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Research Article

Marine Brown Algae for Diabetic Foot Ulcer

Antibacterial Potential of Marine Brown Algae *Turbinaria Conoides* Against Bacteria Associated with Diabetic Foot Ulcer

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Abstract: The marine brown algae *Turbinaria conoides* was extracted with different solvents, viz., acetone, ethanol, methanol and distilled water. The extracts were screened for the presence of various phytochemicals and examined for antibacterial activity by agar well diffusion method. Diabetic foot ulcer (DFU) associated bacteria and their respective standard strains, such as *Staphylococcus aureus* ATCC 5923, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 700603 and *Enterococcus faecal* ATCC 25922 were used as test bacteria. Among the different solvent extracts, the aqueous extract possessed the maximum zone of inhibition against both test and standard bacterial strains. The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of the aqueous extract against test pathogens were 128-4µg. The eluent mixture for separating the active fraction was selected by thin layer chromatography (TLC), which was found to be n-hexane: ethylacetate (6:4) with a maximum of four spots. In column chromatography, 25th -27th-minute fractions showed maximum antibacterial activity against all the DFU bacterial strains. Our study aims to find the use of *T. conoides* for treating DFU-associated bacterial infections. The objectives include exploring different solvent extracts of *T. conoides* for antibacterial activity and characterization of bioactive metabolite.

Keywords: Marine brown algae, *Turbinaria conoides,* solvent extracts, antibacterial, agar well diffusion method, diabetic foot ulcer.

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1. INTRODUCTION

Marine organisms serve as a promising source for pharmacologically potential bioactive secondary metabolites[1]. Due to their enormous molecular diversity and fascinating pharmacological effects, marine natural products constitute a unique source of bioactive molecules that have attracted the attention of drug developers in recent years [2]. Some marine compounds have entered the market and are currently being utilized as medicines providing a valuable road map for further translational initiatives[3]. The marine algae are broadly classified into microalgae that consist of dinoflagellates, blue-green algae, bacillariophyte, and diatoms found in coastal, benthic and throughout the sea[4]. Further, macroalgae (Seaweeds) are classified into three different types such as red algae (Rhodophyta), brown algae (Phaeophyta) and green algae (Chlorophyta), which are found in coastal regions[5]. Macro algae are eukaryotic organisms and are the primary source of microbial diversity[6]. They are exposed to harsh environmental conditions in the marine ecosystem and produce metabolites to challenge their survival[7]. Marine algae are mainly used for industrial manufacturing hydrocolloids like agar-agar, alginate, and carrageenan, not for health reasons[8]. They are reported to produce a wide range of bioactive metabolites, including alkaloids, anthocyanin, reducing sugars, terpenoids, quinones, and flavonoids with antimicrobial, antioxidant, antiinflammatory and cytotoxic activity[9]. Among the marine macroalgae, brown algae (Phaeophyceae)are the second most abundant group[10]. The brown algae *Turbinaria* belong to the family *Sargassaceae* (brown algae). The order of Fucales consists of 22 species. The highest diversity of the genus *Turbinaria* was found in southwest Asia akin to India, Srilanka with 14 species[11] and available throughout different seasons[12]. The chemical constituents of *Turbinaria conoide*s have yet to be explored much that may serve as a captivating source of active chemicals[13]. Further, *T.conoides* found in the south Pacific Ocean have been exploited less for bioactive metabolites. There have been numerous reports on the antioxidant property of *T.conoides*, but information on antibacterial activity is very scarce. Hence, the present study focused on utilizing algal extracts for compacting DFU-associated bacterial pathogens. Finding novel pharmacological molecules, particularly antibacterial and antimicrobial, has recently attracted much interest from many experts since antibiotic resistance poses a severe problem in the ultra-modern therapeutic era [14]. The risk of bacterial infections increases due to antibiotic resistance, increasing morbidity and death. About 19% to 34% of those with diabetes mellitus are susceptible to developing DFU[15]. According to the World Health Organization (WHO), the diabetic foot is an infection, ulceration, or destruction of deep tissues in the lower leg accompanied by neurological and varied degrees of peripheral vascular disease[16]. The most common pathogenic bacteria associated with DFU include gram-positive organisms such as *Staphylococcus aureus and Enterococcus faecalis* and gram-negative organisms like *Pseudomonas aeruginosa, Escherichia coli, Klebsiella sp, Proteus sp*, etc.,[17]. The bacterial association in DFU develops multidrug resistance, increasing morbidity and mortality. Hence, there is increasing demand for novel drugs, especially antibacterial agents, to compact antibiotic resistance. The present study includes characterizing the bioactive metabolite and investigating the antibacterial activity of various solvent extracts of *T. conoides* to treat bacterial infections related to DFU. The methods used for investigation include (i) collection, authentication and preparation of marine seaweed extracts, (ii) phytochemical analysis, (iii) determination of antibacterial activity by agar well diffusion and micro broth dilution method, (iv) purification of bioactive metabolite. The present work shows that the aqueous extract of marine seaweed *T. conoides* possesses excellent antibacterial activity against both common and DFU-associated bacteria that might have possible applications in biomedical sectors.

2. MATERIALS AND METHODS

2.1. Collection of marine seaweed Turbinaria conoides

The marine seaweed *Turbinaria conoides* was collected from Krusadai Island in the Gulf of Mannar. Their taxonomic naming was confirmed by comparing it with the acknowledged collection in the Herbarium of Botany department, Queen Mary's College, Chennai[18]. Further, the collected sample was authenticated by Dr E. Palanisamy, Scientist – E, Central National Herbarium, Botanical Survey of India, Howrah, with voucher reference no. CNH/Tech. II/2022/141/1230. The samples were cleaned and rinsed using sterile distilled water to remove adhering debris. Samples were dried under a sunshade for seven days and ground in an electric mixer. The powdered samples were used for further studies.

2.2. Preparation of marine seaweed extracts

The powdered seaweed (25g) was extracted with acetone, ethanol, methanol and distilled water using a soxhlet apparatus. The resulting organic extracts were concentrated to dryness under reduced pressure at 30 - 35°C with a rotary evaporator. The residues (crude extracts) were collected and stored at –20°C in airtight vials until further use[19]. The yield of 25 g powder was calculated by using the formula.

Yield = Amount of product/Amount of sample ×100

2.3. Phytochemical analysis of Turbinaria conoides

The phytochemical constituents including alkaloids, anthocyanin, combined anthraquinones, cardiac glycosides, coumarins**,** flavonoids, glycosides, phenols, phlorotannins, quinones, reducing sugar, saponins, steroids, tannins and terpenoids of *T. conoides* was tested[20- 23].

2.4. Determination of antibacterial activity

2.4.1. Bacterial strains

The multidrug-resistant isolates of diabetic foot ulcer (DFU) such as *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis* with the appropriate control strain (*Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 700603 and *Enterococcus faecal*is ATCC 25922) were acquired from Dr A. Suresh, Scientist, Meenakshi Medical College Hospital & Research Institute, Enathur, Kanchipuram were used. The strains were sustained on nutrient agar slants at 4°C[24].

2.4.2. Preparation of inoculums

The bacterial strains were cultured on Muller Hinton broth (MHB) and incubated at 37°C for 24 h. The cultures were diluted with fresh MHB to achieve O.D. corresponding to 0.5 (i.e., 10^5 to 10^6 CFU/ml using Mac Farland's standard).

2.4.3. Agar well diffusion assay

The antibacterial activity of the brown algae *T. conoides* was evaluated using agar well diffusion. The bacterial test inoculum was seeded separately onto the surface of Muller Hinton agar (MHA) plates using a sterile cotton swab. About five wells of 6.0 mm diameter were aseptically made using the wide end of the sterile corn borer on the MHA plates seeded with target microorganisms. The crude extracts were reconstituted with dimethylsulfoxide (DMSO) to a final concentration of 0.5 mg/ml. The wells were then filled with 100 µl of each section. The DMSO was used as a negative control. Amoxicillin (10µg) was used as a positive control. The plates were left for two hrs for complete diffusion and then incubated overnight at 37±1°C. The diameter of inhibition zones, including the diameter of the well (6 mm), was measured and compared to that of the negative control. Each test was made in triplicate, and the average of the three replicates for each extract was calculated [25].

2.4.4. Evaluation of minimum inhibitory and bactericidal concentration

The MIC and MBC of the algal extract against the standard and bacterial test strains were carried out using the micro broth dilution method. Algal extracts were serially diluted with dimethyl sulfoxide to obtain concentrations of 256, 128, 64, 32, 16, 8, 4, and 2 µg/100µl. Each well was inoculated separately with 5 µl of standardized inoculums (10^8 CFU/ml) of the test bacteria and incubated at 37°C for 18 h. The experiment included a growth control well (without an antimicrobial agent) and a negative control well (un-inoculated). The MIC was defined as the lowest concentration at which no visible growth was observed. In addition, MBC was evaluated based on the development in the lowest dilution of the well after streaking on MHA plates. The experiment was performed in triplicates [26].

2.5. Purification of the bioactive metabolite from aqueous extract of marine seaweed

2.5.1. Analytical Thin layer chromatography

An aliquot of crude aqueous extract of *T. conoides* was spotted onto the silica gel plate and allowed to dry for a few minutes. The chromatogram was run using different solvent systems such as chloroform: methanol; dichloromethane: methanol; n-hexane: ethyl acetate and ethyl acetate: acetone in the ratio of 1:2, 7:3, 6:4, and 2:1, respectively. The developed plate was dried under normal air, and the spots were visualized under U.V. light. The retention factor values of isolated compounds were calculated.

2.5.2. Column chromatography

The active fraction was further extracted from crude extract using a silica gel column in stepwise elution mode. The natural section of *T. conoides* was loaded onto an open preparative chromatography column (30 cm X 7.5 cm i.d.) and eluted at a flow rate of 1 ml/s with a gradient mode of mixture n-hexane: ethylacetate (6:4, v/v). The fractions eluted were collected at the interval of 10 mins. The particles were collected and tested for antibacterial activity using the agar well diffusion method against test bacterial strains.

3. STATISTICAL ANALYSIS

Results were presented as mean ± standard error mean (SEM) of three replicates. The statistical analysis was performed using Graph Pad Prism software (version 8). Data obtained were analyzed statistically to determine the degree of significance using a one-way analysis of variance (ANOVA) with Tukey's multiple comparisons tests.

4. RESULTS AND DISCUSSION

4.1. Collection, Identification and Extraction of seaweed

The algal samples were collected from the Gulf of Mannar and were aseptically transferred to the laboratory. Ramesh *et al.,*[27] fields investigated the Gulf of Mannar and suggested *Turbinaria* sp. must be exploited for bioactive metabolites of industrial importance. In the current study, the collected algae showed characteristic leaves (blades) of *Turbinaria conoides* with longitudinal, lateral ridges. In addition, the blisters were predominant, making the leaf's distal end inflated (Figure 1). Rohfritsch*et al.,*[28] distinguished the characteristic features of two morphologically similar species of *Turbinaria*, including *T. conoides* and *T. ornate* and described the inflated appearance of blades with no marginal teeth as the main characteristics of *T. conoides.* The shade-dried sample was powdered and extracted using acetone, ethanol, methanol and distilled water. The extraction yield from *T. conoides* is illustrated in Table 1 with different solvents that yielded different amounts of crude extract, with aqueous extract achieving the highest outcome. This resulted from seaweeds consisting of many polar constituents, including carbohydrates, which can account for almost 20% of the dry weight, followed by proteins and lipids [29].

4.2. Phytochemical analysis of Turbinaria conoides

Due to their abundance of secondary metabolites of potentially bioactive chemicals such as terpenoids, phlorotannins, phenolics, flavonoids, fucoidans, sterols, and glycolipids, brown seaweeds constitute a significant source of renewable resources for the commercial sector[30].In our study, Phytochemical data of brown seaweed *T. conoides* (Table 2) shows distinct patterns of chemical compositions in constituents of the extracts. It was found that phenol and coumarins were present in all the sections. The aqueous extract contains phytoconstituent phenol endorsed as algal cells' maximum significant chemical additive. It may have a stimulating or inhibiting influence on microbial growth depending on their establishment and concentration[31]. In addition, phenol compounds possess radical scavenging, metal chelating, and hydrogen-donating properties[32]. The terpenoids from *Dictyotacea*e exhibit bioactivities along with inhibition of herbivores and antifungal, antibiotic, anti-inflammatory, cytotoxic, insecticidal and antiviral activities[31]. Phlobatannins have proven to possess anti-inflammatory, antioxidant and wound-healing properties[33]. The aqueous extract includes anthraquinones, coumarins, reducing sugars and saponins of

algae broadly used in the pharmaceutical industry and, thus an essential source of bioactive natural substances.

4.3 Determination of antibacterial activity

The inhibitory effects of *Ulva lactuca* (Chlorophyta), *Dilophus spiralis* (Phaeophyta), and *Jania Rubens* (Rhodophyta) against gram-positive and gram-negative bacteria were examined by Saleh and Al-Mariri[34]. It was hypothesized that Phaeophyta extracts were more effective against tested isolates than Chlorophyta and Rhodophyta because they contained more phenolic compounds. Several authors endorsed that among different algal divisions, Phaeophyta possesses maximum antibacterial activity[35,36,37,38]. In our study, the antibacterial activity of *T.conoides* solvent extract was in the order of aqueous extract>acetone>methanol>ethanol (Table 3 and Figure 2). The aqueous extract was found to possess broad-spectrum antibacterial activity. It showed a maximum zone of inhibition against standard *Enterococcus faecalis* (25.80±0.28 mm), *Pseudomonas aeruginosa* (21.17±0.37 mm) and DFU-associated *Enterococcus faecalis* (28.57±0.34 mm), *Pseudomonas aeruginosa* (20.33±0.08 mm). The last zone of clearance with aqueous extract was recorded against both standard and DFU-associated *Staphylococcus aureus* with the area of inhibition of 12.17±0.18 and 15.10±0.15 mm, respectively. The intermediate zone of inhibition with aqueous extract was recorded against both standard and DFU-associated *Klebsiella pneumoniae* with the area of inhibition of 14.77±0.14 and 18.9±0.3 mm. It was found that acetone extract of *T. conoides* inhibits all test bacteria with the maximum zone of inhibition recorded against *Pseudomonas aeruginosa* ATCC 27853 (18.73±0.38 mm) and DHU associated *Enterococcus faecalis* (18.65±0.15 mm). The methanol extract was found to inhibit with the most miniature zone of inhibition against all the test bacteria. However, ethanol extract showed no zone of inhibition against standard *Staphylococcus aureus* and DFU-associated *Staphylococcus aureus* and *Pseudomonas aeruginosa.* Bochum *et al.*[39] also reported *that T. conoides* aqueous extract possesses better antibacterial and antifungal activity than ethanol extract. Ganapathy *et al.,*[40] also extracted bioactive fucoidan using the hot water extraction method. Shibu and Dhanam [41] collected *T. conoides* from the coastal area of Mandapam, Gulf of Mannar, Tamil Nadu in India and reported antifungal activity of the extracts. The MIC and MBC of aqueous extract of *T. conoides* tested by micro broth dilution technique against standard *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis* were found to be 64/128 µg, 128/128 µg, 32/64µg, 16/16 µg. DFU bacterial strains *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis* showed MIC/MBC of 32/64 µg, 64/64 µg, 16/32 µg, and 8/16µg, respectively (Table 4). The antibacterial activity of *T. conoides* may be related to secondary metabolites like phenol and terpenoids that inhibit bacterial metabolism and growth[42].

4.4 Characterization of the active metabolite

In TLC maximum of four different spots with Rf value ranging from 0.36-0.78 when n-hexane: ethylacetate (6:4) is used as a solvent system (Figure 3). This eluent mixture was selected for column chromatography since it produced more spots. Zubair *et al.,*[43] selected n-hexane: ethyl acetate (7:3) as an eluent mixture for seaweed *Turbinaria ornate* methanol extract. In column chromatography, 25-27 minute fractions showed maximum antibacterial activity against all the DFU bacterial strains.

Table 1: Extraction yields of *Turbinaria conoides*, extracted using different solvents

Solvent	Average % yield
Distilled water	35±0.3
Methanol	22±0.5
Ethanol	16±0.6
Acetone	12±0.2

Data (n=3) is presented as the mean ± SEM.

The average percentage yield was found to be high in water, i.e., aqueous extract, due to the presence of more polar constituents and the lowest work was obtained in acetone extract. The intermediate yield was achieved in methanol and acetone extract of *Turbinaria conoides.*

Table 2: Phytochemical analysis of different solvent extracts of *T. conoides*

S.No	Phytochemical compounds	Acetone	Ethanol	Methanol	Aqueous
1	Alkaloids	+	+	-	-
2	Anthocyanin	-	-	-	-
3	Combined anthraquinones	-	-	+	+
4	Cardiac glycosides	-	-	-	-
5	Coumarins	+	+	+	+
6	Flavonoids	-	-	+	-
7	Glycosides	-	+	+	-
8	Phenols	+	+	+	+
9	Phylobatannins	-	-	-	+
10	Quinones	-	+	+	-
11	Reducing sugar	-	-	-	+
12	Saponins	-	+	+	+
13	Steroids	-	+	-	-

14	Tannins	-	-	-	-
15	Terpenoids	-	+	+	+

+ indicates the presence of phytochemical and – means the absence of phytochemical

The presence of anthraquinones, coumarins, phenols, phlorotannins, reducing sugars, saponins and terpenoids in aqueous extract indicates its bioactive potential.

Table 3: Antibacterial activity of *T.conoides* solvent extracts against the standard and DFU-associated bacteria

Test strains	Zone of inhibition (mean ± SEM)				
	Amoxicillin 10µg	Acetone 50 µg	Ethanol 50 µg	Methanol 50 µg	Aqueous50 µg
Staphylococcus aureus ATCC 25923	31.7±0.33	$10.23^{a5c5d2e4}$ ±0.14	-	$9.3^{a5b2c5e5}$±0.15	$8.5^{a5b4c5d5}$ ±0.18
Pseudomonas aeruginosa ATCC 27853	-	$18.73^{a5c5d5e4}$±0.38	$14.23^{a5b5d5e}$5 ±0.14	$10.33^{a5b5c5e5}$ ±0.08	$21.17^{a5b4c5d5}$ ±0.37
Klebsiella pneumoniae ATCC 700603	31.53±0.60	$11.83^{a5c4d4e4}$ ±0.26	$10.07^{a5b2d1e5}$ ± 0.17	$8.92^{a4b2c4e5}$±0.29	$14.77^{a5b4c5d5}$ ±0.14
Enterococcus faecal ATCC 25922	19.27±0.54	$9.133^{a5c1d1e5}$±0.23	$8.78^{a5b1d1e5}$±0.28	$8.63^{a5b1c1e5}$±0.23	$25.80^{a5b5c5d5}$ ±0.28
Multidrug-resistant DFU bacterial pathogens					
Staphylococcus aureus	21.17±0.37	$11.20^{a5c5d4e5}$±0.23	-	$9.36^{a5b4c5e5}$±0.08	$15.10^{a5b5c5d5}$±0.15
Pseudomonas aeruginosa	-	$15.33^{a5b5d5e5}$±0.26	-	$8.16^{a5b5c5e5}$±0.08	$20.33^{a5b5c5d5}$±0.08
Klebsiella pneumoniae	8.2±0.2	$15.2^{a5b5d5e5}$±0.23	$8.93^{a1b5d3e5}$±0.46	$7.03^{a2b5c3e5}$±0.14	$12.17^{a5b5c5d5}$±0.3
Enterococcus faecal	12.6±0.17	$18.65^{a5c5d5e5}$±0.15	$15.10^{a5b5c5d5}$±0.15	$11.87^{a1b5c5d5}$±0.2	$28.57^{a5b5c5d5}$±0.34

- No activity

Values are expressed as Mean ± SEM (n=3); analysis was performed with One-Way ANOVA followed by Tukey test with Post Hoc multiple comparisons; [a] compared to the positive control, [b] compared to acetone, [c] compared to ethanol, [d] compared to methanol, [e] compared to aqueous; [1] p ≥ 0.05, [2]p< 0.05, [3]p<0.01, [4] p<0.001, [5]p<0.0001 The antibacterial activity of the different solvent extract against standard and DFU associated bacteria was tested by agar well diffusion method. Amoxicillin (10 µg) was used as the positive control, and DMSO was used as a negative control. Among the solvent extract, the aqueous extract of *T. conoides* was found to possess broad-spectrum antibacterial activity.

Table 4: MIC and MBC of aqueous extract of *T. conoides* against the standard and DFU-associated bacteria

Test Strains	Aqueous extract of *T. conoides*	
	MIC (µg)	**MBC** (µg)
S. aureus ATCC 25923	64	128
P. aeruginosa ATCC 27853	128	128
K. pneumoniae ATCC 700603	32	64
E. faecalis ATCC 25922	16	16
Multidrug Resistant Diabetic Foot Ulcer Isolates		
S. aureus	32	64
P. aeruginosa	64	64
K. pneumoniae	16	32
E. faecalis	8	16

The MIC and MBC of *T. conoides aqueous* extract were tested by micro broth dilution. The concentration tested include 256, 128, 64, 32, 16, 8, 4, and 2 µg/100µl of aqueous extract. The aqueous extract of *T. conoides* was found to effectively inhibit the growth and kill the common and multidrug-resistant diabetic foot ulcer bacterial isolates. The standard and drug-resistant isolates of *Enterococcus faecalis* were found to be sensitive in the minor concentration, whereas *Pseudomonas aeruginosa* was inhibited at the highest concentration.

Fig. 1: *Turbinaria conoides* collected from Krusadai Island in the Gulf of Mannar

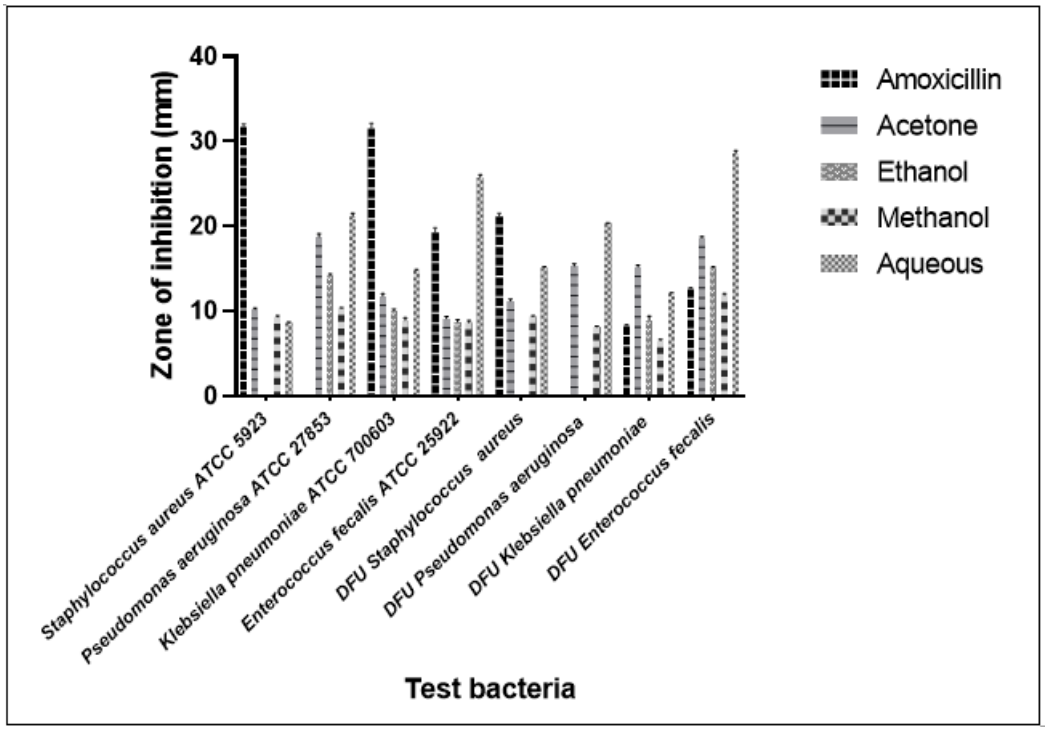

Fig.2. Antibacterial activity of *T.conoides* against common and DFU-associated bacteria was tested by agar well diffusion method.

The bar graph illustrates the zone of inhibition pattern of acetone, ethanol, methanol and aqueous extract of *T.conoides* against test bacteria. The error bar indicates the mean ±SEM.

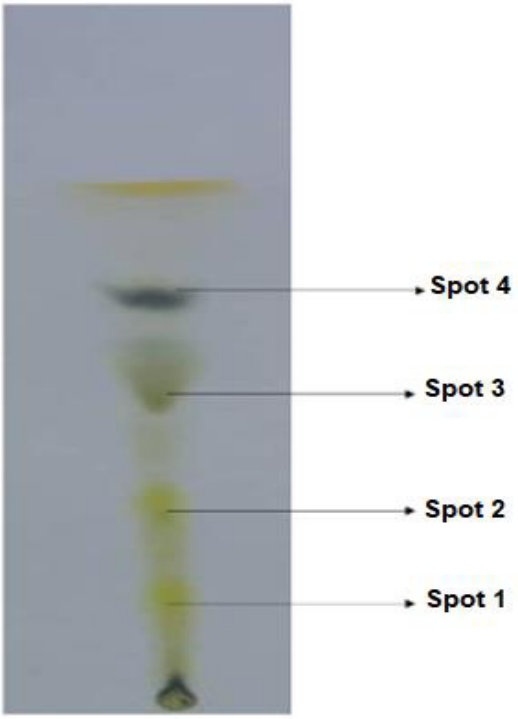

Fig. 3. TLC chromatogram showing four spots with Rf value ranging from 0.36-0.78 using n-hexane: ethylacetate (6:4) as solvent system

5. CONCLUSION

The present study concludes that the aqueous extract of marine brown algae *T. conoides* have promising broad-spectrum antibacterial activity against DFU-associated bacterial pathogens. However, further investigation is necessary to understand better the bioactive compound and pathway involved in antibacterial activity.

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7. AUTHORS CONTRIBUTION STATEMENT

T. Chitrikha Suresh – performed the experiments, T. V. Poonguzhali – designed and directed the project, and V. Anuradha, - helped supervise the project. S. Bharathi- Formal analysis, B. Ramesh and G. Suresh - Data curation. All the authors discussed the methodology and result and contributed to the final version of the manuscript.

7. CONFLICT OF INTEREST

Conflict of interest to declared none.

8. REFERENCES

1. Al-Saif SSA, Abdel-Raouf N, El-Wazanani HA, Aref IA. Antibacterial substances from marine algae isolated from the Jeddah coast of Red Sea, Saudi Arabia. Saudi J Biol Sci. 2014;21(1):57-64. doi: 10.1016/j.sjbs.2013.06.001, PMID 24596500.
2. Moni SS, Alam MF, Safhi MM, Jabeen A, Sanobar S, Siddiqui R et al. Potency of nano-antibacterial formulation from Sargassum binders against selected human pathogenic bacteria. Braz J Pharm Sci. 2018;54(4):1-9. doi: 10.1590/s2175-97902018000417811.
3. Arizza V. Marine biodiversity as a source of new drugs. Ital J Zool. 2013;80(3):317-8. doi: 10.1080/11250003.2013.830370.
4. Nayak G, Sahu A, Bhuyan SK, Bhuyan R, Kumar A, Kar D. Review on biomedical applications of marine algae-derived biomaterials. Univers J Public Health. 2022;10(1):15-24. doi: 10.13189/ujph.2022.100102.
5. Agarwal PK, Dangariya M, Agarwal P. Seaweed extracts: potential biodegradable, environmentally friendly resources for regulating plant defence. Algal Res. 2021; 58:102363. doi: 10.1016/j.algal.2021.102363.
6. Remya RR, Samrat AV, Kumar SS, Mohanavel V, Karthick A, Chinnaiyan VK et al. Bioactive potential of brown algae. Adsorption Sci Technol. 2022;2022: Article ID 9104835, 13 pages. doi: 10.1155/2022/9104835.
7. Alves C, Silva J, Pinteus S, Gaspar H, Alpoim MC, Botana L.M. et al. From marine origin to therapeutics: the antitumor potential of marine algae-derived compounds. Front Pharmacol. 2018; 9:777. doi: 10.3389/fphar.2018.00777, PMID 30127738.
8. Fayaz M, Namitha KK, Murthy KNC, Swamy MM, Sarada R, Khanam S Et al. Chemical Composition, Iron Bioavailability, and Antioxidant Activity of Kappaphycus alvarezzi (Doty). J Agric Food Chem. 2005;53(3):792-7. doi: 10.1021/jf0493627.
9. Cabrita MT, Vale C, Rauter AP. Halogenated compounds from marine algae. Mar Drugs.

2010;8(8):2301-17. doi: 10.3390/md8082301, PMID 20948909.
10. Davis TA, Volesky B, Mucci A. A review of the biochemistry of heavy metal biosorption by brown algae. Water Res. 2003;37(18):4311-30. doi: 10.1016/S0043-1354(03)00293-8, PMID 14511701.
11. Arumugam G, Rajendran R, Khaleelullah NS, Ramanathan S. Anti-candidal and anti-virulence efficiency of selected seaweeds against azole resistance Candida albicans. Biocatal Agric Biotechnol. 2019; 20:101195. doi: 10.1016/j.bcab.2019.101195.
12. Chakraborty K, Joseph D. Antioxidant potential and phenolic compounds of brown seaweeds *Turbinaria conoides* and *Turbinaria ornata* (class: *Phaeophyceae*). J Aquat Food Prod Technol. 2016;25(8):1249-65. doi: 10.1080/10498850.2015.1054540.
13. Chakraborty K, Dhara S. First report of substituted 2H-pyrenoids from brown seaweed Turbinaria conoides with antioxidant and anti-inflammatory activities. Nat Prod Res. 2020;34(24):3451-61. doi: 10.1080/14786419.2019.1578761, PMID 30835545.
14. World Health Organization. Lack of new antibiotics threatens global efforts to contain drug-resistant Infections; 2020. Available from: https://www.who.int/news-room/detail/17-01-2020-lack-of-new-antibiotics-threatens-global-efforts-to-contain-drugresistant-Infections [cited 11/1/2023].
15. Huang ZH, Li SQ, Kou Y, Huang L, Yu T, Hu A. Risk factors for the recurrence of diabetic foot ulcers among diabetic patients: a meta-analysis. Int Wound J. 2019;16(6):1373-82. doi: 10.1111/in.13200, PMID 31489774.
16. Diabetic foot. In: Liapis CD, Balzer K, Benedetti-Valentini F, Fernandes E, Fernandes J, editors. Vascular surgery. Berlin, Heidelberg: Springer Berlin Heidelberg; 2007. p. 501-21.
17. Banu A, Noorul Hassan MMN, Rajkumar J, Srinivasa S. Spectrum of bacteria associated with diabetic foot ulcer and biofilm formation: A prospective study. Australas Med J. 2015;8(9):280-5. doi: 10.4066/AMJ.2015.2422, PMID 26464584.
18. Valentina J, Poonguzhali TV, Josmin Laali Nisha LL. Mosquito larvicidal and suicidal activity of seaweed extracts against *Aedes aegypti*, *Anopheles stephensi* and *Culex*. Int J Mosq Res. 2015;2(4):54-9.
19. Rhimou B, Hassane R, José M, Nathalie B. The antibacterial potential of the seaweeds (*Rhodophyceae*) of the Strait of Gibraltar and the Mediterranean Coast of Morocco. Afr J Biotechnol. 2010;9(38):6365-72.
20. Mehdinezhad N, Ghannadi A, Yegdaneh A. Phytochemical and biological evaluation of some Sargassum species from the Persian Gulf. Res Pharm Sci. 2016;11(3):243-9. PMID 27499794.
21. Obouayeba AP, Diarrassouba M, Soumahin EF, Kouakou TH. Phytochemical analysis, purification and identification of *hibiscus* Anthocyanins. J Pharm Chem Biol Sci. 2015;3(2):156-68.
22. Deyab M, Elkatony T, Ward F. Qualitative and quantitative analysis of phytochemical studies on brown seaweed, *Dictyota dichotoma*. Int J Eng Dev Res. 2016;4(2):674-78.
23. Gul R, Jan SU, Faridullah S, Sherani S, Jahan N. Preliminary phytochemical screening, quantitative analysis of alkaloids, and antioxidant activity of crude plant extracts from *Ephedra intermedia* indigenous to Balochistan. ScientificWorldJournal. 2017; 2017:5873648. doi: 10.1155/2017/5873648, PMID 28386582.
24. Chitrikha Suresh TS, Poonguzhali TV, Anuradha V, Ramesh B, Suresh G. Aqueous extract of Turbinaria Conoides (J.Agardh) Kützing mediated fabrication of silver nanoparticles used against bacteria associated with a diabetic foot ulcer. Mater Today Proc. 2021;43(5):3038-43. doi: 10.1016/j.matpr.2021.01.376.
25. Elnabris KJ, Elmanama AA, Chihadeh WN. Antibacterial activity of four marine seaweeds collected from the coast of the Gaza Strip. Palestine Mesopot. J Mar Sci. 2013;28(1):81-92.
26. El Shafay SM, Ali SS, El-Sheekh MM. Antimicrobial activity of some seaweed species from the Red Sea against multidrug-resistant bacteria. Egypt J Aquat Res. 2016;42(1):65-74. doi: 10.1016/j.ejar.2015.11.006.
27. Ramesh C, Koushik S, Shunmugaraj T, Murthy MVR. Bioinvasive Seaweed Genus, Turbinaria in coral Reefs of Gulf of Mannar. J Life Sci Research. 2019;6(1):1-4. doi: 10.20448/journal.504.2019.61.1.4.
28. Rohfritsch A, Payri C, Stiger V, Bonhomme F. Molecular and morphological relationships between two closely related species, *Turbinaria ornata* and *T. Conoides* (Sargassaceae, Phaeophyceae). Biochem Syst Ecol. 2007;35(2):91-8. doi: 10.1016/j.bse.2006.09.002.
29. O'Keeffe E, Hughes H, McLoughlin P, Tan SP, McCarthy N. Antibacterial activity of seaweed extracts against plant pathogenic bacteria. J Bacteriol Mycol. 2019;6(3):1105.
30. Chakraborty K, Praveen NK, Vijayan KK, Rao GS. Evaluation of phenolic contents and antioxidant activities of brown seaweeds belonging to *Turbinaria spp.* (Phaeophyta, Sargassaceae) collected from the Gulf of Mannar. Asian Pac J Trop Biomed. 2013;3(1):8-16. doi: 10.1016/S2221-1691(13)60016-7, PMID 23570010.
31. Nawas PMA, Sujatha R. Phytochemical analysis and antibacterial potential of marine algae against human and aquatic pathogens. Int J Pharm Sci Rev Res. 2019;58(1):164-70.
32. Proestos C, Boziaris IS, Nychas G-JE, Komaitis M. Analysis of flavonoids and phenolic acids in Greek aromatic plants: investigation of their antioxidant capacity and antimicrobial activity. Food Chem. 2006;95(4):664-71. doi: 10.1016/j.foodchem.2005.01.049.
33. Wadood A Phytochemical Analysis of Medicinal Plants Occurring in Local Area of Mardan. Biochem Anal Biochem;02(4). doi: 10.4172/2161-1009.1000144.
34. Saleh B, Al-Mariri A. Antimicrobial activity of the marine algal extracts against selected pathogens. J Agric Sci Technol. 2017; 19:1067-77.
35. Padmakumar K, Ayyakkannu K. Seasonal variation of antibacterial and antifungal activities of the extracts of marine algae from Southern coasts of India. Botanica Marina. Mar 1997;40(1-6):507-15. doi: 10.1515/botm.1997.40.1-6.507.
36. Caccamese S, Azzolina R. Screening for antimicrobial activities in marine algae from eastern Sicily. Planta Med. 1979;37(4):333-9. doi: 10.1055/s-0028-1097346, PMID 538107.

37. Pesando D, Caram B. Screening of marine algae from French Mediterranean coast for antibacterial and antifungal activity. *Bot Mar.* 1984; 27:381-6.
38. Manivannan K, Karthikai Devi G, Anantharaman P, Balasubramanian T. Antimicrobial potential of selected brown seaweeds from Vedalai coastal waters, Gulf of Mannar. Asian Pac J Trop Biomed. 2011;1(2):114-20. doi: 10.1016/S2221-1691(11)60007-5, PMID 23569739.
39. Boonchum W, Peerapornpisal Y, Kanjanapothi D, Pekkoh J, Amornlerdpison D, Pumas C Et al. Antimicrobial and anti-inflammatory properties of various seaweeds from the gulf of Thailand. Int J Agric Biol. 2011; 13:100-4.
40. Ganapathy S, Lingappa S, Naidu K, Selvaraj U, Ramachandran S, Ponnusamy S Et al. Isolation and bioactive potential of fucoidan from marine macroalgae *Turbinaria conoides*. ChemistrySelect. 2019;4(48):14114-9. doi: 10.1002/slct.201903548.
41. Shibu A, Dhanam S. *In vitro* antifungal activity of *Turbinaria conoides* collected from Mandapam coast, Tamil Nadu, India Journal of Experimental Sciences. 2016; 7:27-30.
42. Ponnan A, Ramu K, Marudhamuthu M, Marimuthu R, Siva K, Kadarkarai M. Antibacterial, antioxidant and anticancer properties of Turbinaria Conoides (J. Agardh) Kurtz. Clin Photos. 2017; 3:1-10.
43. Zubair MS, Ardiana, Nugrahani AW. Antifungal Activity of Methanolic Extract of the Brown Seaweed Turbinaria ornata (Turner) J. Agardh, from Tomini Bay against Candida albicans. IOP Conf Ser.: Earth Environ Sci;253. doi: 10.1088/1755-1315/253/1/012020.