ORIGINAL ARTICLE

Preparation and *In Vitro* Evaluation of a Simple Ointment Containing Ethanolic Extract of *Turbinaria ornata*, a Brown Alga from Red Sea, Jazan, KSA

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Abstract

Objective: The purpose of this work is to investigate the antibacterial activity of *Turbinaria ornata* (*Turner*) J. Agardh, a rare brown alga belonging to the Sargassaceae family collected from Red Sea, Jazan, KSA. The study also focuses on the formulation of a topical ointment for healing wounds. **Materials and Methods:** The phytochemical extraction was performed using the Soxhlet apparatus by a hot continuous percolation technique, with ethanol as the solvent. The crude extract was analyzed to find out various phytoconstituents. The extract was screened for antibacterial potency against selected human pathogenic bacteria, namely *Staphylococcus aureus, Streptococcus pyogenes, Bacillus subtilis, Klebsiella pneumonia, Escherichia coli, and Pseudomonas aeruginosa.* In this study, the extract was further formulated into a 1% w/v ointment which was screened against the test bacteria. **Results:** The ethanol extract of *T. ornata* has shown the presence of carbohydrates, proteins, amino acids, alkaloids, saponin, fixed oil, fat, tannins, and flavonoids. The crude extract and ointment elicited good antibacterial effect against *S. aureus, S. pyogenes, B. subtilis,* and *E. coli* but were inactive against *Kl. pneumonia* and *P. aeruginosa.* However, the antibacterial effect was more pronounced in the ointment compared to the crude extract. **Conclusion:** *T. ornata* has shown potent antibacterial properties against selected human pathogenic bacteria. The present study may lead to new antibacterial formulations for topical applications.

Key words: Antibacterial action, brown alga, red sea, seaweed

INTRODUCTION

The antimicrobial properties of various seaweeds belonging to *Phaeophyceae*, *Rhodophyceae*, and *Chlorophyceae* have been reported by many researchers across the world.[1-3] An earlier report had demonstrated the antioxidant and antiproliferative ability of *Turbinaria ornata*.[4] Vijayabaskar and Shiyamala, in 2011,[5] reported the antibacterial activity of *T. ornata* against various bacteria. Topical antibacterial therapy offers many potential advantages to treat skin-based infections, especially diabetic wounds.[6] However, wound infections persist as a common public health problem due to the misuse of antibiotics as a prophylactic measure, leading to the resistant character of bacteria. Therefore, the development of antibiotic resistance is a significant hurdle in treating wounds.[7] In connection with the earlier cited problem, the present study focuses on a seaweed of Jazan origin. Accordingly, the

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present study is on *T. ornata* (*Turner*) J. Agardh, a brown alga belonging to the Sargassaceae family, collected in March and April 2017, in the Red Sea, Al Murjan beach, Jazan, Saudi Arabia, which is not yet investigated for its potential as a pharmaceutical agent. Thus, the present research is focused on the study of the antibacterial activities of *T. ornata's* ethanolic extract and the development of a topical ointment, aiming to increase the spectrum of activity of phytoextracts, thereby creating a formulation that will be useful in topical wound healing.

MATERIALS AND METHODS

Materials

All chemicals used in this study were manufactured by Scharlau, Spain, and purchased from Somatco, Jeddah, KSA.

Collection, identification, and processing

T. ornata (*Turner*) J. Agardh, a rare species, was collected from the seashore as well as the intertidal region of Al Murjan Beach, Red sea, Jazan, KSA, during March–April 2017. After the collection, the samples were washed thoroughly to remove the extraneous matter attached to the seaweeds. After the cleaning, the sample was air-dried, packed in airtight plastic bags, and transported to the laboratory for further processes. The collected sample was identified in the herbarium of Jazan University (JAZUH), and voucher specimens were also deposited for future reference. The air-dried sample was further dried in the shade at room temperature for 10 days. Then, the dried seaweed sample was cut into small pieces and powdered using a grinder to get a fine powder, which was collected and stored at room temperature in an airtight container.

Solvent extraction and phytochemical screening

Hot continuous percolation of the sample was performed using ethanol as a solvent for extracting the phytochemical constituents. Briefly, 200 g of dried seaweed powder and 500 ml of ethanol were taken in a Soxhlet apparatus. The total assembly was set up in a heating mantle, and it was run at 60°C continuously for 4 h. After the extraction, the extract was transferred into a separate glass beaker individually and kept open for solvent evaporation. The extract was allowed to dry completely, and the extracted sample was collected by scrapping form glass beaker, and it was pooled and weighed. Based on the obtained sample, the percentage yield was calculated using the following formula:

$$\text{Percentage yield} = \frac{\text{Weight of dried extract}}{\text{Weight of seaweed powder utilized for extraction}} \times 100$$

The bioactive principles were analyzed by performing the chemical test as established by Sivakumar *et al.*, 2013; 2008.[8,9] As ascertained earlier by Sivakumar *et al.*, 2013,[8] the extracts were screened for antibacterial effect in various gradient concentrations from 50 µg/100 µl to 500 µg/100 µl. In this study, 500 µg/100 µl of ethanolic extract concentration was used for the preparation of ointment.

Preparation of 1% w/w ointment

The required quantity of the ethanolic extract of *T. ornate* (500 µg/100 µl) was blended with white soft paraffin and yellow beeswax using ointment spatula on a porcelain tile. The physical properties of the ointment, such as the color, odor, pH, and spreadability, were evaluated as established by Rajasree *et al.*, 2012.[10] A known quantity of the ointment was dissolved in dimethly sulfoxide to get 1% w/v of the sample for antibacterial screening.

Bacterial strains used and antibacterial studies

24 h cultures of *Staphylococcus aureus, Streptococcus pyogenes, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa* were employed after the standardization of the cultures by finding the colony-forming unit (CFU) of working stock culture. The antibacterial effect was screened by performing the agar well diffusion technique.[8,11] Briefly, 100 µl of 24 h standardized bacterial cultures were spread on Muller Hinton (MH) agar plates individually using sterile L-shaped rods. After spreading the cultures on MH agar plates, three wells (n = 3) were formed using standard borer, and the extract was placed in the respective wells. The plates were incubated at 37°C for 24 h and observed for the development of inhibitory zones after 24 h of incubation. The zones of inhibition were measured and tabulated. The antibacterial spectrum of activity of the crude bioactive principles and their ointment dosage form was well-compared with the potentiality of streptomycin disc (10 µg/ml).

Statistical analysis

The experiment conducted for antibacterial screening was performed 6 times (n = 6), and the data were subjected to one-way analysis of variance; the level of significance is P <0.0001 (very high significant); P < 0.001 (highly significant); and P < 0.005 (significant) using GraphPad Instat software system. The test values were compared with standard drug values using Dunnett's test (*post hoc* test).

RESULTS

The ethanolic extract of *T. ornata* showed the presence of carbohydrates, proteins, amino acids, alkaloids, saponin, fixed oil, fat, tannins, and flavonoids. In the present study,

1% w/v of yield was obtained after the extraction process. The physical parameters of the prepared ointment have shown various features, such as pale-yellow color, smooth texture, homogeneity, 6.8pH, and the spreadability time of 10 s [Table 1]. Unique CFUs were observed for individual organisms after the standardization of the bacterial cultures. The results summarized in Table 1 demonstrate that the extract as well as the ointment has shown maximum activity against *E. coli* followed by *B. subtilis*, *S. aureus,* and *S. pyogenes*. However, there was no activity against *Kl. pneumonia* and *P. aeruginosa.*

DISCUSSION

The bacterial resistance of antibiotics in treating diabetic foot ulcer is a big challenge because of the poor penetration of the drug through the tissues. Unfortunately, new antibiotics are not discovered promptly and regularly due to the huge investment involved by pharmaceuticals. Moreover, there is no assurance that new antibiotics will not develop resistance. Therefore, the topical antibiotic therapy of infected diabetic foot wounds can focus locally at the site and can avoid the adverse effects of systemic antibiotics. Finding new antimicrobial molecules has been focused on marine resources for the past three decades.[8,12,13] In the current study, the presence of various bioactive principles, such as carbohydrates, proteins, amino acids, alkaloids, saponin, fixed oil, fat, tannins, and flavonoids, is noticed. Paramasivam *et al.*, 2017,[14] reported that the n-hexane and aqueous extracts of *T. ornata* had shown the presence of saponin, alkaloids, amino acids, fixed oils and fats, tannins, flavonoids, and total phenol. The physical features of the prepared ointment are satisfactory and acceptable for human use. In this study, the crude extract and its ointment showed the spectrum of activity against *E. coli*, *B. subtilis*, *S. aureus,* and *S. pyogenes*. However, *Kl. pneumonia* and *P. aeruginosa* were resistant to the crude extract. Interestingly, the activity did not improve even after the preparation of the ointment. However, the ointment preparation enhanced the activity of the extract against the rest of the organisms that were screened. The earlier report revealed that the petroleum ether extract of *Trochomorpha conoides* exhibited excellent activity against *P. aeruginosa,* i.e., more than standard amoxicillin.[15] Arumugama *et al.*, 2017,[16] reported that the ethanolic extract of *T. conoides* did not show the presence of alkaloids. The investigators demonstrated that the ethyl acetate fraction of *T. conoides* showed the highest antibacterial, antioxidant, and anticancer activity. Similarly, in our study, the ethanolic extract of *T. ornata* did not show the presence of alkaloids. Earlier studies also proved the importance of tannins as antibacterial agents against different organisms.[17,18] In this study, the spectrum of antibacterial activity is due to the presence of tannins is proved. Wang *et al.*, 2009,[19] reported that the antibacterial activity of phlorotannins was due to its inhibition of oxidative phosphorylation and the binding on the cell membrane that leads to cell lysis. In 2005, Rattaya *et al.*[20] demonstrated the antioxidant and antibacterial properties of the methanolic extract of *T. oranta* against *S. aureus* and the

Table 1: Physical characterization and antibacterial studies on simple ointment containing ethanolic extract of *T. ornata*

Physical characterization					Antibacterial studies			
Colour	Texture	Homogeneity	pH	Spreadability (sec)	Bacterial organisms	Bacterial concentration (CFU/ml)	Zone of inhibition (mm)	
							Ethanolic extract	Simple ointment	Streptomycin (10 µg/disc)
Pale yellow	Smooth	Yes	6.8	10	*S. aureus*	10^{-4}	17.83±0.75***	20.3±1.03ns	23.5±1.77
					S. pyogenes	10^{-5}	16±0.89 ***	18.3±0.51*	22±1.265
					B. subtilis	10^{-5}	17.6±0.81***	19.3±1.03***	25.66±1.9
					E. coli	10^{-6}	21±0.89***	22±1.6***	27.33±1.63
					Kl. pneumonia	10^{-3}	-	-	25.6±1.033
					P. aeruginosa	10^{-4}	-	-	21.16±1.32

*Each value is the mean of six batches with standard deviation the test values were compared with standard drug value by Dunnett's post hoc test. ***$P<0.001$ extremely significant, *$P<0.05$ is significant; ns: Non-significant, CFU: Colony-forming unit, *S. aureus: Staphylococcus aureus, S. pyogenes: Streptococcus pyogenes, B. subtilis: Bacillus subtilis, E. coli: Escherichia coli, Kl. pneumonia: Klebsiella pneumonia, P. aeruginosa: Pseudomonas aeruginosa, T. ornate: Turbinaria ornata*

lack of activity against *B. subtilis* and Gram-negative bacteria. In contrast to their study, the ethanolic extract of *T. oranta* exhibited a good spectrum of activity against Gram-positive bacteria *S. aureus, S. pyogenes,* and *B. subtilis* and Gram-negative bacteria *E. coli,* but there was no activity against Gram-negative bacteria *Kl. pneumonia* and *P. aeruginosa.* Furthermore, in this study, by formulating the ointment dosage form of the obtained crude extract, the antibacterial activity against the screened organisms was improved except for *Kl. pneumonia* and *P. aeruginosa.* Thus, the ointment will be topically beneficial, and therefore, further *in vivo* study should be done on its wound healing properties, especially in diabetic wound ulcers, which is a foremost problem in the present therapeutic era.

CONCLUSION

The research work demonstrated that the ethanolic extract of *T. ornata* and its 1% w/w of simple ointment showed good antibacterial activity against certain human pathogenic bacterial strains. A further detailed study is under process to understand the impact of the seasonal variations on the phytoconstituents and their antibacterial effects.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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