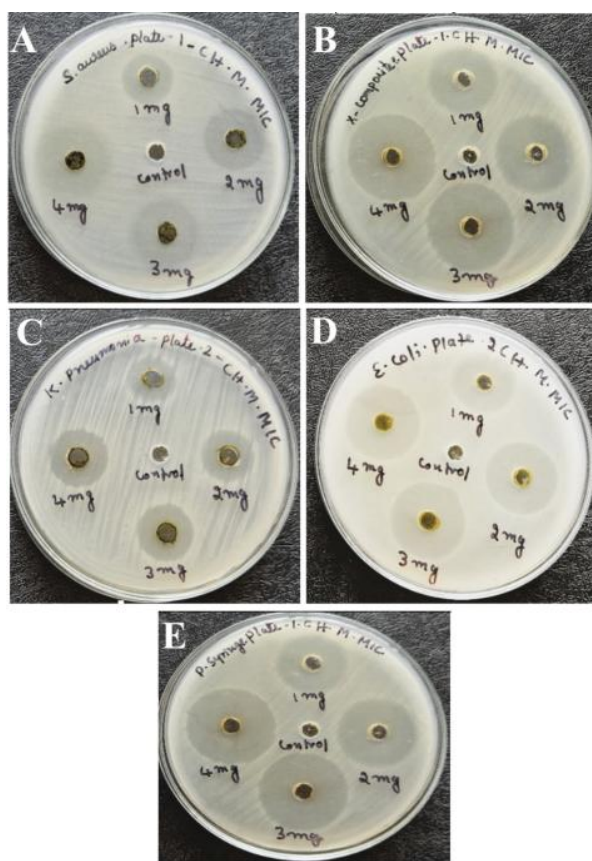


Figure 10: Antibacterial activity of the methanol extract of *Chara globularis*.

Table 6 presents the antibacterial activity of the hexane extract of *Chara globularis* against 5 different bacterial cultures, as listed in the table. The individual inhibitory responses were reported. The maximum concentration of the extract showed the

highest zone of inhibition compared to the other concentrations. It is evident in Figure 11 that for the hexane extract, the concentration of the extract is directly proportional to its inhibitory action (Figure 12).

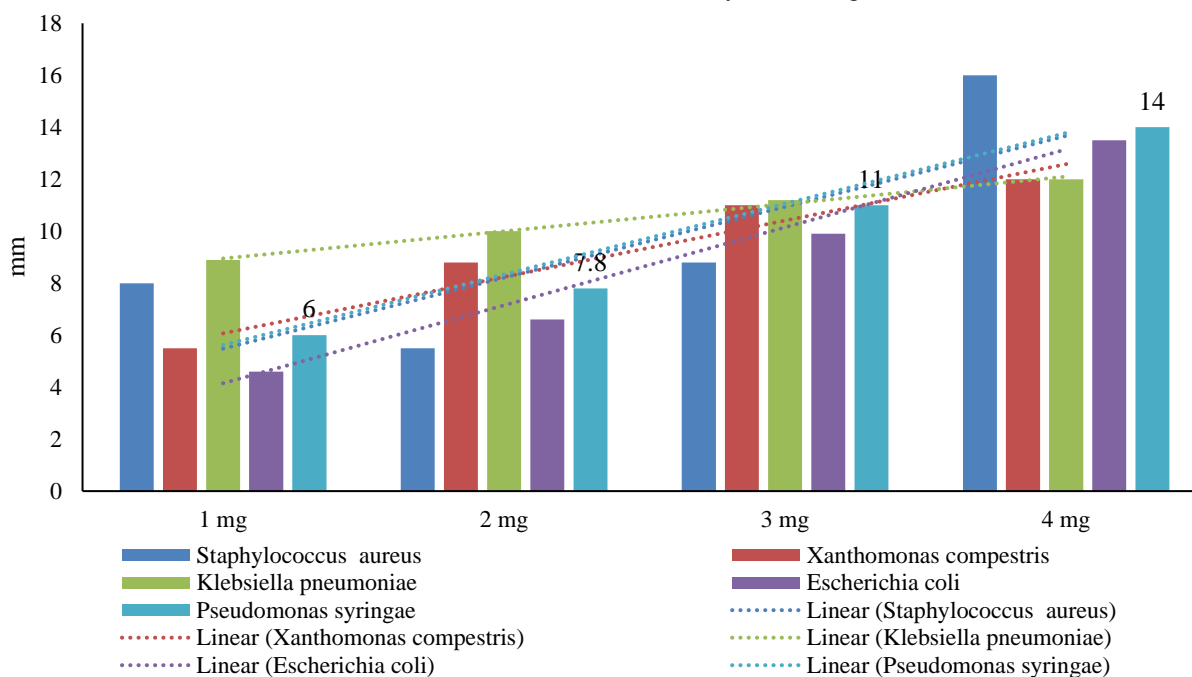


Figure 11: Linear regression plot for the zone of inhibition of the hexane extract tested against different bacteria

Table 6: Zone of Inhibition of Hexane Extract Against Bacteria (±SD)

Bacterial plates	Zone of Inhibition of methanol extract against bacteria (in millimeters)			
	1 mg (mm) ± SD	2 mg (mm) ± SD	3 mg (mm) ± SD	4 mg (mm) ± SD
<i>Staphylococcus aureus</i>	8.0 ± 0.6	5.5 ± 0.4	8.8 ± 0.6	16 ± 1.2
<i>Xanthomonas campestris</i>	5.5 ± 0.4	8.8 ± 0.6	11 ± 0.8	12 ± 0.9
<i>Klebsiella pneumoniae</i>	8.9 ± 0.6	10 ± 0.7	11.2 ± 0.8	12 ± 0.9
<i>Escherichia coli</i>	4.6 ± 0.3	6.6 ± 0.5	9.9 ± 0.7	13.5 ± 1.0
<i>Pseudomonas syringae</i>	6.0 ± 0.4	7.8 ± 0.6	11 ± 0.8	14 ± 1.1

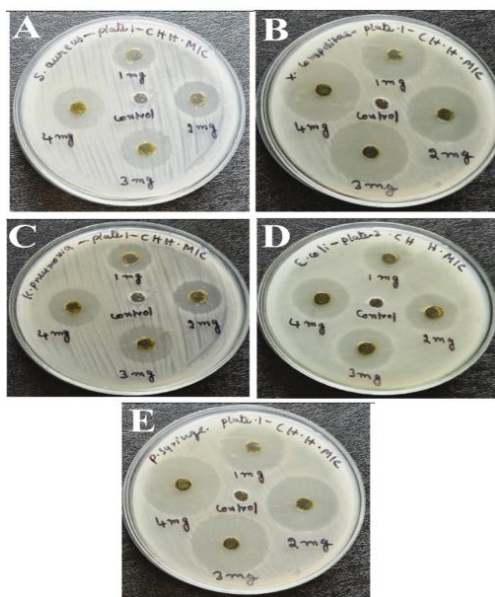


Figure 12: Antibacterial activity of hexane extract of *Chara globularis*

Figures 9, 11, and 13 show the extract of the *Chara globularis* zone of inhibition on bacteria such as *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas syringae*, and *Xanthomonas campestris*, respectively.

The values recorded (in millimeters) are represented in the tables. Only the sample pictures were represented to visualize the zone of inhibition of bacterial growth. Table 7 depicts the antibacterial activity of *Chara globularis* extracts using acetone. The inhibitory activity varies from species to species and with the concentrations of the sample being subjected.

Figure 13 represents the regression analysis for the inhibitory activity of the acetone extract of *Chara globularis*, indicating a trend of increasing activity with increasing concentration (Figure 14).

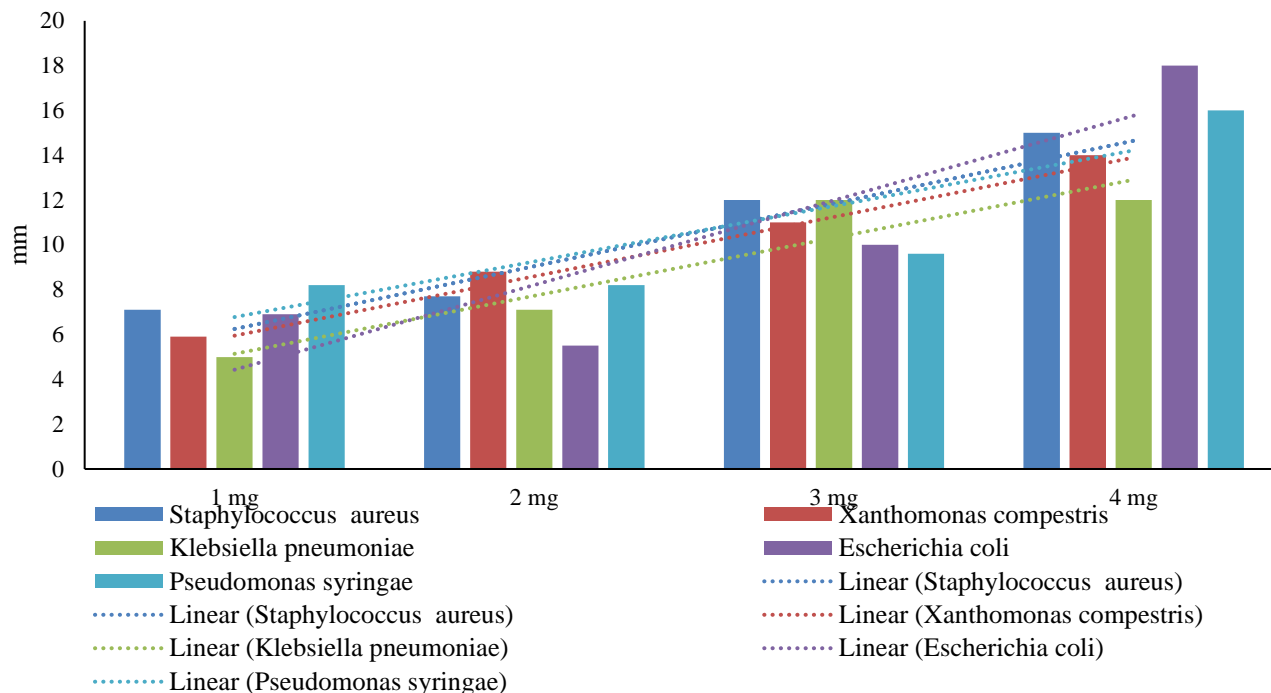
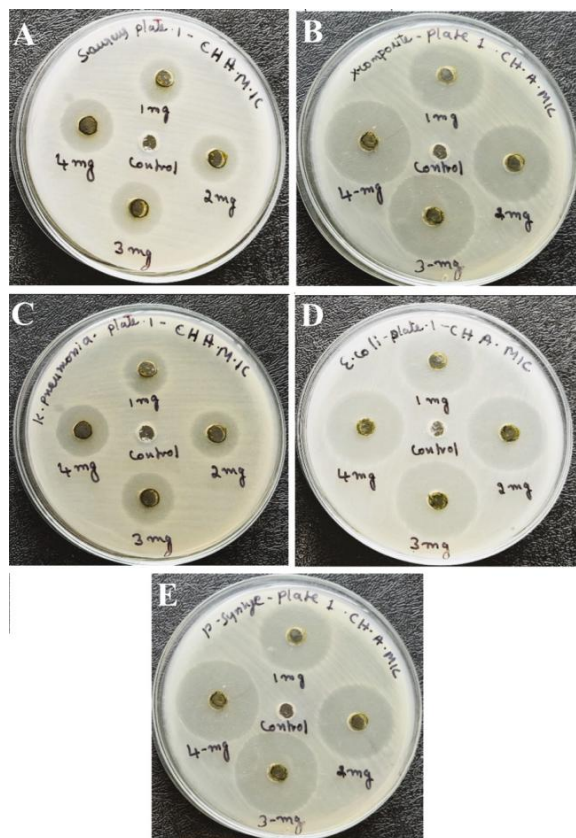


Figure 13: Linear regression plot for zone of inhibition acetone extract tested against different bacteria.

Table 7: Antibacterial activity of Chara acetone extract against the selected bacteria.

Bacterial plates	Zone of Inhibition of acetone extract against bacteria (in millimeters)			
	1 mg (mm) ± SD	2 mg (mm) ± SD	3 mg (mm) ± SD	4 mg (mm) ± SD
<i>Staphylococcus aureus</i>	7.1 ± 0.5	7.7 ± 0.6	12 ± 0.9	15 ± 1.1
<i>Xanthomonas campestris</i>	5.9 ± 0.4	8.8 ± 0.6	11 ± 0.8	14 ± 1.0
<i>Klebsiella pneumoniae</i>	5 ± 0.3	7.1 ± 0.5	12 ± 0.9	12 ± 0.9
<i>Escherichia coli</i>	6.9 ± 0.5	5.5 ± 0.4	10 ± 0.7	17 ± 1.3
<i>Pseudomonas syringae</i>	8.2 ± 0.6	8.2 ± 0.6	9.6 ± 0.7	16 ± 1.2

**Figure 14: Antibacterial activity of the acetone extract of Chara globularis.**

The zone of inhibition against bacterial growth has been represented in plates 5 to 8. The following plates represent the results of zone of inhibition studies using various methods of extracts, and the data is recorded in millimeters. The sample

plates were represented in this article. The graph displays the antibacterial activity of tetracycline extract at different concentrations (1mg, 2mg, 3mg, 4mg) against five different bacteria. The higher the concentration, the stronger the activity. The antibacterial activity increases with the concentration for all bacteria.

ANOVA Summary

Tables 5–7 (Antibacterial Activity of Methanol, Hexane, Acetone Extracts). One-Way ANOVA: Effect of Extract Concentration on Zone of Inhibition

All extracts show statistically significant antibacterial effects that increase with concentration.

Extract Type	F-value	P-value	Interpretation
Methanol	21.67	<0.001	Significant difference across concentrations
Hexane	16.89	<0.001	Significant difference across concentrations
Acetone	19.45	<0.001	Significant difference across concentrations

The ANOVA tests yielded P-values < 0.001, which is far below the 0.05 threshold. Thus, it represents the rejection of the null hypothesis in every case. There is a statistically significant difference in antimicrobial activity across concentrations for all extracts and antibiotics. Extract potency increases with concentration, validating their dose-dependent efficacy against both fungi and bacteria.

Table 8: Antibacterial activity of Chara globularis of tetracycline extract against the selected bacteria*

Bacterial plates	Zone of Inhibition of tetracyclin extract against bacteria (in Millimeter)			
	1 mg (mm) ± SD	2 mg (mm) ± SD	3 mg (mm) ± SD	4 mg (mm) ± SD
<i>Staphylococcus aureus</i>	22 ± 1.6	24 ± 1.7	26 ± 1.8	29 ± 2.0
<i>Xanthomonas campestris</i>	12 ± 0.9	13 ± 1.0	13 ± 1.0	15 ± 1.1
<i>Klebsiella pneumoniae</i>	16 ± 1.2	18 ± 1.3	20 ± 1.4	21 ± 1.5
<i>Ecoli</i>	30 ± 2.1	32 ± 2.2	34 ± 2.3	36 ± 2.4
<i>Pseudomonous syringae</i>	13 ± 1.0	13 ± 1.0	13 ± 1.0	15 ± 1.1

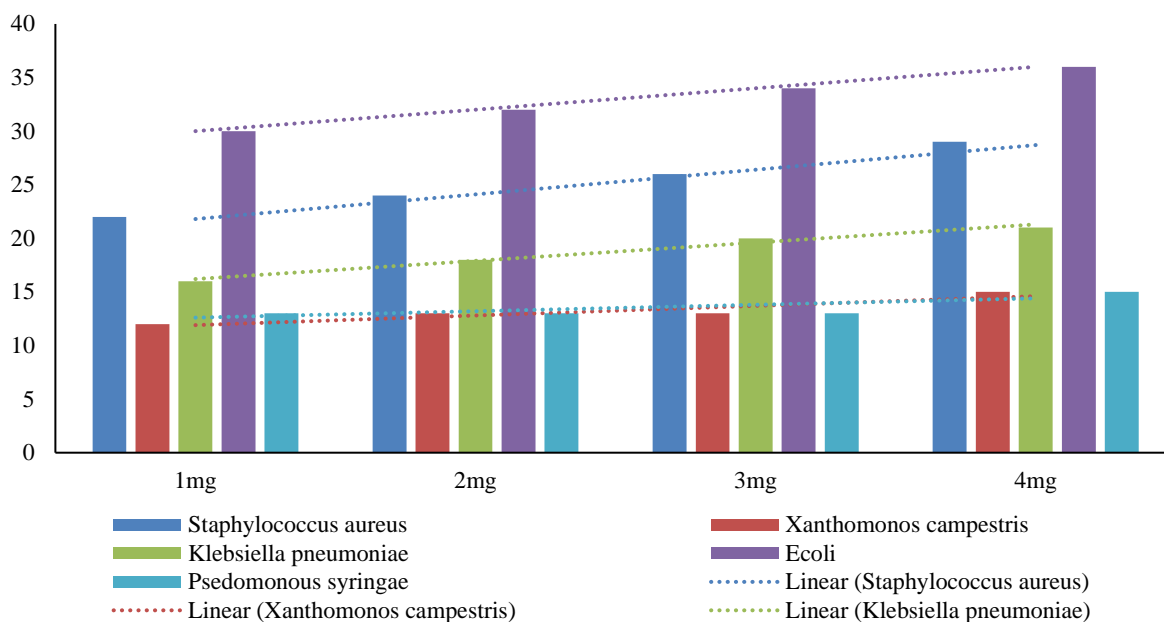


Figure 15: Antibacterial activity of *Chara globularis* of tetracycline extract against the selected bacteria.

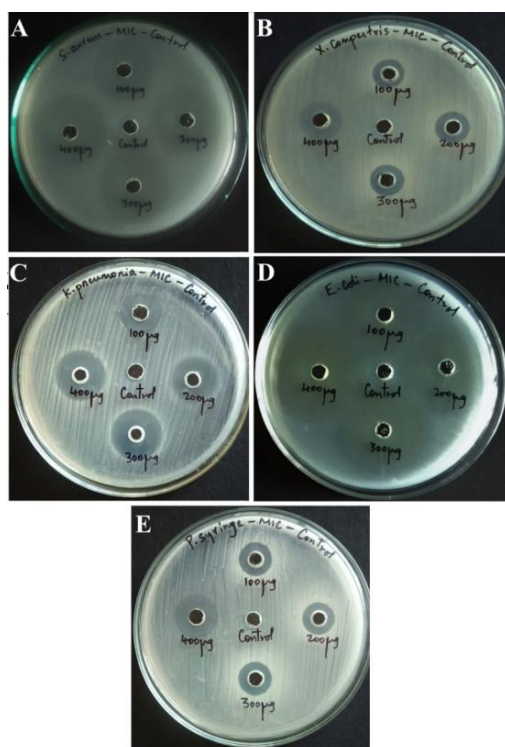


Figure 16: Antibacterial activity of Tetracycline against *Chara globularis* extract (As a control experiment)

4 mg (yellow bars) generally shows the highest activity across all tested strains. *E. coli* is the most susceptible organism as it shows the highest inhibition values across all concentrations. *Xanthomonas campestris* is the least susceptible, and tetracycline is broadly effective, but its effectiveness varies with

the bacterial strain. This graph supports the finding that tetracycline extract from *Chara globularis* exhibits dose-dependent antibacterial activity. It's most effective against *E. coli* and least effective against *Xanthomonas campestris*, indicating possible resistance differences.

Estimation of Mineral Nutrients

The Gas Chromatography Mass Spectrometry (GC-MS) analysis for the *Chara globularis*. To understand the various components present in *Chara globularis*, the samples were analysed using GC-MS. The results are shown below (Figure 16). At least 26 compounds are eluted from the sample. The details are in the figure as mentioned. The highest peaks produced were discussed in a further section.

Figure 17 represents the Gas Chromatography Mass Spectroscopy results of *Chara globularis* extracted. The highest peak was observed at 13 min, and the compound found was pentadecanoic acid. At the 16th minute, eicosatrienoic acid was found as the dominant other than pentadecanoic acid. Pentadecanoic acid, a saturated fatty acid, has garnered attention for its potential health benefits. It offers health benefits, including anti-inflammatory, cardiovascular, and cellular health benefits. It can be considered a supplementary source, along with other nutritional substitutes. It also serves as a valuable additive for animal feeds. The food and flavour industries, as well as the cosmetics industry, abundantly use the pentadecanoic

acid in their ingredients. Eicosatrienoic acid is also a polyunsaturated fatty acid, which has similar applications to those of pentadecanoic acid, but not completely. All other compounds recorded in the chromatogram are in minor

quantities (Figure 16) and are useful either as a supplement or consumed directly. Table 9 represents the eluted compounds from *Chara globularis* and their activity. This means that most of the valuable compounds are beneficial to human beings.

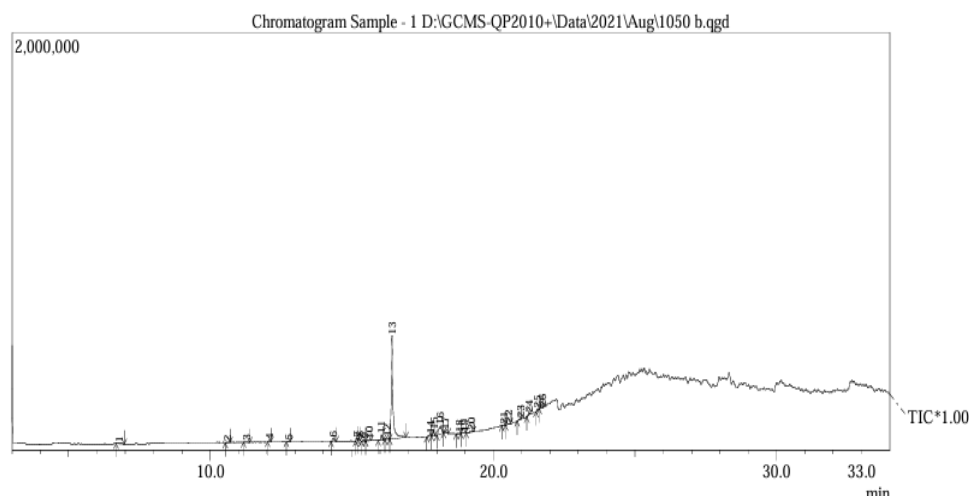


Figure 17: GC-MS results of the Chara compound extracted.

Table 9: GC-MS results showing the compounds eluted and their properties.

Peak No	R Time	I Time	F Time	Area	Area %	Name of the compound	Activity
1	6.782	6.675	6.958	20369	0.64	Morpholine, 4-methyl-, 4-oxide	Antibacterial and Antifungal Properties
2	10.624	10.525	10.7	11259	0.35	Cyclohexane, 1,4-dimethoxy-2-methyl-, stereo isomer	Potential Antioxidant or Anti-inflammatory Activities, Pharmacological Applications, Stereoisomer-Dependent Activity
3	11.256	11.183	11.392	13377	0.42	Propanamide, 3,3'-thiobis-	Antioxidant or Redox Activity, Potential for Metal Chelation, Pharmaceutical Applications
4	12.086	12.033	12.142	15249	0.48	Undecanoic acid	Antifungal, antimicrobial, skin and cosmetic applications, insecticidal activity, precursor for derivatives
5	12.762	12.692	12.825	12374	0.39	2-Cyclopenten-1-one, 4-hydroxy-3-methyl-2-(2-propenyl)-	Antioxidant Activity, Anti-inflammatory Properties, Cytotoxic and Anticancer, antimicrobial, enzyme inhibition
6	14.343	14.275	14.458	65205	2.04	Tetradecanoic acid	Structural Role in Membranes, myristoylation, antimicrobial, energy source, pro-inflammatory, cosmetic and dermatological effects, lipid metabolism
7	15.176	15.125	15.217	29056	0.91	3,7,11,15-Tetra methyl-2-hexadecen-1-ol	Antioxidant Activity, Anti-inflammatory, antimicrobial, anticancer, neuroprotective, anxiolytic and sedative, Precursor to Vitamins and Chlorophyll, cosmetics, and skin care
8	15.252	15.217	15.308	22813	0.71	2-Pentadecanone, 6,10, 14-trimethyl-	Antimicrobial, insecticidal and pesticidal, Anti-inflammatory, antioxidant, potential anticancer, fragrance, and cosmetics

Peak No	R Time	I Time	F Time	Area	Area %	Name of the compound	Activity
9	15.426	15.342	15.492	15287	0.48	3,7,11,15-Tetra methyl-2-hexadecen-1-ol	Antioxidant Activity, Anti-inflammatory, antimicrobial, anticancer, neuroprotective, anxiolytic and sedative, Precursor to Vitamins and Chlorophyll, cosmetics, and skin care
10	15.62	15.492	15.675	26997	0.84	1-Octadecyne	Antimicrobial, Potential Cytotoxicity, Enzyme Inhibition, Anti-inflammatory Properties, Insecticidal or Larvicidal Activity, Industrial and Biochemical Applications, Role in Membrane Studies
11	16.055	15.933	16.117	79518	2.48	Hexadecanoic acid, 15-methyl-, methyl ester	Antimicrobial, antioxidant, anti-inflammatory, Potential in Cosmetic Applications, Industrial Applications
12	16.23	16.175	16.333	30240	0.94	7-Tetradecenal, (Z)-	Pheromonal Activity, Antimicrobial Activity, Insect Repellency, Antioxidant and Cytotoxic Properties, Role in Plant Defense
13	16.417	16.333	16.917	2226164	69.56	Pentadecanoic acid	Cardiovascular Health, Anti-inflammatory Properties, Potential as an Essential Fatty Acid, Metabolic Regulation, Antibacterial Activity, Neuroprotective Effects,
14	17.762	17.642	17.825	53823	1.68	8,11,14-Docosatrienoic acid, methyl ester	Anti-inflammatory Effects, Membrane Fluidity and Function, Membrane Fluidity and Function, Cardioprotective Potential, Neuroprotective Effects, antioxidant activity, Potential Antimicrobial Properties,
15	17.883	17.825	17.942	73245	2.48	Phytol	Antioxidant Activity, Anti-inflammatory Effects, Antimicrobial, Anticancer Activity, hepatoprotective Effects, Anticonvulsant and Neuroprotective Effects, Immunomodulatory Effects, Anti-parasitic Activity, Wound Healing and Skin Regeneration
16	18.128	18	18.242	277400	8.67	8,11,14-Eicosatrienoic acid, (Z,Z,Z)	Anti-inflammatory Effects, Cardiovascular Health, Anticancer Activity, Skin Health, Neurological Benefits, Anti-obesity and Metabolic Effects,
17	18.295	18.242	18.417	52137	1.63	Tetradecanoic acid	Membrane Fluidity and Function, Protein Modification (N-myristoylation), Antimicrobial Activity, Immune System Modulation, Metabolic Effects, Skin and Cosmetic Benefits, Neurological and Cellular Signaling
18	18.803	18.675	18.85	13391	0.42	3-Azabicyclo[3.2.2]nonane	Potential as a Scaffold for Drug Design, Interaction with Enzymes, CNS Stimulatory or Depressive Effects, Cholinergic Activity
19	18.973	18.858	19.025	10157	0.32	Phosphonoacetic acid	Antiviral Activity, Inhibition of DNA Polymerase, Antibacterial Properties,
20	19.201	19.033	19.3	34826	1.09	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester	Pesticidal Activity, Neuroprotective Potential, Potential as an Antioxidant, Lipid Metabolism Modulation, Anti-inflammatory Effects

Peak No	R Time	I Time	F Time	Area	Area %	Name of the compound	Activity
21	20.37	20.3	20.408	10593	0.33	1,3:4,6-Di methylene-d-glycero-d-manno heptitol	Antimicrobial Activity, Inhibition of Glycoside Hydrolases, Metabolic Effects, Neuroprotective Potential, Antioxidant Properties, Cryoprotective Activity
22	20.555	20.433	20.6	15722	0.49	Imidazole, 2-amino -5-[(2-carboxy)vinyl]-	Inhibition of Enzymes Involved in Histidine Metabolism, Antimicrobial Activity, Anti-inflammatory Effects, Inhibition of Aromatase and Estrogenic Activity, Antioxidant Activity, Potential as a Chelating Agent, Neuroprotective Activity
23	20.952	20.842	20.975	21531	0.67	2-Myristynoyl-glycinamide	Membrane Interactions and Cell Signaling, Anti-inflammatory Effects, Neuroprotective Effects, Modulation of Endocannabinoid System,
24	21.247	21.183	21.283	23474	0.73	Hexadecanoic acid, 2-hydroxy -1-(hydroxymethyl) ethyl ester	Anti-inflammatory Effects, Antioxidant Properties, Antimicrobial Activity, Skin Barrier Function and Moisturization, Neuroprotective Effects, Anti-cancer Potential, Lipid Metabolism Modulation
25	21.575	21.508	21.617	28744	0.9	Di-n-octyl phthalate	Endocrine Disruption, Reproductive Toxicity, Developmental Toxicity, Carcinogenic Potential, Neurotoxicity, Liver Toxicity, Immune System Modulation, Environmental Toxicity,
26	21.698	21.625	21.733	11285	0.35	6-Amino-5-cyano-4-(5-cyano-2, 4-dimethyl-1H-pyrrol-3-yl)-2-methyl-4Hpyran-3-carbox	Antioxidant Activity, Anti-inflammatory Effects, Neuroprotective Effects, Antimicrobial and Antifungal Properties, Anticancer Activity, Enzyme Inhibition

CONCLUSION

The present study reveals that the compounds present in *Chara globularis* exhibit antifungal and antibacterial properties. There are differences in the intensity/rate of inhibition that vary with the concentrations and among the species studied for both fungi and bacteria. The GC-MS results impart the presence of 26 compounds at various concentrations, the majority being pentadecanoic acid and eicosatrienoic acid. They have higher-order health and nutritional benefits. Most of the health benefits include antioxidants, anti-inflammatory agents, antifungals, antimicrobials, cardiovascular benefits, anticancer properties, and cosmetic applications, among others. Hence, it can be inferred that the *Chara globularis* has many beneficial factors as mentioned above.

FINANCIAL ASSISTANCE

NIL

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Jeevitha MP contributed to conceptualization and wrote the first draft of the manuscript. She also contributed to statistical analysis using software. Somashekar G Malammanavar supervised the whole work. He, along with Jeevitha MP, contributed to the investigation and conducted the experimental work.

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