

# *Tubastraea coccinea*: A Non-Indigenous Coral (Cnidaria, Scleractinia) Collected at Arvoredo Island, South of Brazil with Potential MRSA and VRE Antimicrobial Activity

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## Abstract

Bacterial infection is considered to be one of the most critical health issues of the world. It is essential to overcome this problem by the development of new drugs. Marine organisms as corals, sponges, seaweeds, and other are an incredible source of novel pharmacologically active compounds. Herein, the antimicrobial activity (extract/fractions) of the invasive stony coral *Tubastraea coccinea* was evaluated by the disk diffusion method against 21 microbial strains (ATCC and clinical strains). Micro broth dilution was used to determinate the MIC of the fractions that showed antimicrobial activity by the disk diffusion method. Bioautography assay was also performed. Our results showed that the *n*-butanol (BF) and aqueous fractions (AF) showed activity against ATCC strains *Staphylococcus aureus* (MIC 31.25 and 250 µg/mL), *Salmonella typhimurium* (MIC 125 and 500 µg/mL), *Escherichia coli* (MIC 62.5 and 500 µg/mL) and *Pseudomonas aeruginosa* (MIC 62.5

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and 500 µg/mL), respectively. Moreover, BF fraction was also effective against the clinical strains *S. aureus* (MIC 62.5 µg/mL), *Klebsiella pneumoniae* Carbapenemase—KPC (MIC 125 µg/mL), Methicillin-Resistant *Staphylococcus aureus*—MRSA (MIC 125 µg/mL) and Vancomycin-Resistant *Enterococcus faecalis*—VRE (MIC 62.5 µg/mL). The ratio MBC/MIC reinforces the bactericidal profile of BF fraction. The bioautography assay of BF fraction showed the presence of antimicrobial components at R<sub>f</sub> 0.55.

## Keywords

Marine Natural Products, *Tubastraea coccinea*, Scleractinia, Antimicrobial Activity, Methicillin-Resistant *Staphylococcus aureus* (MRSA), Vancomycin-Resistant *Enterococcus faecalis* (VRE)

## 1. Introduction

The increasing global resistance of pathogenic bacteria has become a public health problem and, in attempts to overcome it, research efforts are now addressing the discovery of novel and efficient antibacterial compounds [1]. Furthermore, the emergence of multidrug-resistant bacteria has created a situation in which there are few or no treatment options for infections with certain microorganisms [2]. In this context, unusual sources, such as natural products from marine organisms, are important for antibacterial drug discovery, since they represent a high potential source of new drugs with diverse and often unique structures [3]-[6].

Corals (phylum Cnidaria) represent a dominant group of benthic marine invertebrates that inhabit all oceans, including Brazilian waters. These organisms are of the great interest to the scientific community, since they are source of marine natural products that possess high potential in terms of chemical novelty and as drug leads, yielding structures with novel modes of action [7]-[9].

*Tubastraea coccinea* Lesson, 1829 (Dendrophylliidae) is a yellow-orange non-indigenous coral found in some regions of Brazil [10], probably first introduced to the Brazilian coast by oil platforms and ships by fouling [11]. A recent report suggests that *T. coccinea* produces chemical defenses by competing against native octocorals [10], and a few data in relation to its ecological significance [12], chemical composition [13] as well as pharmacological properties are reported.

Thus, the aim of this work was to evaluate the antimicrobial potential of extract and related fractions (HF, BF and AF) from the invasive stony coral *T. coccinea*, against several microorganism strains as well as multi-drug resistant bacteria by the disk diffusion, microdilution assay and by bioautography.

## 2. Material and Methods

### 2.1. Collection and Extractions

Coral collection and extraction: Specimens of *T. coccinea* were collected by SCUBA at Arvoredo Island, in the state of Santa Catarina, Brazil, at depths of 3 to 10 m, in February 2012. The coral material was manually cleaned to remove associated organisms, and immediately frozen and kept at -20°C until processed. A voucher specimen (CC UFSC 0351) was deposited at the invertebrate collection of the Department of Ecology and Zoology of Universidade Federal de Santa Catarina. The material (160 g, wet weight) was blended in EtOH 92% (7 days), filtered and dried under reduced pressure, providing the ethanol crude extract (ECE). The ECE was partitioned with *n*-hexane/H<sub>2</sub>O and *n*-BuOH/H<sub>2</sub>O, yielding the *n*-hexane (HF), *n*-butanol (BF) and the aqueous residue (ARF) fractions, respectively. Then, ARF was submitted to XAD-4, yielding the AF (aqueous fraction).

### 2.2. Microorganism Strains

Twenty one microbial species were analyzed, including 14 ATCC strains [*Candida albicans* (ATCC 10231); *Candida tropicalis* (ATCC 13803); *Clostridium sporogenes* (ATCC 11437); *Enterobacter cloacae* (ATCC 13047); *Enterococcus faecalis* (ATCC 29212); *Escherichia coli* (ATCC 25922); *Klebsiella pneumoniae* (ATCC 13883); *Pseudomonas aeruginosa* (ATCC 27853); *Salmonella typhimurium* (ATCC 14028); *Shigella flexneri* (ATCC 12022); *Staphylococcus aureus* (ATCC 25923); *Staphylococcus epidermidis* (ATCC 12228); *Strepto-*

*coccus pneumoniae* (ATCC 49619); *Streptococcus pyogenes* (ATCC 19615)], and 7 clinical strains isolated from patients in São José Hospital, Criciúma city/Brazil [*Acinetobacter baumannii*, *Enterococcus faecium*, *Klebsiella pneumoniae*, *Klebsiella pneumoniae* carbapenemase (KPC), *Staphylococcus aureus*, Methicillin-resistant *Staphylococcus aureus* (MRSA) and Vancomycin-resistant *Enterococcus faecalis* (VRE)].

### 2.3. Disk Diffusion Method

The antimicrobial activities were evaluated by the disk diffusion method, as previously described by De Oliveira *et al.* [14] with minor modifications. Standard antibiotic disks were selected according to the sensitivity of the microorganism tested. Thus, ampicillin (10 µg), ceftazidime (30 µg), chloramphenicol (30 µg), doxycycline (30 µg), fluconazole (25 µg), imipenem (10 µg), levofloxacin (5 µg), oxacilline (1 µg), polymyxin b (300 µg) and vancomycin (30 µg) were used [15].

### 2.4. Determination of the Clinical Strains Sensitivity to Antibiotics

Antibiotic sensitivity testing of the clinical isolates was performed using disc diffusion method [16]. Antibacterial agents of different classes were used, including amikacin; ampicillin; ampicillin/sulbactam; ciprofloxacin; levofloxacin; cephepime; chloramphenicol; erythromycin; gentamicin; imipenem; oxacillin; piperacillin/tazobactam; sulphazotrim; vancomycin and clindamycin.

### 2.5. Micro Broth Dilution

The microdilution assay was performed according to the CLSI guidelines [16]. Briefly, each well of a 96-well microplate was coated with 100 µL of Muller Hinton broth together 100 µL of active fractions in serial dilutions (2000 µg/mL - 3.9 µg/mL). After 5 µL suspension, the test strain (equivalent to 0.5 McFarland standard) was replaced into wells. The microplates were incubated at 35°C for 18 h and the MIC was defined as the lowest concentration of fractions in which the microorganism tested did not show visible growth. The MBC was performed on nutrient agar plates, and was defined as the lowest concentration that showed negative growth.

### 2.6. Bioautography

Thin-layer chromatography (TLC) plates (10 × 10 cm) were loaded with 20 µL of the extract (BF) solutions at a concentration of 100 mg/mL. The plates were developed using *n*-BuOH/AcOH/H<sub>2</sub>O (6:2:1) as mobile phase. After the solvent had evaporated from the TLC plates (24 h), Muller Hinton agar (HIMEDIA®) was deposited over the plates, and after solidification, a microorganism suspension at 1.5 × 10<sup>8</sup> CFU/mL was added over the culture medium. The plates were incubated at 35°C ± 1°C, and after 18 h, the bioautogram was sprayed with an aqueous solution of 2,3,5-triphenyltetrazolium chloride (TTC) (Vetec®) and incubated at 35°C ± 1°C for 4 h. Inhibition zones indicated the presence of bioactive compounds [15] [17].

## 3. Results and Discussion

Among the fractions assayed, BF and AF showed moderate activity against ATCC strains of *S. aureus* (10.5 mm and 9 mm of inhibition zones, respectively), and weak activity against *S. typhimurium* (8 mm and 8 mm), *E. coli* (8.5 mm and 7.5 mm), and *P. aeruginosa* (7 mm and 7 mm). The HF fraction did not show activity against all bacteria or fungi assayed. Concerning the clinical strains, only BF fraction showed moderate activity against *S. aureus* (12 mm), KPC (11 mm), MRSA (10 mm) and VRE (10 mm) (Table 1).

The fractions that showed antibacterial activity by the disk diffusion method were further tested by the microplate assay, to determine the minimum inhibitory concentration (MIC). In addition, the wells that showed negative visible growth after 18 h of incubation were replated on agar nutrient plates, to obtain the minimum bactericidal concentration (MBC) of the fractions. The MIC values of the BF and AF fractions were, respectively: 31.25 and 250 µg/mL (*S. aureus*), 125 and 500 µg/mL (*S. typhimurium*), 62.5 and 500 µg/mL (*E. coli*), and 62.5 and 500 µg/mL (*P. aeruginosa*) (Table 2). On the other hand, BF and AF fractions showed MBC values 62.5 and 500 µg/mL (*S. aureus*), 250 and 1000 µg/mL (*S. typhimurium*), 125 and 1000 µg/mL (*E. coli*), and 125 and 1000 µg/mL (*P. aeruginosa*).

It is important to emphasize that the BF fraction showed activity against *P. aeruginosa*, which has, in clinical cases, a high degree of resistant against multiple classes of antibiotics [18]. Moreover, in contrast to the weak

**Table 1.** Antibacterial and antifungal activity of fractions of the coral *Tubastraea coccinea* by the disk diffusion method.

ATCC strains	Fractions assayed		
	HF	BF	AF
<i>Candida albicans</i> (ATCC 10231)	–	–	–
<i>Candida tropicalis</i> (ATCC 13803)	–	–	–
<i>Clostridium sporogenes</i> (ATCC 11437)	–	–	–
<i>Enterobacter cloacae</i> (ATCC 13047)	–	–	–
<i>Enterococcus faecalis</i> (ATCC 29212)	–	–	–
<i>Escherichia coli</i> (ATCC 25922)	–	+	+
<i>Klebsiella pneumoniae</i> (ATCC 13883)	–	–	–
<i>Pseudomonas aeruginosa</i> (ATCC 27853)	–	+	+
<i>Salmonella typhimurium</i> (ATCC 14028)	–	+	+
<i>Shigella flexneri</i> (ATCC 12022)	–	–	–
<i>Staphylococcus aureus</i> (ATCC 25923)	–	++	++
<i>Staphylococcus epidermidis</i> (ATCC 12228)	–	–	–
<i>Streptococcus pneumoniae</i> (ATCC 49619)	–	–	–
<i>Streptococcus pyogenes</i> (ATCC 19615)	–	–	–
Clinical strains	HF	BF	AF
<i>Acinetobacter baumannii</i>	–	–	–
<i>Enterococcus faecium</i>	–	–	–
<i>Klebsiella pneumoniae</i>	–	–	–
KPC	–	++	–
<i>Staphylococcus aureus</i>	–	++	–
MRSA	–	++	–
VRE	–	++	–

(–): not active; (+): 7 - 8.5 mm; (++) : 9 - 12 mm; (+++) : 13 - 16 mm; (++++): 17 - 20 mm. Positive controls: for ATCC strains were used ampicillin (10 µg), ceftazidime (30 µg), chloramphenicol (30 µg), doxycycline (30 µg), fluconazole (25 µg) and oxacilline (1 µg); for clinical strains were used imipenem (10 µg), levofloxacin (5 µg), oxacilline (1 µg), polymyxin b (300 µg) and vancomycin (30 µg). HF: *n*-hexane fraction; BF: *n*-BuOH fraction; AF: aqueous fraction; KPC: *Klebsiella pneumoniae* carbapenemase; MRSA: Methicillin-resistant *Staphylococcus aureus*; VRE: Vancomycin-resistant *Enterococcus faecalis*.

**Table 2.** Results of MIC (µg/mL), MBC (µg/mL) and MBC/MIC ratio of BF fraction of the coral *Tubastrea coccinea*.

ATCC strains	BF fraction		
	MIC (µg/mL)	MBC (µg/mL)	MBC/MIC
<i>Escherichia coli</i> (ATCC 25922)	62.5	125	2
<i>Pseudomonas aeruginosa</i> (ATCC 27853)	62.5	125	2
<i>Salmonella typhimurium</i> (ATCC 14028)	125	250	2
<i>Staphylococcus aureus</i> (ATCC 25923)	31.25	62.5	2
Clinical strains	MIC (µg/mL)	MBC (µg/mL)	MBC/MIC
<i>Staphylococcus aureus</i>	62.5	125	2
KPC	125	125	1
MRSA	125	125	1
VRE	62.5	62.5	1

Samples with MIC > 1000 µg/mL were considered inactive; MIC from 500 - 1000 µg/mL antibacterial activity was considered weak; MIC from 100 to 500 µg/mL the antibacterial activity was considered moderate; MIC is equal or smaller than 100 µg/mL, the antibacterial activity was considered significant. KPC: *Klebsiella pneumoniae* carbapenemase; MRSA: Methicillin-resistant *Staphylococcus aureus*; VRE: Vancomycin-resistant *Enterococcus faecalis*.

activity detected by the disk diffusion assay, the MIC of the BF fraction against *P. aeruginosa*, was higher when compared among all the samples tested (62.5 µg/mL), except against *S. aureus*, which showed MIC 31.25 µg/mL.

The BF fraction also exhibited significant growth inhibitory activities against the four clinical strains with MICs ranging from 62.5 to 125 µg/mL. Further, this fraction showed the same value of MBC (125 µg/mL) against *S. aureus*, KPC and MRSA. The VRE was susceptible to BF fraction with MBC of 62.5 µg/mL (**Table 2**).

The minimum bactericidal concentrations (MBC) for each sample were compared with the MIC values. Low MBC/MIC ratios ( $\leq 4$ ) demonstrate bactericidal activity, while ratios higher than four indicate the bacteriostatic mode of action [19]. Our results reinforce the bactericidal activity detected for BF fraction in the MBC assay for the all strains available (**Table 2**).

These findings demonstrated that the BF fraction has a higher potential antimicrobial activity, including clinical multi resistant strains, suggesting that this activity should be higher when the compounds responsible for the activity are isolated.

In addition, the most active fraction (BF) was submitted to the bioautography assay for *S. aureus*, *S. typhimurium*, *E. coli*, *P. aeruginosa* and four clinical strains (*S. aureus*, KPC, MRSA and VRE). In all bioautography analyses, the inhibition zone of the bioactive compounds was detected at Rf. 0.55 [TLC developed with *n*-BuOH/AcOH/H<sub>2</sub>O (6:2:1)] (data not shown). Since the bioactive compound showed inhibition zone at Rf. 0.55, we decide to develop a TLC chemical characterization of this compound using the same chromatographic conditions. The TLC fingerprint of the bioactive fraction showed the presence of compounds with alkaloids profile after Dragendorff nebulization (data not shown). This result is in agreement to literature data that reports stony scleractinian corals, *Tubastraea* (Dendrophylliidae) genus as a source of biologically active alkaloid derivatives including aplysinopsin and (bis)-indole alkaloids [20]-[23].

Regarding the pharmacological properties of this coral genus, there is only one report of antimicrobial activity from methanol extract of *Tubastraea faulkneri*, against *Vibrio alginolyticus*, *Vibrio harveyi*, *Photobacterium damsela*, *Alteromonas rubra*, *Staphylococcus aureus* and *Synechococcus* sp. [24]. Additionally, from the same species, aplysinopsin, 6-bromoaplysinopsin, 6-bromo-29-de-N-methylaplysinopsin and its dimer have also been reported [25]-[27]. Moreover, Zoraghi and colleagues [28], reported that MRSA pyruvate kinase is a potential target for (bis)-indole alkaloids with antibacterial activity.

## 4. Conclusion

In this work we described the antimicrobial potential of the extract and fractions obtained from Scleractinia coral *T. coccinea*. Our results showed that the *n*-butanol fraction (BF fraction) was effective against Gram-positive and Gram-negative bacterial, including multi-resistant clinical strains. Moreover, it is relevant to emphasize the importance of marine biodiversity as sources of secondary metabolites with potential pharmacological activities, which could be used on development of new drugs and/or as lead compound. Further studies are in progress in our laboratory, to isolate these bioactive compounds.

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## Conflicts of Interest

The authors declare no conflict of interest.

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