Indonesian Journal of Life Sciences Vol. 02 | Number 02 | September (2020)
http://journal.i3l.ac.id/ojs/index.php/IJLS/

REVIEW ARTICLE

A Review on the Antimicrobial Properties of Giant Barrel Sponge-*Xestospongia* sp.

Gisella Edny[1], Jeannifer Rebecca[1], Sheryl[1], Audrey Amira Crystalia[1], Leonny Yulita Hartiadi[1*]
[1]Department of Pharmacy, Indonesia International Institute for Life Sciences, Indonesia

**corresponding author. Email: leonny.hartiadi@i3l.ac.id*

ABSTRACT

Indonesia sits in the heart of the largest biodiversity hotspot -Indo-Pacific region. Indonesia has access to endless resources of bioactive compounds from marine animals and plants. Marine sponges have been extensively studied over the years due to their nature of being exposed to various microorganisms. Xestospongia sp. establishes a symbiotic relationship with diverse microorganisms, leading to the synthesis of abundant bioactive resources which capable of inhibiting the growth of pathogenic bacteria. Publications from the last ten years were retrieved from PubMed and included in this review article. Bioactive compounds produced by *Xestospongia* sp. were effective in inhibiting gram-negative bacteria-*P. aeruginosa*, *A. baumanii*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. epidermis*, *S. typhi*- and gram-positive bacteria -*M. Intracellulare*, *S. aureus*, *S. pneumoniae*, *B. subtilis*, *V. anguaillarum*. In addition, extracts were able to inhibit the growth of multidrug-resistance *P. aeruginosa* and methicillin-resistant *S. aureus* (MRSA). *C. albicans*, *C. tropicalis*, *C. neofarmans*, *A. niger*, *Epidermophyton* sp., *M. gypseum*, *T. rubrum*, *T. mentagrophytes* were susceptible to *Xestospongia* sp. extracts. The growth of chloroquine-resistant and susceptible strains of *P. falciparum* were inhibited by *Xestospongia* sp. with similar zones of inhibitions. The antimicrobial properties were contributed by the composition of chemically complex compounds such as phenolics, steroids and alkaloids; each of which exhibits a unique mechanism of action. The vast range of antimicrobial activity exhibited by *Xestospongia* sp. extracts implies their promising role in clinical settings for the treatment of infectious diseases including tuberculosis and malaria.

Keywords: *Xestospongia* sp.; Antimicrobial; Antibacterial; Antifungal; Antimalarial

INTRODUCTION

Indonesia is considered as one of the world's richest countries in terms of its biodiversity because Indonesia is an archipelago made up of more than 17.000 islands, each of them containing unique ecosystems. Almost 78% of Indonesian territory is covered in water, making it a vast resource of marine biodiversity. Marine biodiversity plays a key role through ecosystems because it is a reservoir of bioactive compounds (Goulletquer, Gros, Boeuf, & Weber, 2014). Indonesia has an ocean-wide resource of bioactive compounds from marine animals, corals, plants and microorganisms. However,

the study of marine biodiversity is not as common as the land biodiversity which suggests that there are still yet endless options of marine life that can be explored and researched.

Sponges are an important part of marine life because they provide a habitat to various range of marine species. Sponges are rich in bioactive compounds because they can produce secondary metabolites. This secondary metabolite is produced as a response of their defense strategies due to their exposure to many microorganisms in the ocean (Hanif, Murni, Tanaka, & Tanaka, 2019). It is known that sponges contain enormous amounts of bacteria within their tissues, around 40-60% of biomass (Laport, Santos, & Muricy, 2009) making sponges among the richest sources of pharmacological products that can provide novel leads against bacterial, fungal, and parasitic disease (Figure 1). Sponges synthesize chemicals such as alkaloids as their secondary metabolites possessing diverse mechanisms of action that contributes to their antimicrobial activity. There are several antimicrobial activities of sponges such as cell division impairment and production of reactive oxygen species (Longeon *et al.*, 2011;Helber *et al.*, 2018). Moreover, sponges can produce other metabolites such as terpenoids, peptides, and polyketides which makes them desirable as antimicrobial sources (Kim & Dewapriya, 2012). Nowadays, researchers are trying to come up with novel antimicrobial drugs because infectious microorganisms develop resistance to existing antimicrobial drugs. Thus, sponges are promising candidates for the elucidation of novel antimicrobial compounds (Laport, Santos, & Muricy, 2009).

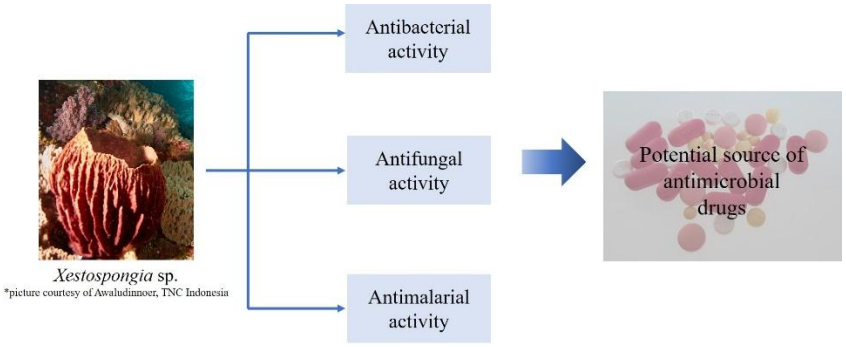

Figure 1. *Xestospongia* sp. as potential source of antimicrobial drugs

Xestospongia sp. "giant barrel sponge" is found in abundance in Indo-Pacific regions (Mcgrath, *et al.*, 2018); the largest marine biodiversity in which Indonesia lies in the heart of this region. Just like any other type of sponges, they can live up to hundred of years making them exposed to various microorganisms. *Xestospongia* sp. has salmon pink to purple color due to the presence of photosynthetic symbiotic cyanobacteria which contain reddish phycoerythrin and blue phycocyanin and this sponge is usually found up to 120 meters below the sea water (Wiedenmayer, 1977). *Xestospongia* sp. maintains organisms and microorganisms by providing food through filtering sea water with pores. Therefore, it opens chances for symbiotic relationships between the sponges and beneficial microorganisms (Brinkmann & Ipek, 2017).

Symbiotic relationships provide support and protection to the microbial symbionts and host organisms. Symbiosis can also contribute towards the host defense mechanisms, where

the compounds produced by symbiotic microorganisms are able to protect themselves and host from pathogens and predators (Brinkmann & Ipek, 2017). For example, symbiotic relationships with microorganisms such as *Micrococcus luteus* R-1588-10 and *Aspergillus versicolor* result in bioactive compounds that exert antimicrobial activity. Another example is symbiotic relationship with *Penicillium cf. montanense* which produces bioactive compounds that exert antifungal activity (Thomas, Kavlekar, & LokaBharathi, 2010). This review focuses on the antimicrobial properties of *Xestospongia* sp. extracts as well as those produced by their symbiotic microorganisms.

ANTIBACTERIAL ACTIVITY

Gram-negative bacteria

Numerous species of *Xestospongia* were tested against gram-negative bacteria. Ankisetty & Slattery (2012) tested different compounds isolated from *Xestospongia* sp. (ID: PN10407137) towards the growth of *Pseudomonas aeruginosa* (Table 1) (Ankisetty & Slattery, 2012). Three compounds were tested ($C_{24}H_{40}O_2$, $C_{22}H_{38}O_2$, $C_{24}H_{40}O_2$) and exhibited inhibition against bacterial growth (IC_{50} < 2 µM). Two other compounds elucidated had already been identified, namely 18-hydroxyrenierin-2 (IC_{50} = 2.6 µM) and strongylodiol A (IC_{50} = 2.9 µM) extracted from *Reniera fulva* and *Strongylophora* sponges, respectively (Cimino & De Stefano, 1977; Watanabe *et al*., 2000). *P. aeruginosa* is a gram-negative bacteria and notoriously known as one of the most virulent among opportunistic pathogens (Maurice, Bedi, & Sadikot, 2018). *P. aeruginosa* causes a wide range of acute and chronic infections, including ventilator-associated pneumonia and acute nosocomial infections (Sadikot, Blackwell, Christman, & Prince, 2005). Some strains of *P. aeruginosa* isolated from hospitals are found to be resistant to many antibiotics, including amikacin, tetracycline and ciprofloxacin (Mohanty, Baliyarsingh & Nayak, 2020). In addition, *P. aeruginosa* isolated from the lungs of cystic fibrosis patients were associated with higher morbidity and mortality compared to other bacterial infections (de Bentzmann & Plésiat, 2011). Hence, treatments against *P. aeruginosa* are urgent and extracts from *Xestospongia sp*. poses as a potent treatment.

Table 1. Antibacterial activity of *Xestospongia* sp. and their symbiotic microorganisms.

Place of origin	Target organism	Compound	Unit of inhibition	Reference
Pohnpei, Federated States of Micronesia at a depth of 40 meters below sea level in a cave	*M. intracellulare ATCC 23068*	$C_{24}H_{40}O_2$	IC_{50} = 9.9 µM	Ankisetty & Slattery, 2012
		$C_{22}H_{38}O_2$	IC_{50} = 7.7 µM	
		$C_{24}H_{40}O_2$	IC_{50} = 14.3 µM	
		18-hydroxyrenierin-2	IC_{50} = 23 µM	
		strongylodiol A	IC_{50} = 17.5 µM	
	P. aeruginosa ATCC 27853	$C_{24}H_{40}O_2$	IC_{50} = 1.7 µM	
		$C_{24}H_{40}O_3$	IC_{50} = 1.9 µM	
		$C24H_{40}O_4$	IC_{50} = 1.8 µM	
		18-hydroxyrenierin-2	IC_{50} = 2.6 µM	
		strongylodiol A	IC_{50} = 2.9 µM	
Sharm Obhur, Jeddah, Saudi	*A. baumannii*	$C_{19}H_{25}O_2Br$	ZOI = 14 mm	Ayyad *et al*., 2015
	E. coli	$C_{19}H_{25}O_2Br$	ZOI = 23 mm	

Arabia	*K. pneumoniae*	$C_{19}H_{25}O_2Br$	ZOI = 15 mm	
	P. aeruginosa	$C_{19}H_{25}O_2Br$	ZOI = 24 mm	
	S. aureus	$C_{19}H_{25}O_2Br$	ZOI = 12 mm	
	MRSA	$C_{19}H_{25}O_2Br$	ZOI = 12 mm	
	S. epidermis	$C_{19}H_{25}O_2Br$	ZOI = 16 mm	
	S. pneumoniae	$C_{19}H_{25}O_2Br$	ZOI = 13 mm	
	A. baumannii	$C_{19}H_{23}O_2Br$	ZOI = 15 mm	
	E. coli	$C_{19}H_{23}O_2Br$	ZOI = 12 mm	
	K. pneumoniae	$C_{19}H_{23}O_2Br$	ZOI = 19 mm	
	P. aeruginosa	$C_{19}H_{23}O_2Br$	ZOI = 19 mm	
	S. aureus	$C_{19}H_{23}O_2Br$	ZOI = 13 mm	
	MRSA	$C_{19}H_{23}O_2Br$	ZOI = 14 mm	
	S. epidermidis	$C_{19}H_{23}O_2Br$	ZOI = 14 mm	
	S. pneumoniae	$C_{19}H_{23}O_2Br$	ZOI = 14 mm	
Extract 1: Gosong Island at a depth of 5-10 m, Riau, Indonesia; Extract 2: Penjaul Island at a depth of 5-10 m, Riau, Indonesia	*S. aureus*	Extract of *Xestospongia* sp. 1	ZOI = 10.2 mm	Putra *et al*., 2016
		Extract of *Xestospongia* sp. 2	ZOI = 16.7 mm	
	B. subtilis	Extract of *Xestospongia* sp. 1	ZOI = 9.3 mm	
		Extract of *Xestospongia* sp. 2	ZOI = 8.6 mm	
	E. coli	Extract of *Xestospongia* sp. 1	ZOI = None	
		Extract of *Xestospongia* sp. 2	ZOI = None	
	V. anguaillarum	Extract of *Xestospongia* sp. 1	ZOI = 8.3 mm	
		Extract of *Xestospongia* sp. 2	ZOI = 10.3 mm	

*ZOI: zone of inhibition

Ayyad *et al*. (2015) elucidated two compounds, -$C_{19}H_{25}O_2Br$ and $C_{19}H_{23}O_2Br$,- through nuclear magnetic resonance and tested them against a variety of gram-negative and gram-positive bacteria. Among gram-negative bacteria tested were opportunistic pathogens *A. baumanii, E. coli, K. pneumoniae, P aeruginosa, and S.* pneumoniae (Ayyad *et al*., 2015). Both compounds produced visible ZOIs against a variety of gram-negative bacteria tested (Table 1). This indicates that the use of *Xestospongia sp*. is not limited to empirical treatment but as a prophylactic treatment against opportunistic infections. Studies done against *E. coli*, *K. pneumoniae*, *S. typhi* and MDR *P. aeruginosa* yielded ZOI within the ranges of 9-15 mm (Table 1). This is an important finding as it was proven that *Xestospongia* sp. extracts were potent against MDR *P. aeruginosa*. Results stated above provide a promising approach of utilizing *Xestospongia* sp. extracts and its symbiotic microbes extracts for the treatment of gram-negative bacteria infections.

Another study observed the secondary metabolites produced by symbiotic fungi inhabiting the surfaces of *Xestospongia*

testudinaria (Aulia et al., 2019). Seven symbiotic fungi were isolated which yielded 7 fungal extracts tested against *P. aeruginosa*. Based on table 2, all fungal extracts generated significant zones of inhibition (ZOI). It was concluded that Xt6 was the most active antibacterial agent. Phytochemical testing was performed on the extracts and it was shown that Xt6 contained phenols and alkaloids (Aulia et al., 2019). Numerous studies have established the potent antimicrobial potential of phenolics and alkaloids. Aulia *et al*. (2019) proposed that the mechanism of action of Xt6 as an antimicrobial agent was contributed by phenolics and alkaloids. Quarterneric aromatic compounds such as alkaloids are natural chelators of DNA and induce inter-strand DNA breaks, leading to bacterial cell death. Phenolics exert antimicrobial properties in a concentration-dependent manner. Phenols penetrate the cell membrane and trigger protein denaturation inside bacterial cells at low concentrations. In elevated concentration, phenols alter the permeability of bacterial cell membranes by coagulating with proteins intra- and extra-cellularly, followed by membrane lysis.

Table 2. Antibacterial activity of *Xestospongia testudinaria* and their symbiotic microorganisms.

Place of origin	Target organism	Isolated compound or extract	Zone of inhibition (mm)	Reference
Mandeh island at a depth of 10- 15 m, West Sumatra, Indonesia	*S. aureus*	Symbiotic fungi extract Xt1	8	Aulia *et al*., 2019
		Symbiotic fungi extract Xt2	16	
		Symbiotic fungi extract Xt3	7	
		Symbiotic fungi extract Xt4	9.5	
		Symbiotic fungi extract Xt5	7.5	
		Symbiotic fungi extract Xt6	15	
		Symbiotic fungi extract Xt7	8	
	P. aeruginosa	Symbiotic fungi extract Xt1	8	
		Symbiotic fungi extract Xt2	13.5	
		Symbiotic fungi extract Xt3	11.5	
		Symbiotic fungi extract Xt4	21.5	
		Symbiotic fungi extract Xt5	9.5	
		Symbiotic fungi extract Xt6	26.5	
		Symbiotic fungi extract Xt7	16.5	

Tanjung Kasuari, Sorong, Papua, Indonesia	*S. aureus*	Xp 4.1; Xp 4.2; Xp 4.3; Xp 4.4; Xp 4.5; Xp 4.6	-	Cita *et al*., 2017
	E. coli	Xp 4.1	19	
		Xp 4.2	24	
		Xp 4.3	-	
		Xp 4.4	23	
		Xp 4.5	18	
		Xp 4.6	-	
	B. subtilis	Xp 4.1	-	
		Xp 4.2	12	
		Xp 4.3	6	
		Xp 4.4	8	
		Xp 4.5	17	
		Xp 4.6	-	
	K. pneumoniae	Xp 4.1	8	
		Xp 4.2	22	
		Xp 4.3	16	
		Xp 4.4	15	
		Xp 4.5	14	
		Xp 4.6	16	
Pasir Putih, East Java, Indonesia	*S. aureus*	*Xestospongia* extract	20.1	Muzaki *et al*., 2017
	Escherichia coli		9.5	
	K. pneumoniae		15.25	
	S. typhi		15.25	
	P. aeruginosa		15.2	
	MDR *MRSA*		17.5	
Vinh Moc at a depth of 15-20 m, Quang Tri, Vietnam	*E. coli*	XT06	20	Nguyen *et al*., 2019
		XT10	20	
		XT19	15	
		XT28	10	
		XT34	10	
		XT39	20	
		XT52	13	
	P. aeruginosa	XT03	20	
		XT13	14	
		XT28	11	
		XT47	22	
	S. aureus	XT01	9	
		XT03	20	
		XT19	17	
		XT32	19	
		XT41	18	
		XT55	10	
	B. subtilis	XT01	12	
		XT10	15	

XT17	10
XT25	10
XT35	15
XT43	15
XT50	10
XT59	20

A similar study was done by Cita *et al*. (2017), however, instead of symbiotic fungi, they successfully isolated 6 symbiotic bacteria and tested the isolated bacteria against gram-negative bacteria (Cita *et al.*, 2017). All isolates except Xp 4.3 exhibited antibacterial activity against both gram-negative bacteria tested as shown with apparent ZOIs (Table 2). Further, isolate Xp 4.2 displayed superior antimicrobial activity with the highest ZOI to *S. aureus* (24 mm) and *K. pneumonia* (22 mm). Hence, the secondary metabolites from isolate Xp 4.2 were subjected to further testing. Phytochemical screening on the compounds showed the presence of alkaloid and steroid compounds in the pool of secondary metabolites which contributed to their antibacterial properties (Cita *et al.*, 2017). Aulia *et al*. and Cita *et al*. shared a common method of extraction which was extraction with ethyl acetate. This implies that the extracted compounds are similar in terms of alkaloidal structures. This raises the question of whether the compounds possessed similar pharmacophores responsible for antibacterial activity. Both studies tested isolates from the same sponge species (*Xestospongia testudinaria*) collected from different locations. Aulia *et al*. harvested sponges from *Pulau Mandeh,* West Sumatra, whereas Cita *et al*. retrieved the sample from *Tanjung Kasuari,* Sorong, Papua. The compounds observed from phytochemical testing were different even though they tested the same sponge species. Steroids were observed in one extract but not the other. This is an interesting finding as the same sponge species extracted with the same method contain different secondary metabolites. This may be possible due to the difference in metabolic pathways of symbiotic microorganisms; different microorganisms have distinct roles in maintaining the survival of both the sponge and the microorganisms itself (Taylor *et al.*, 2007).

Similar to Cita *et al*. (2017), Nguyen *et al*. (2019) isolated twenty different strains of symbiotic bacteria from *X. testudinaria* and tested these bacteria against *E. coli*, *P. aeruginosa, S. aureus* and *B. subtilis.* Out of 20 isolates, 7 isolates exhibited antibacterial properties against *E. coli* with a range of ZOI between 10-20 mm (Table 2). Only 4 isolates displayed an activity against *P. aeruginosa* with a ZOI ranging between 11-22 mm (Table 2). Additionally, the study identified the bioactive compounds-producing strains through 16s rRNA analysis. The strains verified were T01 (*B. subtilis*), XT19 (*B. lincheniformis*), XT34 (*P. fluvialis*), XT35 (*V. panuliri*), XT41 (*S. ascomycinius*), XT50 (*S. glebosus*), and XT55 (*Streptomyces* sp.). Microorganisms living within a symbiotic relationship with the sponge are also capable of synthesizing bioactive compounds. These bioactive compounds are able to ward off pathogenic bacteria which could otherwise jeopardize the survival of the sponge. Therefore, it is common for symbiotic bacteria to synthesize bioactive compounds to ward off pathogenic species.

Gram Positive Bacteria

In addition to the activities of *Xestospongia* sp. extracts towards gram-negative bacteria, assays were also conducted against gram-positive bacteria with different clinical implications. $C_{24}H_{40}O_2$, $C_{22}H_{38}O_2$, $C_{24}H_{40}O_2$, 18-hydroxyrenierin-2, and strongylodiol A revealed an IC_{50} of 2.6, 9.9, 7.7, 14.3, and 23 µM against *Mycobacterium intracellulare,* respectively (Table 1). Compared to the activity of compounds extracted from *Xestospongia* sp. towards gram-negative bacteria, activities against gram-positive bacteria were moderate (Ankisetty & Slattery, 2012). This could be due to differences in the morphology of the gram-negative and gram-positive bacteria. *Mycobacterium* genus is a gram-positive bacteria with a high incidence of intrinsic resistance due to relative impermeability of mycobacterial cell wall (Rodriguez, Garcia-Pachon, Ruiz, & Royo, 2006). *M. tuberculosis*, a tuberculosis associated strain, is a deadly pathogen that affects over two million people each year (Smith, 2003). Despite innovations in live attenuated vaccines and antibiotics for the treatment of *M. tuberculosis*, tuberculosis is still a serious condition in developing countries where sanitation and hygiene is still lacking. Therefore, novel treatments against Mycobacteria are crucial to reduce mortality rates world-wide and *Xestospongia* sp. extracts delivers promising results for the treatment of gram-positive bacteria.

Xestospongia sp. extracts were also tested against a variety of opportunistic gram-positive bacteria. Aulia *et al*. (2017) performed assays against *S. aureus* and obtained ZOIs between 7-16 mm (*Table 1*). ZOIs differences against gram-positive and gram-negative bacteria were observed where the extracts tested had lesser efficacy against gram positive bacteria as shown by smaller ZOI values. This result may arise from the fact that gram-positive bacteria are more resilient to influx of foreign compounds due to the presence of cell walls. This prominent observation was replicated in the studies done by Ayyad *et al*. (2015) and Cita *et al*. (2017). $C_{19}H_{25}O_2Br$ was tested against *S. aureus,* methicillin-resistant *S. aureus* (MRSA), *S. pneumoniae* yielded ZOI values of 12, 12 and 13 mm, respectively. $C_{19}H_{23}O_2Br$ was relatively more effective against *S. aureus,* MRSA and *S, pneumoniae* with ZOIs at 13, 14 and 14 mm. This suggests $C_{19}H_{23}O_2Br$ has better antibacterial activity against both gram-negative and gram-positive bacteria compared to $C_{19}H_{25}O_2Br$. Similarly, Muzaki *et al*. (2017) observed antibacterial activity of *X. testudinaria* extracts against *S. aureus* and MRSA with ZOI values of 20 and 17.5 mm, respectively (Table 2). Studies by Ayyad *et al*. (2015) and Muzaki *et al*. (2017) demonstrated the efficacy of *Xestospongia* sp. extracts against MRSA and drug resistant strains of *P. aeruginosa*. *Xestospongia* sp. extracts and secondary metabolites produced by their symbiotic microorganisms were effective against drug resistant strains of *P. aeruginosa* and MRSA. This provides insightful implications for the treatment of drug resistant strains of bacteria. MRSA strains are resistant to most β-lactam antibiotics (Guignard, Entenza, & Moreillon, 2005). MRSA constitutes over 44% of nosocomial infections in Europe and 18,000 deaths per year was recorded in the United States (Lawhon, 2016). Despite their alarming infections, most antibiotics are ineffective against MRSA. However, results from Ayyad *et al*. (2015) displayed optimistic potentials of the sponge's extract in clinical settings (Ayyad *et al*., 2015).

Nguyen *et al*. (2019) successfully isolated 20 different symbiotic microorganisms from *X. testudinaria* that were effective against *E. coli, P. aeruginosa, S. aureus* and *B. subtilis* with ZOI

values ranging from 9-20 mm (Table 2) (Nyuyen *et al*., 2019). Interestingly, secondary metabolites produced from the bacterial isolates exhibited similar ZOIs against gram-positive and gram-negative bacteria. This leads to the implication that different microorganisms with an established symbiotic relationship with the sponge possess similar roles in warding off both gram-negative and gram-positive bacteria as mentioned previously. Secondary metabolites produced by *Xestospongia* sp. are not necessarily potent against both gram-negative and gram-positive bacteria. Nevertheless, the establishment of a complicated niche composed of the sponge and symbiotic microorganisms creates a reservoir of chemically complex bioactive compounds. This is in line with a study done by Weis *et al*. (2001) where they concluded that the relationship between sponges and symbiotic microorganisms provides protection to both the host and microorganisms (Weis *et al*., 2001). Therefore, bioactive compounds responsible for antimicrobial activity are not solely produced by the sponges itself, but also by symbiotic microorganisms which synthesize biologically active compounds as well (Paul *et al.*, 2007).

ANTIFUNGAL ACTIVITY

C. albicans is one of very few fungal species causing disease in humans. It is a member of the healthy microbiota, but an alteration in host microbiota, changes in the host immune response, or variations in the local environment enable *C. albicans* to overgrow, develop virulence and result in infection (Lopez *et al*., 2015; Nobile & Johnson, 2015). Aulia *et al*. (2019) isolated *X. testudinaria* from Maneh Island, West Sumatra and tested it against *C. albicans*. Seven symbiotic fungi from *X. testudinaria* were isolated and purified. After incubation, all fungi symbiotic extracts showed an antifungal activity with ZOI ranging from 7.5-19 mm (Table 3).

Table 3. Antifungal activity of *Xestospongia testudinaria* and their symbiotic microorganisms.

Place of origin	Target organism	Isolated compound or extract	Zone of inhibition (mm)	Reference
Mandeh island at a depth of 10- 15 m, West Sumatra, Indonesia	*C. albicans*	Symbiotic fungi extract Xt1	7.5	Aulia *et al*., 2019
		Symbiotic fungi extract Xt2	9	
		Symbiotic fungi extract Xt3	8.5	
		Symbiotic fungi extract Xt4	8	
		Symbiotic fungi extract Xt5	8	
		Symbiotic fungi extract Xt6	18	
		Symbiotic fungi extract Xt7	9.5	
VinhMoc at depth	*C. albicans*	XT06	10	Nguyen *et*

of 15 - 20 m, Quang Tri, Vietnam	XT13	15	*al*., 2019
	XT28	18	
	XT34	21	
	XT39	17	
	XT47	14	
	XT52	13	

Nguyen *et al*. (2019) isolated *X. testudinaria* in Vinh Moc, Quang Tri (Nguyen et al., 2019). Several compounds were isolated from *X. testudinaria* but only seven compounds were used for antifungal activity test against *C. albicans* (Table 3). Those seven compounds showed antifungal activity toward *C. albicans*, as evident by ZOI of 10, 15, 18, 21, 17, 14, and 13 mm, respectively. Therefore, it can be inferred that several compounds in *X. testudinaria* has antifungal activity with different potency.

Ayyad *et al*. (2015) has isolated several compounds from *Xestospongia* sp. isolated from Sharm Obhur, Jeddah, Saudi Arabia (Ayyad *et al*., 2015). The compounds, $C_{19}H_{25}O_2Br$ and $C_{19}H_{23}O_2Br$, were tested against several bacteria and fungi, including *C. albicans* and *C. tropicalis* (Table 4). *C. tropicalis* is the second most pathogenic Candida species after *C. albicans*. *C. tropicalis* belongs to the human microbiota and is present on skin, gastrointestinal, genitourinary, and respiratory tracts of humans (Zuza-Alves, Silva-Rocha, & Chaves, 2017). *C. tropicalis* causes disease in humans, including superficial mucosal infection (oral thrush and vulvovaginitis), meningeal, and pneumonia (Yesudhason & Mohanram, 2015). The study showed that $C_{19}H_{25}O_2Br$ was only able to inhibit the growth of C. albicans and *Aspergillus niger* with MIC of 2.2 and 2.5 µM, respectively. However, $C_{19}H_{25}O_2Br$ did not showing antifungal activity against *C. tropicalis, C. neoformans, Epidermophyton* sp., *M. gypseum, T. rubrum,* and *T. mentagrophytes*. Another compound, $C_{19}H_{23}O_2Br$, displayed a broader activity against different types of fungus. $C_{19}H_{23}O_2Br$ showed antifungal activity towards both *C. albicans, C. tropicalis, C, neoformans, A. niger, Epidermophyton sp., M. gypseum, T. rubrum, T. mentagrophytes*, with ZOI values of 24, 17.4, 15.8, 13, 14, 17.2, 16, and 19.4 mm, respectively (Table 4). Therefore, it can be inferred that $C_{19}H_{23}O_2Br$ has more potent effect compared to $C_{19}H_{25}O_2Br$, as $C_{19}H_{23}O_2Br$ showed higher inhibition zone value and able to inhibit growth of many species of fungi.

Table 4. Antifungal activity of *Xestospongia* sp. and their symbiotic microorganisms.

Place of origin	Target organism	Isolated compound or extract	Unit of inhibition	Reference
Sharm Obhur, Jeddah, Saudi Arabia	*C. albicans*	$C_{19}H_{25}O_2Br$	MIC = 2.2 µM	Ayyad *et al*., 2015
	C. tropicalis	$C_{19}H_{25}O_2Br$	MIC = none	
	C. neoformans	$C_{19}H_{25}O_2Br$	MIC = none	
	A. niger	$C_{19}H_{25}O_2Br$	MIC = 2.5 µM	
	Epidermophyton sp	$C_{19}H_{25}O_2Br$	MIC = none	
	M. gypseum	$C_{19}H_{25}O_2Br$	MIC = none	

	T. rubrum	$C_{19}H_{25}O_2Br$	MIC = none	
	T. mentagrophytes	$C_{19}H_{25}O_2Br$	MIC = none	
	C. albicans	$C_{19}H_{23}O_2Br$	ZOI = 24 mm	
	C. tropicalis	$C_{19}H_{23}O_2Br$	ZOI = 17.4 mm	
	C. neoformans	$C_{19}H_{23}O_2Br$	ZOI = 15.8 mm	
	A. niger	$C_{19}H_{23}O_2Br$	ZOI = 13 mm	
	Epidermophyton sp	$C_{19}H_{23}O_2Br$	ZOI = 14 mm	
	M. gypseum	$C_{19}H_{23}O_2Br$	ZOI = 17.2 mm	
	T. rubrum	$C_{19}H_{23}O_2Br$	ZOI = 16 mm	
	T. mentagrophytes	$C_{19}H_{23}O_2Br$	ZOI = 19.4 mm	
Penjaul Island at a depth of 5-10 m, Riau, Indonesia	*C. albicans*	Extract of *Xestospongia* sp. 1	ZOI = 13.9 mm	Putra *et al*., 2016
		Extract of *Xestospongia* sp. 2	ZOI = 11.2 mm	
	A. niger	Extract of *Xestospongia* sp. 1	ZOI = 11.6 mm	
		Extract of *Xestospongia* sp. 2	ZOI = 12.4 mm	

Putra *et al*. (2016) isolated 2 extracts from *Xestospongia* sp*., i. e.* extract of *Xestospongia* sp. 1 and *2,* in Penjaul Island (Table 4; Putra *et al*., 2016). The antifungal activity of the extracts was assessed using *C. albicans* and *A. niger* and it was shown that both extracts had antifungal activity, as evidenced by the ZOI values against the fungi tested.

All studies conducted by different researchers showed that *Xestospongia* sp*.* has antifungal activity. *Xestospongia* sp. contains a long chain of polyacetylenic alcohols with chemotaxonomic markers and polyacetylenes with antimicrobial, cytotoxic, antitumour, antiviral, and immunosuppressant bioactivity. One of the polyacetylenes isolated from *Xestospongia* sp. is xestospongiamide, a secondary metabolite (Ayyad, *et al.,* 2015; Deshmukh, *et al.,* 2018), which elicit antifungal activity (Putra, Hadi, & Murniasih, 2016). However, the exact mechanism of action of xestospongiamide remains elusive (Mayer, *et al,* 2019).

ANTIPARASITIC ACTIVITY

Antiparasitic activity in *Xestospongia* sp. has also been studied but to a much lesser extent than the other antimicrobial activity. Most of these studies focus on antimalarial activity, specifically assessing the activity of *Xestospongia* sp. extracts against *Plasmodium Falciparum*. *P. falciparum* is one of the most common parasites that causes malaria. Resistance emerged to all classes of antimalarial drugs (Buffet *et al*., 2010). *P. falciparum* is highly resistant to chloroquine and several known antimalarial drugs. Although the mechanism of resistance is still less studied, most likely it is probably due to mutation. The most common resistance of *P*. *falciparum* is to chloroquine, which occurs via mutation in *P. falciparum* Multidrug Resistance gene (PFMDR1) and *P. falciparum* Chloroquine

Resistance Transporter (PfCRT). In the PfMDR1, there are 10 transmembrane domains with known polymorphism that appear on the chloroquine resistance. From all cases of chloroquine resistance, K76T mutation is the vital mutation that is found in all chloroquine mutations, indicating that this mutation might be responsible for the resistance on *P. falciparum* (Dajem & Al-Qahtani, 2010; 39. Diakité *et al*., 2019). The exact mechanism on how mutation in this gene can cause resistance on the parasites have not been fully elucidated. Nevertheless, it is known that mutation in the transporter may cause drug efflux, preventing chloroquine to enter the food vacuole (Griffin *et al*., 2012).

Longeon *et al*. (2010) conducted a study on antiparasitic activity of *X. testudinaria* isolated from the South Pacific ocean towards *P. falciparumn* (Longeon *et al*., 2010). The study was done in 96 well plates and chloroquine was used as the positive control. From the study, they took one sample *X. testudinaria* from the Solomon Islands and 2 samples of *Xestospongia* sp. from Fiji Islands to investigate whether different environments would yield a different compound. Two new analogs were isolated and identified as xestosaprol C methylacetal and halenaquinol from extraction done in Solomon Islands and 5 others known constituents named 3-ketodaciaquinone A, 3-ketodaciaquinone B, tetrahydrohalenaquinone A and B, and Halenaquinol sulfate. 3-ketodaciaquinone A, 3-ketodaciaquinone B and orhalquinone were the most active compounds (Table 5). This study used two different strains of *P. falciparum*, FcB1 being chloroquine-resistant and 3D7 is the chloroquine-sensitive strain. No significant differences in IC_{50} of the compounds was observed between the 2 strains of Plasmodium falciparum (Table 5). This signifies the versatility of the extract in treating resistant *P. falciparum* strains. Chloroquine use was prevalent among third-world countries such as Indonesia where the incidence of malaria is still high, as a first line and prophylaxis treatment (Elyazar, Hay, & Baird, 2011). In 2004, the treatment of malaria with chloroquine was officially abandoned due to resistance and replaced by artemisinin-based combination therapy (Sutanto *et al.,* 2010). Even with this artemisinin-based combination therapy, it still can cause resistance cases. Hence, the study performed by Longeon *et al*. (2010) suggests critical implications of *Xestospongia* sp. where the extracts may be used as an alternative treatment to both chloroquine sensitive and resistant strain of *P. falciparum*.

Table 5. Antiparasitic activity of *Xestospongia testudinaria* and their symbiotic microorganisms.

Place of origin of sponge	Target organism	Isolated compound	IC_{50} (μM)	Reference
South Pacific Ocean	*P. falciparum* FcB1	Halenaquinone	> 30	Longeon *et al*., 2010
		3- ketoadociaquinone A	1.08	
		3- ketoadociaquinone B	3.89	
		Tetrahydrohalenaquinone A	> 29	
		Tetrahydrohalenaquinone B	> 29	
		Halenaquinol sulfate	> 24	
		Xestosaprol C	> 21	

	methylacetal	
	Orhalquinone	9.22
P. falciparum	Halenaquinone	> 30
3D7	3- ketoadociaquinone A	1.67
	3- ketoadociaquinone B	4.12
	Tetrahydrohalenaquinone A	> 29
	Tetrahydrohalenaquinone B	> 24
	Halenaquinol sulfate	> 21
	Xestosaprol C methylacetal	> 21
	Orhalquinone	10.94

CONCLUSION

Extracts of *Xestospongia* sp. were found to be effective against various strains of gram-negative and gram-positive bacteria, exerting ZOIs above 5 mm. Extracts were composed of different bioactive compounds including phenolics, alkaloids and steroids responsible for exhibiting antimicrobial activity. Fungal strains were also susceptible to bioactive compounds synthesized by *Xestospongia* sp. Studies against *P. falciparum* revealed important clinical implications for the treatment of chloroquine-resistant strains. This provides an alternative treatment for malarial infections. Extracts were not limited to only those produced by the sponge itself but by symbiotic microorganisms with an established relationship with the sponge.

ACKNOWLEDGMENT

The authors would like to thank Awaludinnoer from TNC Indonesia who has provided a picture of *Xestospongia* sp.

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