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ANTIBACTERIAL ACTIVITY OF THE CRUDE EXTRACT OF *TRIDIDEMNUM SAVIGNII* (HERDMAN 1886) AGAINST CLINICAL PATHOGENS

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ABSTRACT:

Antibacterial activity of the ascidian, *Tridemnum savignii* was tested against human clinical pathogens by agar well diffusion method. Different concentrations of 0.5mg/ml and 1mg/ml of the crude extract were analysed in this experiment. The crude methanol extract showed more active and broad-spectrum of antibacterial activity than the ethanol extract. The maximum inhibition zone (14mm) was observed against *Escherichia coli* at 1mg/ml concentration while the minimum inhibition zone of 2mm was noticed in 1mg/ml of ethanol extract against *Vibrio parahaemolyticus*. Highest MIC and MBC (1.10) were observed in methanol extract of 1mg/ml concentration than the ethanol extract. The result indicates that the crude extract of *Tridemnum savignii* have an excellent antibacterial activity against the tested clinical pathogens. Further studies will fulfil the purification and the structural elucidation of antibacterial drugs and secondary metabolites from this ascidian.

KEYWORDS:

Antibacterial activity, ascidian, Tridemnum savignii, crude extracts, MIC and MBC.

INTRODUCTION

The number of natural products isolated from marine organisms increasing rapidly in recent years and reached more than hundreds of novel compounds .The natural products of marine organisms, a large proportion have been extracted from invertebrates especially, sponges, ascidians, bryozoans, molluscs and most of them are currently in preclinical and clinical trials [1]. Ascidians are marine invertebrate's ranks second with gifted source of drugs [2]. They are the richest source of biologically active compounds and ranked third for their overall activities, next to sponges and bryozoans [3]. The biologically active compounds identified from marine source, comprised of various derivatives of peptides and alkaloids. Still the natural products found out from marine organisms only less than 1% has been analysed for pharmacological deeds [4]. Although research on bioactive compounds from ascidians were recently started the first marine natural product Didemnin B is entering into human clinical trial and is an ascidian metabolite. But unfortunately it was failed in the further trials [5]. Ascidians such as *Botryllus species, Didemnum species, and Tridemnum solidum* were proved for producing anti cancer drugs [2, 6]. The potent anticancer bioactive compound Ecteinascidin-743 was isolated from Caribbean tunicate *Ecteinascidia turbinate* [7].

Many other highly potent bioactive compounds were isolated from marine organisms so that they are consumed as food in many East Asian countries. From this mechanism the researchers got an idea to develop an alternate natural medicine for human beings from ascidians [8]. In the arena of marine habitat the colonization process is affected by organic metabolites produced by the host organism. Such metabolites may affect bacteria in number of ways, ranging from the induction of chemo tactic responses to the inhibition of bacterial growth or cell death [9]. Secondary metabolite plays a vital role as a chemical defence against epibosis [10]. The present study was carried out to evaluate the antibacterial properties of the secondary metabolites derived from the ascidians on the expansion of some selected human bacterial pathogens.

MATERIALS AND METHODS

Specimen collection and identification

Ascidians were collected during the low tide of the intertidal area at Palk Bay region (Latitude 9º 55' 10º 45'E and Longitude 78º 58' 79º 55'N), Southeast coast of India during June, 2013. To remove sand, mud, salt and the overgrowing organisms from the collected samples, they were thoroughly washed with autoclaved sea water at the site of sample collection. The samples were preserved in the mixture of methanol and ethanol solvents (1:2) and transported to the laboratory aseptically. The collected samples were identified by using the standard literature and shade dried.

Sample extraction

Sample extraction process followed by the standard procedure [11]. Ten grams of the sample was dried and powdered using homogenizer. The homogenized samples were soaked in the above mentioned solvents for 48hrs. The extracts were then filtered through Whatman No. 1 filter paper and the solvents were concentrated by rotary evaporator. The resultant residues were stored at 4ºC for further analysis.

Antibacterial assay

Antibacterial activity was carried out by using standard agar well diffusion method [12]. Bacterial strains such as *Escherichia coli, Streptococcus aureus, Vibrio parahaemolyticus* and *Vibrio vulnificus* were used for this assay. Muller Hinton agar plates were prepared by pouring 15ml of the medium and allowed to solidify. The petri plates were swabbed with 24 hrs old culture of the four selected bacterial strains. Wells were made on the agar plates seeded with isolated human pathogens by using gel puncture and the extracts were loaded in the wells and incubated at 37ºC for 24hrs. Susceptibility of the clinical pathogens was determined by the zone of inhibition around each well.

Minimum Inhibitory Concentration (MIC):

Minimum inhibitory concentration was determined by Collins method [13].

Minimum Bactericidal Concentration (MBC):

Minimum bactericidal concentration was experimented after the MIC in freshly prepared agar plates was followed by the standard method [14].

RESULTS AND DISCUSSION

In-vitro antibacterial screening of ascidian, *Trididemnum savignii* against selected clinical isolates was performed and the zone of inhibition is given in the **Table 1**. Maximum inhibition zone of 14 mm was observed against *E.coli* (**Figure 1**) in crude methanol extract (1mg/ml). In methanol extract, the range of inhibition of the clinical pathogens varied from 5-14 mm. This is consistent with the findings [15] where the crude methanol extract of *Didemnum psammathodes* exhibited strong antibacterial activity and the zone ranged from 2-15 mm with an average of 4.3mm. On the other hand, reported that the crude methanol extract of *Phallusia arabica* showed the zone of inhibition varied from 4-12 mm with an average of 7 mm. For ethanol extract of *Phallusia arabica*, the range of zone of inhibition varied from 3-10 mm [16]. In the present study the crude methanol extract of *Trididemnum savignii* showed the maximum antibacterial activity in 1mg/ml concentration against *E. coli*, followed by *V. vulnificus, S. aureus* and *V. parahaemolyticus.* The minimum activity was noticed in *V. parahaemolyticus* in 0.5mg/ml concentration of the same extract. Crude ethanol extract showed the maximum antibacterial activity in *E. coli*, followed by *V. vulnificus, S. aureus* and *V. parahaemolyticus* at 1mg/ml concentration and the minimum activity was observed in *V. parahaemolyticus* (**Figure 2**) of 0.5mg/ml concentration.

FIGURES

Figure 1 Antibacterial activity of *Trididemnum savignii* against *E.coli*

Figure 2 Antibacterial activity of *Trididemnum savignii* against *V.parahaemolyticus*

Table: 1 Antibacterial activity of *Tridemnum savignii* (Herdman 1886)

Zone of Inhibition(mm)				
Clinical pathogens	**Methanol extract**		**Ethanol extract**	
	0.5mg/ml	**1mg/ml**	**0.5mg/ml**	**1mg/ml**
S.aureus	6	8	3	5
V.parahaemolyticus	5	8	-	2
V.vulnificus	7	9	6	9
E.coli	10	14	8	10

Table: 2 MIC and MBC of different concentration of crude extracts of *Tridemnum savignii* (Herdman 1886)

Clinical pathogens	**Ethanol extract MIC (0.5mg/ml)**	**MIC (1mg/ml)**	**Ethanol extract MBC (0.5mg/ml)**	**MBC (1mg/ml)**	**Methanol extract MIC (0.5mg/ml)**	**MIC (1mg/ml)**	**Methanol extract MBC (0.5mg/ml)**	**MBC (1mg/ml)**
S.aureus	0.75	0.85	0.95	1.05	0.70	0.90	0.80	0.95
V.parahaemolyticus	-	0.80	-	0.90	0.85	1.10	0.80	0.90
V.vulnificus	0.85	0.95	0.90	1.05	-	0.80	-	0.85
E.coli	-	0.90	-	1.05	-	0.85	-	1.10

The minimum inhibitory concentration (MICs) and minimum bactericidal concentration (MBCs) of the crude extracts are showed in Table. 2. Range of MIC varied between 0.75-1.10 in 0.5 mg/ml and in 1mg/ml against all the bacterial strains. Minimum bacterial concentration (MBC) ranges between 0.80-1.10 in 0.5mg/ml and in 1mg/ml against all the bacterial strains. The MIC was more (0.95) in ethanol extract at 1mg/ml concentration and methanol extract (1.10) at 1mg/ml concentration. The highest MBC (1.05) was observed in ethanol extract at 1mg/ml concentration and methanol extract (1.10) at 1mg/ml concentration. The MIC was low (0.75) in ethanol extract at 0.5mg/ml concentration and methanol extract (0.70) at 0.5mg/ml concentration. The MBC was low (0.90) in ethanol extract at 0.5mg/ml concentration and methanol extract (0.80) at 0.5mg/ml concentration. The minimum inhibitory concentrations (MIC) and minimum bactericidal concentration (MBC) of the methanol extract of *Polyclinum madrasensis* and the MIC was varied between 0.70-0.95 mg/ml and MBC ranges between 0.85-1.10 mg/ml against all the microbes tested [17]. Methanol extracts of ascidians were evaluated and well documented as a potent antibacterial metabolite [18]. Ascidians are already reported for rich nitrogenous source with a wide range of biological activities [19].

The preliminary screening of nine species of ascidian indicated the presence of antibacterial activity of the three different solvents was tested (methanol, methylene chloride and hexane). Among them methylene chloride extracts showed maximum activity followed by methanol and hexane [20]. The evaluation of antibacterial activity against *Agrobacterium tumifaciens*, *E. coli*, *P. aeruginosa* and *S. aureus* from the extracts of Morocco Atlantic sea ascidian,*Cynthia savignyi* [21]. It showed a good antibacterial activity except the dichloromethane extract. In that study *A. tumifaciens* was more sensitive against the ascidian extract. Activity of hexane and diethyl ether extracts of this ascidian against *A. Tumifaciens* was somewhat less, but higher than the activity of *Lissoclinum fragile* extracts. Ascidian has the potential to yield novel compounds of ecological, chemical and also biomedical interest [22]. Specifically, the cosmopolitan genus, *Tridemnum* is renowned for the variability of its metabolites. From ascidians, *Tridemnum solidum* the first marine compound entered into human cancer clinical trial as a purified natural product [6]. This class of cyclic peptide provides important structural lead for a variety of antiviral, cytotoxic, anticancer and immunosuppressant activities [23]. A new pentacyclic alkaloid Shermilamine A from purple colonial ascidian, *Tridemnum sp*. [24]. However a wide range of natural products has been isolated from tunicates little is known about the ecological roles of most of these metabolites and their distribution within ascidian tissues [25, 26]. Marine genus synthesizes active constituents which are used in traditional and complementary medicine [27]. Overall, an antibacterial compounds from natural resources would be an alternative to overcome the resistant of clinical pathogens. From this study the ascidian, *Tridemnum savingnii* seems to be a promising source of antibacterial compounds which could be used in pharmacological research.

CONCLUSION

The present study deduces that the progressing and overwhelming contributions of ascidians metabolites for the progress of new pharmaceuticals are clearly evident and need to be explored. After taking this consideration the immense side effects of synthetic drugs, great attention has to be paid for the discovery of new drugs from marine natural products.

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