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Phytochemical Characterization and *In Vitro* Antibacterial Activity of *Turbinaria Conoides* Against Clinical Pathogens

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Abstract

Marine macroalgae are recognized as valuable sources of structurally diverse bioactive metabolites with potential pharmaceutical and biotechnological applications. The present study investigated the phytochemical composition and in vitro antibacterial activity of the brown macroalga *Turbinaria conoides* collected from the Pamban coastal region of Tamil Nadu, India. The collected biomass was thoroughly washed, shade-dried, powdered, and subjected to aqueous and acetone extraction. Aqueous extraction was performed by thermal treatment, while acetone extraction was carried out using a Soxhlet apparatus. The resulting extracts were evaluated for extraction yield and qualitatively screened for major phytochemical classes. Their antibacterial activity was subsequently assessed against *Escherichia coli* using the agar well diffusion method. The acetone and aqueous extraction procedures yielded 2.1% and 5.2%, respectively. The acetone extract showed positive reactions for phenols, tannins, flavonoids, glycosides, terpenoids, alkaloids, and phytosterols, whereas saponins and steroids were not detected. In contrast, the aqueous extract contained tannins, saponins, alkaloids, and proteins, while phenols, flavonoids, glycosides, terpenoids, phytosterols, and steroids were not detected. Both extracts exhibited inhibitory activity against *E. coli*, indicating measurable antibacterial potential. The observed differences in phytochemical profiles between the extracts demonstrate the influence of extraction solvent on the recovery of bioactive constituents from *T. conoides*. Overall, the findings provide preliminary evidence supporting *T. conoides* as a potential source of naturally derived antibacterial metabolites. Further investigations involving quantitative phytochemical analysis, chromatographic and spectroscopic characterization, minimum inhibitory concentration determination, broader pathogen screening, and mechanistic studies are required to identify the compounds responsible for the observed antibacterial activity and establish their biological significance.

Keywords: Acetone extract, Aqueous extract, Brown macroalgae, *Escherichia coli,* Phytochemical screening, *Turbinaria conoides.*

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1. Introduction

The marine environment represents a rich reservoir of chemically diverse natural products with considerable biological and pharmacological significance. Marine macroalgae are particularly important sources of structurally diverse primary and secondary metabolites that contribute to ecological interactions and chemical defence. Their extracts have attracted considerable research interest because of reported antioxidant, antibacterial, antifungal, anti-inflammatory, and other biological properties. Among the major algal groups, brown macroalgae (Phaeophyceae) are recognized for their diverse chemical composition, which includes phenolic compounds, flavonoids, terpenoids, steroids, phlorotannins, and other biologically active constituents (Gogineni *et al*., 2019). The occurrence and abundance of these metabolites can vary considerably with species, geographical origin, environmental conditions, season, tissue characteristics, and extraction methodology.

Turbinaria conoides is a marine brown macroalga belonging to the family Sargassaceae and order Fucales. The species is distributed predominantly in tropical and subtropical marine environments and commonly occurs in rocky, reef-associated, and shallow coastal habitats. *T. conoides* is characterized by a robust, branched thallus with distinctive blade-like structures and represents an ecologically and biotechnologically relevant member of tropical brown algal communities. Along the Indian coast, including the Gulf of Mannar region, *T. conoides* is an important component of the marine macroalgal flora. In addition to its ecological significance, previous investigations have reported biological activities associated with different extracts and fractions of *T. conoides*, including antibacterial effects against selected pathogenic bacteria (Noorjahan *et al*., 2022).

The biological properties of macroalgal extracts are closely associated with the nature and concentration of their extractable metabolites. Brown algae contain metabolites with different physicochemical characteristics, and consequently, their recovery can vary substantially according to solvent polarity and extraction conditions. Solvents such as water and acetone differ in their ability to extract polar, moderately polar, and relatively less-polar constituents from algal biomass. Comparative extraction using solvents of different polarities can therefore provide useful information regarding the distribution of phytochemical constituents and their potential relationship with biological activity. Preliminary qualitative phytochemical screening is widely used as an initial approach for detecting major classes of secondary metabolites in crude plant and algal extracts and can provide a basis for subsequent chemical characterization (Franco *et al*., 2008).

Antibacterial screening of marine macroalgae is of particular interest because naturally derived metabolites may provide chemical scaffolds for the development of alternative or complementary antimicrobial agents. However, antibacterial activity reported for a given seaweed species may differ considerably among studies because of differences in extraction procedures, solvent systems, extract concentrations, target organisms, assay conditions, and geographical origin of the biomass. Therefore, standardized evaluation of solvent-specific extracts is important for establishing reproducible evidence of the antibacterial potential of *T. conoides*. Previous studies have indicated that extracts of *T. conoides* contain biologically active constituents and exhibit antibacterial activity against selected microorganisms, supporting further investigation of this species as a source of marine-derived bioactive compounds (Noorjahan *et al*., 2022; Suresh *et al*., 2023).

Despite these reports, comparative information on the phytochemical profiles and antibacterial activity of aqueous and acetone extracts of *T. conoides* collected from the Pamban coastal region remains limited. Differences in extraction efficiency and solvent selectivity may result in distinct phytochemical profiles and consequently influence the observed antibacterial response. A comparative assessment of these extracts can therefore provide useful preliminary information on the relationship between extraction solvent, phytochemical composition, and antibacterial activity.

Accordingly, the present study aimed to characterize aqueous and acetone extracts of *T. conoides* collected from the Pamban coastal region of Tamil Nadu, India. The study specifically evaluated extraction yield, qualitatively profiled major phytochemical classes, and assessed the in vitro antibacterial activity of the extracts against *Escherichia coli* using the agar well diffusion method. The findings provide preliminary information on the solvent-dependent phytochemical composition and antibacterial potential of *T. conoides* and establish a basis for subsequent quantitative chemical characterization and evaluation of its bioactive constituents.

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2. Materials and Methods

2.1 Sample collection and Preparation

The brown macroalga *Turbinaria conoides* was collected from the Pamban coastal region of Tamil Nadu, India. The collected biomass was transferred to the laboratory in clean, conditioned plastic bags and processed for extraction. The algal material was thoroughly washed several times with water to remove adhering sand, epiphytes, and other surface-associated debris. The cleaned biomass was subsequently shade-dried at room temperature for seven days and homogenized into a fine powder using a laboratory grinder. The powdered material was stored in airtight containers at room temperature until further extraction and analysis (Kavitha *et al*., 2019).

Fig 1 ***Turbinaria conoides***

2.2 Preparation of Acetone Extract

The acetone extract of *T. conoides* was prepared by Soxhlet extraction. Briefly, 10 g of the dried algal powder was transferred into a cellulose extraction thimble and extracted with 100 mL acetone, maintaining a sample-to-solvent ratio of 1:10 (w/v). Soxhlet extraction was carried out continuously for 6 h. The resulting extract was filtered and concentrated under reduced pressure at 40 °C using a rotary evaporator to obtain the crude acetone extract. The concentrated extract was weighed, and the extraction yield was calculated. The extract was subsequently stored at 4 °C in a sealed container until further phytochemical and antibacterial analyses (Perumal *et al*., 2026).

2.3 Preparation of Aqueous Extract

For aqueous extraction, 50 g of dried *T. conoides* powder was mixed with 500 mL of distilled water, corresponding to a 1:10 (w/v) sample-to-solvent ratio. The mixture was heated at approximately 100 °C for 60 min with intermittent stirring to facilitate the extraction of water-soluble constituents. After completion of the extraction, the mixture was allowed to cool to room temperature and was initially filtered through sterile muslin cloth to remove coarse algal residues. The filtrate was subsequently passed through Whatman No. 1 filter paper. The resulting aqueous filtrate was concentrated in a water bath at 50 °C to obtain the crude aqueous extract. The concentrated extract was weighed to determine the extraction yield and stored at 4 °C until further analysis (Perumal *et al*., 2026).

2.4 Determination of Extraction Yield

The extraction yield was determined gravimetrically and expressed as a percentage of the initial dry algal biomass. The percentage yield was calculated using the following equation:

Extraction yield (%) = (Weight of dried crude extract / Weight of dry algal powder) × 100

The calculated yields of the aqueous and acetone extracts were used for comparative evaluation of extraction efficiency.

2.5 Preliminary Phytochemical Screening

The aqueous and acetone extracts were subjected to preliminary qualitative phytochemical screening to detect major classes of bioactive constituents. The extracts were screened for phenols, tannins, flavonoids, glycosides, saponins, terpenoids, steroids, alkaloids, carbohydrates, proteins, and phytosterols using standard qualitative chemical reactions. The presence or absence of each phytochemical class was determined based on characteristic colour development, precipitate formation, or foam formation following the respective test procedures. The phytochemical screening was performed according to the experimental procedures described by Gutiérrez *et al.* (2018). The results were recorded qualitatively as positive or negative reactions.

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2.6 Preparation of Bacterial Inoculum

The antibacterial activity of the *T. conoides* extracts was evaluated against *Escherichia coli*. A pure culture of *E. coli* was inoculated into sterile nutrient broth and incubated at 37 °C for 12 h. Following incubation, the bacterial suspension was adjusted to approximately 0.5 McFarland turbidity standard to obtain a standardized inoculum for the agar diffusion assay.

2.7 In Vitro Antibacterial Assay

The antibacterial activity of the aqueous and acetone extracts was determined using the agar well diffusion method with minor modifications to the procedure reported by Gumgumjee *et al.* (2018). Mueller–Hinton agar plates were prepared under sterile conditions, and the standardized *E. coli* suspension was uniformly spread over the agar surface using a sterile cotton swab. After allowing the inoculated surface to equilibrate, sterile wells of approximately 8 mm diameter were aseptically prepared in the agar.

The crude aqueous and acetone extracts were reconstituted in 1% Tween 20 to obtain working concentrations of 80 and 100 mg/mL. Approximately 50 µL of each extract preparation was carefully introduced into the respective wells. Ciprofloxacin was used as the positive control, while 1% Tween 20 was used as the negative control. The plates were incubated at 37 °C for 18–24 h. Following incubation, antibacterial activity was assessed by measuring the diameter of the clear inhibition zones surrounding the wells using a ruler or calibrated measuring scale, and the results were expressed in millimetres (mm).

All treatments, including the controls, were evaluated in triplicate, and the mean inhibition-zone diameter was recorded for comparative analysis. The antibacterial response was interpreted based on the inhibition-zone diameter produced by each extract relative to the corresponding controls.

3. Results

3.1 Collection and Extraction of *Turbinaria conoides*

Turbinaria conoides biomass collected from the intertidal region of Rameshwaram, Tamil Nadu, was successfully processed for subsequent extraction and phytochemical analysis. Following thorough washing to remove adhering sand, debris, and epiphytic materials, the biomass was shade-dried and pulverized to obtain a fine, homogeneous powder. The final dried biomass obtained after processing was 374.1 g.

The powdered biomass was subjected to aqueous and acetone extraction using the procedures described in Section 2. The aqueous extraction yielded approximately 240 mL of filtrate, while Soxhlet extraction with acetone produced approximately 40 mL of crude extract. The resulting extracts were subsequently used for qualitative phytochemical screening and in vitro antibacterial evaluation. The overall workflow from collection and processing of T. conoides to preparation of the extracts is presented in Fig. 3.

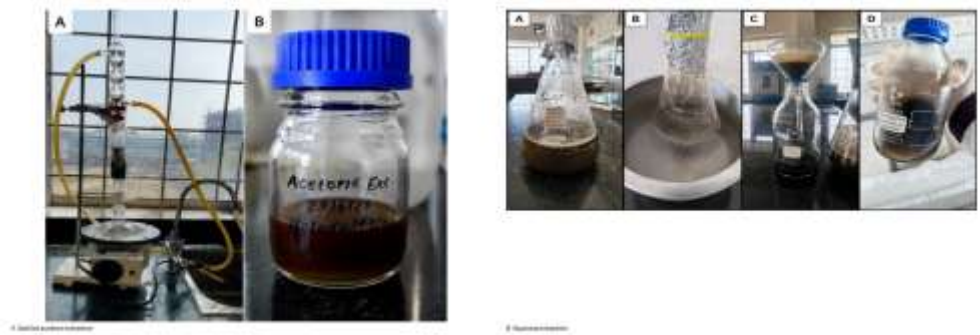

Fig 3 Processing and Extraction of *Turbinaria conoides*

3.2 Qualitative Phytochemical Profile

Qualitative phytochemical screening demonstrated clear differences in the detectable phytochemical profiles of the acetone and aqueous extracts of *Turbinaria conoides*. The acetone extract showed positive reactions for phenols, tannins, flavonoids, glycosides, terpenoids, alkaloids, and phytosterols, whereas saponins, steroids, and carbohydrates

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were not detected. In contrast, the aqueous extract showed positive reactions for tannins, saponins, and alkaloids, while phenols, flavonoids, glycosides, terpenoids, phytosterols, steroids, and carbohydrates were not detected. Overall, the acetone extract exhibited a broader qualitative phytochemical profile than the aqueous extract, whereas the aqueous extract was predominantly characterized by tannins, saponins, and alkaloids. The comparative phytochemical screening results are presented in **Table 1.**

Phytochemical test	**Acetone extract**	**Aqueous extract**
Phenols	+	_
Tannins	+	+
Flavonoids	+	_
Glycosides	+	_
Saponins	_	+
Terpenoids	+	_
Alkaloids	+	+
Phytosterols	+	_
Steroids	_	_
Carbohydrates	_	_
Proteins	_	+

Table 1. Preliminary phytochemical screening of acetone and aqueous extracts of *Turbinaria conoide*.

3.3 Antibacterial Activity

The aqueous and acetone extracts of *Turbinaria conoides* exhibited antibacterial activity against *Escherichia coli*, with the magnitude of inhibition varying between the extraction solvents. At a concentration of 500 µg/mL, the aqueous extract produced an inhibition zone of 8.00 ± 1.0 mm, whereas the acetone extract showed a comparatively higher inhibition zone of 16.00 ± 1.0 mm. Thus, the acetone extract produced a two-fold greater inhibition-zone diameter than the aqueous extract under the tested conditions. The comparatively higher activity observed for the acetone extract may be associated with more effective extraction of antibacterial constituents from the algal biomass. However, inhibition-zone diameter is influenced by both antimicrobial activity and the diffusion characteristics of extract constituents in agar. The antibacterial activity of the *T. conoides* extracts against *E. coli* is presented in **Table 2.**

Extract	**Test organism**	**Result**
Turbinaria conoides aqueous extract	*Escherichia coli*	8.00 ± 1.0 mm inhibition zone at 500 µg/mL
Turbinaria conoides acetone extract	*Escherichia coli*	16.00 ± 1.0 mm inhibition zone at 500 µg/mL

Table 2. Antibacterial Activity of *Turbinaria conoides* Extracts Against *Escherichia coli*

4. Discussion

The present study demonstrated clear differences in the phytochemical composition and extraction characteristics of the aqueous and acetone extracts of *Turbinaria conoides*. The acetone extract showed positive reactions for phenols, tannins, flavonoids, glycosides, terpenoids, alkaloids, and phytosterols, whereas the aqueous extract showed positive reactions for tannins, saponins, and alkaloids. The broader range of phytochemical classes detected in the acetone extract indicates that solvent selection substantially influenced the recovery of detectable constituents from the algal biomass. Similar solvent-dependent differences in the recovery of secondary metabolites have been reported for marine macroalgae, where the polarity of the extraction solvent affects the solubility, diffusion, and recovery of chemically diverse constituents (Franco *et al*., 2008). Such differences are particularly relevant for brown algae because their chemical composition includes compounds with markedly different physicochemical properties, including phenolic compounds, terpenoids, sterols, flavonoids, and other secondary metabolites (Gogineni *et al*., 2019).

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The broader phytochemical profile of the acetone extract may be associated with the ability of acetone to recover moderately polar and relatively less-polar constituents from the algal matrix. The detection of phenols, flavonoids, terpenoids, phytosterols, and glycosides in the acetone extract suggests that these metabolite classes were more readily extracted under the conditions employed. In contrast, the aqueous extract was characterized predominantly by tannins, saponins, and alkaloids. Water preferentially extracts highly polar and water-soluble constituents, and consequently the composition of an aqueous crude extract may differ substantially from that obtained using an organic solvent. However, the absence of a particular phytochemical class in a qualitative test should not necessarily be interpreted as its complete absence from the algal biomass, because detection is influenced by extraction efficiency, compound concentration, assay sensitivity, and possible interactions among constituents.

The extraction yield further demonstrated a marked difference between the two extraction systems. The aqueous extraction produced a yield of 5.2%, whereas the acetone extraction yielded 2.1%. The higher yield obtained with water indicates greater recovery of water-soluble material under the conditions used. However, extraction yield represents the total mass of recovered material and does not directly reflect the concentration or biological potency of individual bioactive constituents. A higher crude extract yield may result from the simultaneous recovery of carbohydrates, proteins, salts, polysaccharides, pigments, and other non-bioactive components. Conversely, an organic solvent may produce a lower overall yield while selectively concentrating compounds with greater biological activity. Therefore, extraction yield should be interpreted together with phytochemical composition and biological activity rather than as an independent indicator of extract quality.

The qualitative detection of phenols and flavonoids in the acetone extract is of particular interest because phenolic compounds are widely reported among brown macroalgae and have been associated with antioxidant and antimicrobial properties. Brown seaweeds are especially recognized for their phlorotannin content, in addition to other phenolic metabolites. These compounds may interact with microbial cell surfaces, proteins, enzymes, and membrane-associated components, potentially contributing to antimicrobial effects. Flavonoids may similarly influence microbial growth through interactions with cellular membranes, proteins, and intracellular metabolic processes. Nevertheless, the present study employed qualitative phytochemical reactions and therefore cannot establish the presence, concentration, or identity of individual phenolic or flavonoid compounds. The observed antibacterial response should consequently be considered a property of the crude extract rather than attributed to a specific compound without further chemical evidence.

Tannins and alkaloids were detected in both aqueous and acetone extracts, suggesting that these metabolite classes may be distributed across fractions with different solvent affinities. Tannins have been associated with protein-binding and membrane-interacting properties that can affect microbial growth, whereas alkaloids represent a chemically diverse group with several reported biological activities. The detection of these compounds in both extracts may partly explain why both preparations exhibited antibacterial activity against *Escherichia coli*. However, the present data do not establish a direct relationship between the presence of these phytochemical classes and the observed inhibition. Bioassay-guided fractionation would be necessary to determine whether tannins, alkaloids, phenolics, flavonoids, or other constituents are responsible for the activity.

The antibacterial assay demonstrated that both *T. conoides* extracts inhibited the growth of *E. coli*, although the magnitude of inhibition differed considerably between the two extraction systems. At 500 µg/mL, the aqueous extract produced an inhibition zone of 8.00 ± 1.0 mm, whereas the acetone extract produced an inhibition zone of 16.00 ± 1.0 mm. Thus, under the experimental conditions of the present study, the acetone extract produced a substantially greater inhibition-zone diameter than the aqueous extract. The stronger response observed for the acetone extract is consistent with its broader qualitative phytochemical profile and may indicate that one or more antibacterial constituents were preferentially recovered in the acetone fraction.

The present observation is also consistent with previous investigations reporting antibacterial activity associated with *T. conoides*. Noorjahan *et al.* (2022) reported antibacterial activity from *T. conoides* extracts against selected bacterial pathogens, supporting the biological potential of this brown macroalga. Although the specific magnitude of antibacterial activity may differ among studies, the repeated observation of antimicrobial effects suggests that *T. conoides* contains extractable constituents capable of interfering with bacterial growth. Such activity may be related to

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the combined effects of phenolic compounds, terpenoids, sterol-related metabolites, flavonoids, alkaloids, and other algal constituents.

The stronger antibacterial response obtained with the acetone extract may also reflect differences in compound diffusion through the agar medium. Agar well diffusion is a convenient preliminary screening method, but inhibition-zone diameter is not determined exclusively by antimicrobial potency. Molecular size, polarity, concentration, diffusion coefficient, extract viscosity, solvent characteristics, and interactions with the agar matrix can all influence the size of the inhibition zone. Consequently, the 16.00 ± 1.0 mm zone observed for the acetone extract should not be interpreted directly as evidence that the extract is twice as potent as the aqueous extract. Quantitative assays such as minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and time-kill analysis would provide more reliable measures of antibacterial potency.

The observed antibacterial activity may arise through several possible mechanisms involving the chemically diverse constituents present in the extracts. Phenolic and flavonoid compounds can potentially alter bacterial membrane permeability and interfere with cellular proteins and enzymes. Terpenoid and phytosterol-related constituents may interact with membrane-associated components, while alkaloids may interfere with essential metabolic processes. Tannins can interact with proteins and other cellular macromolecules and may contribute to growth inhibition. Because the present investigation did not isolate individual compounds or examine bacterial cellular responses, these mechanisms remain proposed explanations rather than experimentally demonstrated mechanisms for *T. conoides*.

The geographical origin of the algal biomass should also be considered when interpreting the results. *T. conoides* collected from the Pamban coastal region may exhibit a phytochemical profile influenced by local environmental conditions. Marine macroalgae are exposed to variations in salinity, temperature, light intensity, nutrient availability, herbivory, epiphytic organisms, and other environmental stresses. These factors can influence the biosynthesis and accumulation of secondary metabolites. Consequently, extracts obtained from the same species at different geographical locations or seasons may exhibit different phytochemical profiles and biological activities. This may partly explain differences between the present findings and those reported in previous studies.

The relationship between solvent selection and biological activity is particularly important for the development of macroalgal-derived antimicrobial preparations. The present study showed that water provided the greater overall extraction yield, whereas acetone produced the broader qualitative phytochemical profile and stronger antibacterial response against *E. coli*. This distinction demonstrates that extraction efficiency and biological activity are not necessarily directly correlated. An extraction procedure optimized solely for maximum yield may not provide the fraction with the greatest biological activity. Therefore, future optimization should consider both recovery efficiency and bioactivity when selecting extraction solvents and processing conditions.

The findings also have relevance to the broader investigation of marine macroalgae as sources of naturally derived antimicrobial compounds. Brown algae are increasingly investigated because their chemical diversity provides opportunities for identifying novel compounds and bioactive fractions. However, crude extract screening represents only the initial stage of natural-product research. A positive agar diffusion response establishes preliminary biological activity but does not demonstrate pharmaceutical efficacy, selectivity, safety, or practical applicability. Further chemical and biological validation are therefore essential before the extracts can be considered for therapeutic or industrial antimicrobial applications.

Several limitations of the present study should be acknowledged. First, the phytochemical analysis was qualitative and based on colour reactions or other preliminary chemical responses. These assays provide information on broad metabolite classes but cannot identify individual molecules or determine their concentrations. Second, antibacterial activity was evaluated against a single bacterial species, *E. coli*. Although *E. coli* is an important Gram-negative model organism and clinically relevant pathogen, testing against a wider panel of Gram-positive and Gram-negative bacteria would provide a more comprehensive assessment of the antimicrobial spectrum. Third, agar well diffusion provides a preliminary measure of inhibition but does not establish MIC or MBC values. Finally, the study did not investigate the mechanism of bacterial inhibition or evaluate the cytotoxicity and selectivity of the extracts.

Future studies should therefore focus on detailed chemical characterization of the active fractions using chromatographic and spectrometric approaches such as HPLC, LC–MS/MS, GC–MS, and, where appropriate, NMR spectroscopy. Quantitative determination of total phenolic and flavonoid contents would further strengthen the

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relationship between phytochemical composition and antibacterial activity. Bioassay-guided fractionation could be employed to identify the fractions responsible for the observed inhibition, followed by purification and structural characterization of individual active constituents. MIC and MBC determination using standardized broth-based methods would provide quantitative evidence of antibacterial potency, while mechanistic studies could investigate effects on bacterial membrane integrity, cellular morphology, enzyme activity, and intracellular processes.

In addition, comparative evaluation against a broader range of bacterial pathogens, including clinically relevant Gram-positive and Gram-negative strains, would help determine the spectrum of activity of *T. conoides*. Cytotoxicity and selectivity assays would be essential if the extracts are intended for pharmaceutical or biomedical applications. Reproducible extraction protocols, appropriate solvent controls, standard antibacterial controls, biological replicates, and statistical analysis should also be incorporated into subsequent investigations. These approaches would allow the biological activity of *T. conoides* to be distinguished from non-specific effects associated with crude extracts or assay conditions.

Overall, the present findings demonstrate that *T. conoides* possesses solvent-dependent phytochemical characteristics and measurable antibacterial activity against *E. coli*. The acetone extract showed a broader detectable phytochemical profile and produced a higher inhibition zone than the aqueous extract, despite the lower extraction yield obtained with acetone. This observation emphasizes the importance of solvent selection in recovering biologically relevant constituents from marine macroalgal biomass. The results are consistent with previous reports describing the bioactive potential of *T. conoides* and other brown macroalgae (Franco *et al*., 2008; Gogineni *et al*., 2019; Noorjahan *et al*., 2022). Nevertheless, the present evidence remains preliminary, and further quantitative chemical characterization, antimicrobial potency testing, mechanistic investigation, and safety evaluation are required to establish the specific contribution and practical significance of *T. conoides*-derived bioactive constituents.

5. Conclusion

The present study demonstrated that *Turbinaria conoides* contains a diverse range of solvent-dependent phytochemical constituents with potential biological relevance. The acetone extract exhibited a broader qualitative phytochemical profile, with phenols, tannins, flavonoids, glycosides, terpenoids, alkaloids, and phytosterols detected, whereas the aqueous extract showed the presence of tannins, saponins, and alkaloids. A higher crude extraction yield was obtained with the aqueous extraction procedure (5.2%) compared with acetone extraction (2.1%), indicating differences in the recovery of extractable constituents between the two solvents. The acetone extract also demonstrated greater antibacterial activity against *Escherichia coli* under the tested conditions, producing an inhibition zone of 16.00 ± 1.0 mm compared with 8.00 ± 1.0 mm for the aqueous extract at 500 µg/mL. These findings support the potential of *T. conoides* as a source of naturally occurring antibacterial constituents. However, the present investigation represents preliminary phytochemical and in vitro antibacterial screening, and the active compounds responsible for the observed activity were not established. Further studies involving quantitative phytochemical analysis, chromatographic and spectrometric characterization, bioassay-guided fractionation, MIC/MBC determination, mechanistic evaluation, toxicity assessment, and validation against a broader range of bacterial pathogens are required to determine the therapeutic and biotechnological significance of *T. conoides*.

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