Original Article

Comparative bioactivity analysis of hard corals: antioxidant and antimicrobial responses three years after the MV Wakashio oil spill in Mauritius

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Abstract
In 2020, the MV Wakashio spilled 1000 tons of low-sulphur fuel oil along the south-east coast of Mauritius. This study aimed to compare the bioactivity of healthy hard corals with those affected by the oil spill, three years after the incident. *Acropora selago, Pocillopora damicornis, Millepora alcicornis*, and *Porites rus* were collected from affected and unaffected regions. Extracts were prepared through solvent extraction and fractionation with ethyl acetate and hexane, followed by antioxidant activity analysis using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay and antimicrobial testing via disc diffusion. All species exhibited significant antioxidant activity ($p < 0.05$), with *P. rus* showing the highest radical scavenging activity (RSA), 9.04 % and crude extracts demonstrating the strongest overall bioactivity. *Bacillus subtilis, Staphylococcus aureus* and *Escherichia coli* were resistant to all extracts. Only crude extracts of *A. selago, M. alcicornis,* and *P. rus* (among other fractions) could inhibit *Pseudomonas aeruginosa*, with *A. selago* exhibiting the largest inhibition zone (10.56 mm). No significant bioactivity differences were found between corals of oil-affected and unaffected regions, possibly due to the floating and rapid dispersion of low-sulphur fuel on the water surface. These findings highlight the pharmaceutical potential of hard corals and suggest further research on species-specific and environmental factors.

Keywords: hard-coral, MV Wakashio oil spill, antioxidant, antimicrobial

Introduction
Coral reefs are equivalent to tropical rainforests due to their rich biodiversity and complex ecosystems (Urban and Ittekkot, 2022), encompassing two primary categories: Soft corals and Hard corals. Central to the vitality of coral ecosystems is their symbiotic relationship with zooxanthellae, photosynthetic algae residing within coral tissues. This mutualistic association consists of corals providing shelter and essential compounds for photosynthesis, while zooxanthellae, in turn, produce oxygen and aid in waste removal (Titlyanov and Titlyanova, 2020).

Mauritius, a small island located in the southwest Indian Ocean, is renowned for its clear lagoons and fine sandy beaches (Elliott *et al.*, 2018). These reefs play a critical role in coastal protection by reducing erosion and acting as a buffer against extreme weather events, while also supporting a rich diversity of marine species. Additionally, they are economically significant to Mauritius, contributing to both the fisheries and tourism sectors (Nazurally and Rinkevich, 2014). Globally, well-managed coral reefs can produce approximately 5 tons of seafood per square kilometre annually (Urban and Ittekkot, 2022). Beyond their ecological and economic value, coral reefs contribute to carbon capture and storage by enhancing carbon sequestration in associated seagrass meadows (Guerra-Vargas *et al.*, 2020). Furthermore, coral reefs hold considerable biomedical potential, with around 245 bioactive compounds identified, which have applications in medicine and biotechnology (Sang *et al.*, 2019).

Hard corals, being sessile organisms, rely on chemical defences, including the production of secondary metabolites, to protect themselves from various biotic and abiotic threats (Mohamadizadeh *et al.*, 2014). This trait has stimulated interest in exploring their bioactive potential. For instance, Hamed and Hussein (2020) demonstrated the antibacterial and antimicrobial properties of six hard coral species: *Acropora hemprichii, Acropora austera, Seriatopora hystrix, Seriatopora pistillata, Pocillopora verrucosa* and *Millepora dichotoma*. In addition, hard corals exhibit antioxidant activities, as observed by Palmer *et al.* (2011), who compared the antioxidant responses of bleached and healthy *Acropora millepora*. In Mauritius, while studies have explored the bioactivity of soft corals (Jahajeeah *et al.*, 2023), research on the bioactivity of hard corals remains largely unexplored. Even globally, studies focusing on the bioactivity of hard corals are relatively scarce compared to those on soft corals.

The coral reefs surrounding Mauritius have experienced significant damage in recent years, primarily due to anthropogenic stressors (Elliott *et al.*, 2018). Climate change has exacerbated this degradation, with notable reductions in coral benthic cover, more specifically, a 40 % decline in hard corals and an 83 % decline in soft corals (McClanahan and Muthiga, 2021). In July 2020, the island's marine ecosystems suffered extensive damage when the MV Wakashio, a 300-meter-long Japanese vessel, collided with a barrier reef near Pointe d'Esny in the southeast of the island, releasing 1,000 metric tons of Very Low Sulphur Fuel Oil (VLSFO) into coral reefs, lagoons, and other critical ecosystems. Oil spills are a significant contributor to the accelerated decline of coral reefs globally (Hughes *et al.*, 2018; Miranda *et al.*, 2022). Even low-level exposure to polycyclic aromatic hydrocarbons (PAHs), the main toxic components of oil, has been linked to chronic detrimental effects on marine life (Seveso *et al.*, 2021). Direct exposure to oil results in histological, biochemical, reproductive, and developmental stress in corals, with species-specific variations in sensitivity and potential lethality depending on the extent of exposure (Miranda *et al.*, 2022).

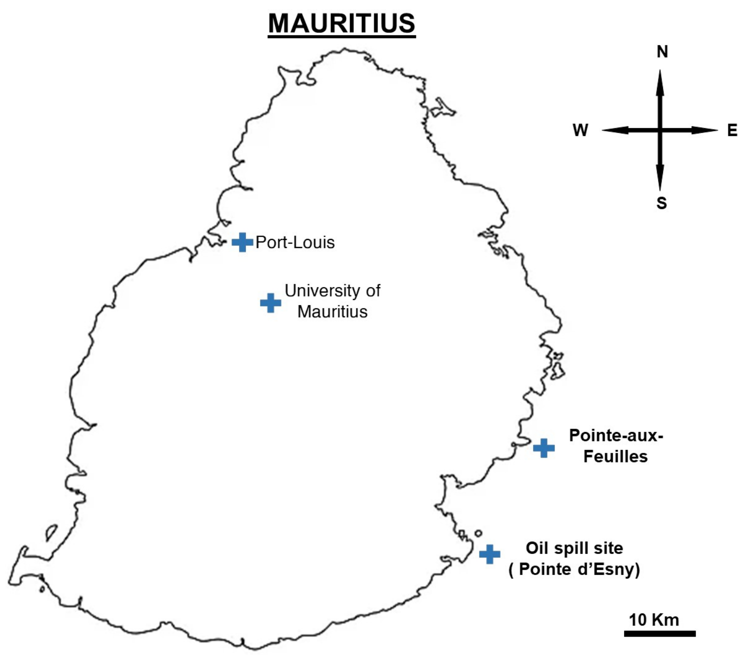

Figure 1. Map of Mauritius showing the site of the MV Wakashio oil spill and the site for sample collection (Pointe aux Feuilles).

This study assessed the residual impact of the MV Wakashio oil spill on the bioactive properties of hard corals, three years after the incident, contributing to a better understanding of environmental disturbances on marine ecosystems. The specific objectives included the collection of four hard coral species—*Acropora selago, Pocillopora damicornis, Millepora alcicornis* and *Porites rus*— from the Wakashio oil spill site at Pointe D'Esny, representing oil-affected corals, and from an unaffected region near Pointe aux Feuilles, Mauritius, representing healthy corals. The antimicrobial and antioxidant potential of these species were compared to assess variations in bioactivity between oil-affected and unaffected corals.

Materials and methods

Site description, sample collection, and morphological identification

Pointe aux Feuilles, on the southern coast of Mauritius, was selected as a collection site for healthy corals for comparison to Pointe D'Esny (Fig. 1). This region is predominantly inhabited by coastal communities and fishermen and is known for its successful coral farming initiatives, with nurseries established near fish farms (Nazurally and Rinkevich, 2014). Corals from the Wakashio oil spill site were also relocated to this region in coral nurseries.

Three years after the incident, in February 2023, eight coral samples were collected by snorkelling: four healthy coral species from Pointe aux Feuilles and four species affected by the Wakashio oil spill (*Acropora selago, Pocillopora damicornis, Millepora alcicornis,* and *Porites rus*). Species identification was conducted based on morphological characteristics. The corals were placed in zip lock bags containing seawater and transported in a cooler box (3-4 ^{0}C) to the University of Mauritius for further morphological analysis, following Moothien *et al.* (2002). The samples were then stored at -80 °C until further analysis.

Extraction procedure

The coral samples were freeze-dried and ground into a fine powder using a pestle and mortar. Ten grams of each sample were macerated in a solvent mixture of dichloromethane and methanol (1:1 ratio, 100 mL of each) for 72 h. After maceration, each sample was vacuum filtered and concentrated with a rotary evaporator. A 10 mL portion of the resulting crude extract was stored at -20 °C for further analysis, while the remainder was allocated for fractionation.

Fractionation

For fractionation, the crude extract was dissolved in 200 mL of distilled water and partitioned with 100 mL of hexane by vigorous shaking to separate the two phases, which were then isolated using a separating funnel. This partitioning process was repeated three times. The aqueous layer was subsequently partitioned three times with ethyl acetate, and each fraction was collected. Both the hexane and ethyl acetate fractions were concentrated using a rotary evaporator, and then stored at -20 °C for subsequent analyses.

Antioxidant assay

A DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was performed on extracts of the four coral species from both regions, following the modified protocol of Kavitha *et al.* (2022). To 0.5 mL of each extract, 2 mL of a 25 µg/mL DPPH solution was added and mixed using a vortex. Ascorbic acid was used as a standard in concentrations ranging from 10–100 µg/mL. The mixtures were incubated in the dark for 1 h, after which absorbance was measured at 517 nm. Each sample was analysed in triplicate, in a factorial design. The percentage radical scavenging activity (RSA) was calculated as:

$$\% \text{ Radical scavenging activity (RSA)} = 100 - (A_C - A_S)/A_C \times 100$$

Where A_C = *Absorbance of control*

A_S = *Absorbance of sample*

Antimicrobial assay

The antimicrobial assay was carried out on the crude extracts and fractions of three coral species (*Acropora selago*, *Millepora alcicornis*, *Porites rus*) from both regions. The Mueller-Hinton agar (MHA) disk diffusion method was performed according to the modified protocol of Tanjung *et al.* (2020). Pure bacterial cultures of *Bacillus subtilis* (ATCC 29213), *Staphylococcus aureus* (ATCC 11778), *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 27853) were standardized to a 0.5 McFarland level. The agar plates were inoculated with the bacterial cultures using a glass spreader. Six mm paper discs were impregnated with 30 µL of the coral extracts, 30 µg of chloramphenicol (positive control), and distilled water (negative control), and allowed to dry. The discs were placed on a bacteria-inoculated agar surface and incubated at 37 °C for 24 h. Each sample was tested in triplicate, and the zone of inhibition was measured.

Statistical analysis

The antimicrobial and antioxidant assays were performed in triplicate, following a factorial design where species, regions, and extract types (crude extract [CE], ethyl acetate fraction [EAF], and hexane fraction [HF]) were treated as independent variables. The dependent variables were the percentage radical scavenging activity (% RSA) for the antioxidant assay and the zone of inhibition (ZOI) for the antimicrobial assay. Data analysis was conducted using Jamovi software (Jamovi, 2021) through ANOVA (analysis of variance). A significance level of $p < 0.05$ was used to determine statistical significance. Post-hoc tests were employed to identify significant differences amongst the independent variables.

Results

DPPH assay

The antioxidant potential of four coral species was assessed using the DPPH assay. Figure 2 shows the percentage radical scavenging activity (% RSA), with values ranging from 0.77 % (*A. selago* from Pointe aux Feuilles) to 15.60 % (*P. rus* from Pointe aux Feuilles). To further analyse the data, the estimated marginal means of % RSA were compared across the independent variables (Fig. 3). No significant difference ($p > 0.05$) was observed in % RSA between corals from the Wakashio oil spill site and those from the unaffected region of Pointe aux Feuilles. The species showed significant differences in antioxidant activities, whereby *P. rus* had the highest marginal mean at 9.04 %, and *M. alcicornis* the lowest at 2.62 % ($p < 0.05$). The crude extract displayed the highest % RSA, at 8.63 %, followed by the ethyl acetate (6.34 %) and the hexane (2.56 %) fractions.

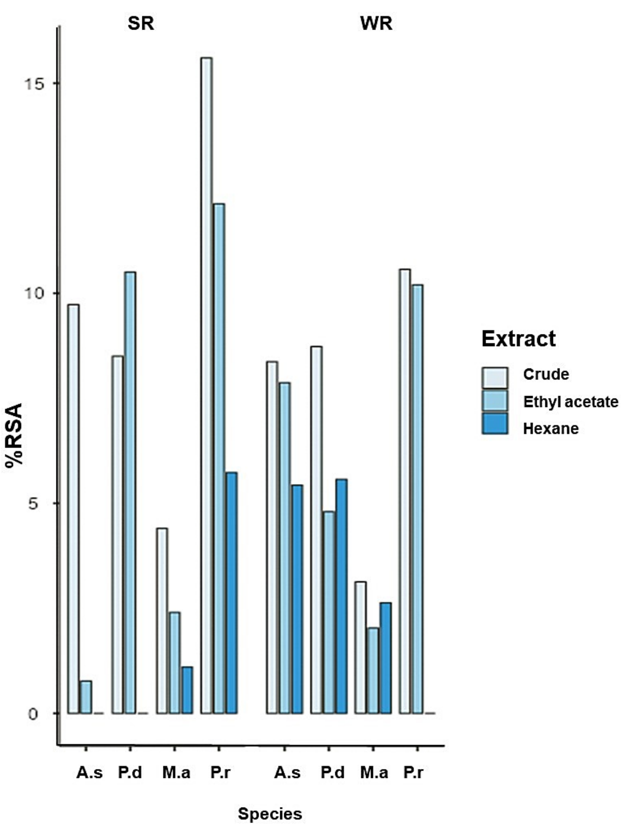

Figure 2. Bar plot representing the % RSA of samples [Key: A.s = *Acropora selago*; P.d = *Pocillopora damicornis*; M.a = *Millepora alcicornis*; P.r = *Porites rus*; SR= Surrounding region of Pointe aux Feuilles; WR= Wakashio oil spill region].

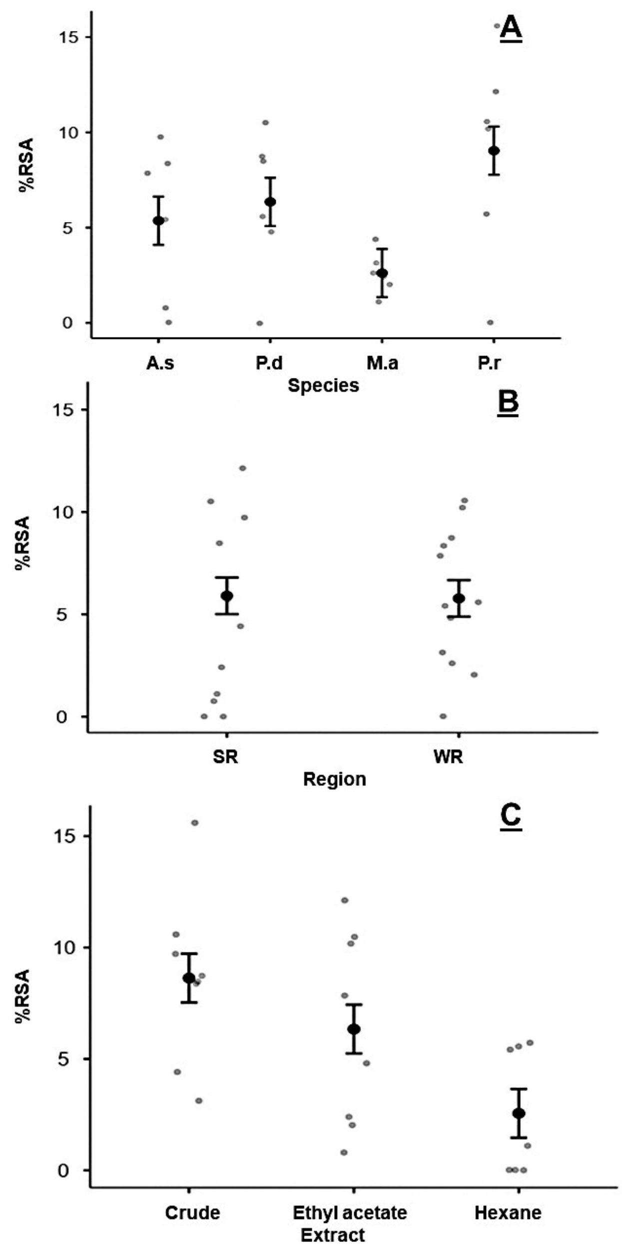

Figure 3. Estimated marginal means of % RSA of region **(A)**, of species **(B)**, and of extract and fractions **(C)** [Key: A.s = *Acropora selago*; P.d = *Pocillopora damicornis*; M.a = *Millepora alcicornis*; P.r = *Porites rus*; SR = Surrounding region of Pointe aux Feuilles; WR= Wakashio oil spill region].

Antimicrobial assay

An antimicrobial assay was carried out by disc diffusion and the zone of inhibition (ZOI) was measured. No ZOI was obtained for the species against *Bacillus subtilis, Staphylococcus aureus*, and *Escherichia coli*, but was for *Pseudomonas aeruginosa.* As for extracts, there was no antimicrobial activity with ethyl acetate and hexane fractions except for the crude extracts. The ZOI of the crude extracts against *P. aeruginosa* is represented in Figure 4 (A). The values ranged from 7.07 mm (*P. rus*) to 10.56 mm (*A. selago*). The estimated marginal means of ZOI of the factors were then compared (Fig. 4B, 4C). There was no statistically significant difference in the ZOI of coral species between the two regions (p>0.05). As for the species, the following trend was obtained: *A. selago*> *M. alcicornis*>*P. rus*.

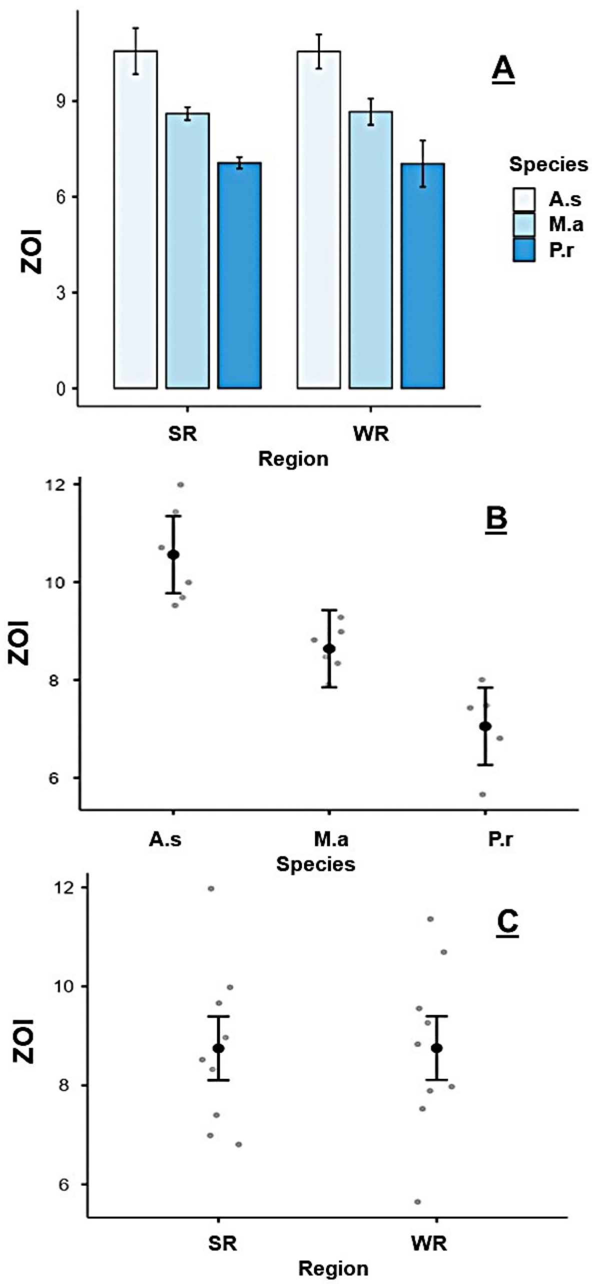

Figure 4. Antimicrobial activity results: **(A)** of crude extracts (CE) against *Pseudomonas aeruginosa*; **(B)** Estimated marginal means - species; **(C)** Estimated marginal means - Region [Key: A.s = *Acropora selago*; M.a = *Millepora alcicornis*; P.r = *Porites rus*; SR= Surrounding region of Pointe aux Feuilles; WR= Wakashio oil spill region].

Discussion

Antioxidant properties

Antioxidants are critical agents that combat excess superoxide radicals in the body, which can contribute to various diseases, including cancer, diabetes, and cardiovascular disorders (Ayoka *et al.*, 2022). Thus, the ongoing search for new sources of antioxidants is essential. Previous research has demonstrated that hard corals can activate antioxidant mechanisms under stress to protect themselves (Marques *et al.*, 2023; Safuan *et al.*, 2021; Samshuri *et al.*, 2023). However, studies focusing on the antioxidant activities of corals in the Western Indian Ocean (WIO) remain absent.

This study demonstrated that all coral extracts exhibited antioxidant activity, with significant variations observed amongst the extracts (p < 0.05). The crude extracts showed the highest radical scavenging (RSA) capacities, with the highest RSA of 8.63 %. The variation in antioxidant activity among extracts may be attributed to the polarity of the solvents used, as suggested by Nawaz *et al.* (2020). In this study, the solvents applied were dichloromethane: methanol (crude extract), ethyl acetate, and hexane, which have decreasing polarity, respectively. The higher free radical scavenging activity observed in the crude extracts could be due to the high affinity of antioxidant compounds towards more polar solvents compared to non-polar ones. For instance, Zhang *et al.* (2019) identified the presence of terpenes, secondary metabolites known for their antioxidant potential (Baccouri *et al.*, 2021), in hard corals. As terpenes are non-polar, they would be expected to concentrate in hexane extracts. Yet, the higher antioxidant activity obtained in this study with the more polar solvent suggests that hard corals contain other polar antioxidant compounds in higher quantities, leading to enhanced activity in polar solvents.

As such, the varying levels of the responsible antioxidant compounds may have led to the observed significant differences in antioxidant activity amongst species (p < 0.05). *Porites rus* exhibited the highest antioxidant activity, consistent with the findings of Strahl *et al.* (2016), who reported that *Porites spp.* possess total antioxidant capacities approximately four times higher than other coral species. Globally, antioxidant activity has been studied more extensively in soft corals than in hard corals, likely due to the perceived higher potential in soft corals. For example, Tanod *et al.* (2019) found that soft corals exhibited DPPH activity between 15-20 %, which is higher compared to most hard corals in this study, where the majority showed antioxidant activity below 10 % except for *P. rus*, which reached 15.60 %.

Antimicrobial properties

Antimicrobial resistance is a growing global health concern, prompting researchers to explore marine resources for new antimicrobial agents (Barbosa *et al.*, 2020). Hard corals, as part of their defence mechanism against pathogens, are known to possess antimicrobial properties (Phartade, 2024). However, studies on the antimicrobial potential of hard corals remain limited, particularly in the WIO region. This study, therefore, investigated the antimicrobial properties of three hard coral species—*A. selago*, *M. alcicornis*, and *P. rus*—from Mauritius.

The results revealed no antimicrobial activity against *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli*. However, crude extracts from all three coral species showed ZOI when tested against the gram-negative bacterium *Pseudomonas aeruginosa*. Hamed and Hussein (2020) also investigated the antimicrobial potential of corals of the *Acropora* and *Millepora* genera. In contrast to the present study, Hamed and Hussein (2020) observed significant inhibition of *S. aureus* (with ZOIs above 12 mm) and no activity against *P. aeruginosa*. These discrepancies could stem from species-specific or geographical variations in the composition of antimicrobial compounds. Similar to this study, *Acropora* spp. in Hamed and Hussein's (2020) work did not inhibit *E. coli*, and both *Acropora* and *Millepora* showed no inhibition of *B. subtilis*.

As for *P. rus*, Mohamadizadeh *et al.* (2014) reported that *Porites* species were unable to inhibit *P. aeruginosa* and *E. coli*, but displayed significant antimicrobial activity against *S. aureus*, both contrasting and supporting this study's results. The lack of inhibition observed with the ethyl acetate and hexane fractions in this study suggests that the antimicrobial compounds responsible for *P. aeruginosa* inhibition in these hard corals are highly polar, as they were only extractable using the most polar solvent mixture (dichloromethane: methanol). This is in line with findings by Qaralleh *et al.* (2014), who also observed that the antimicrobial components of other hard corals tend to be polar. Conversely, Kim (1994) demonstrated that non-polar solvents exhibited greater antimicrobial potential in gorgonian corals.

Regional comparison

No significant differences in antioxidant or antimicrobial activities were observed between corals collected from the oil spill site and those from an unaffected region ($p > 0.05$), suggesting the Wakashio oil spill may not have exerted a lasting impact on the bioactive properties of hard corals. In contrast, previous studies have documented increased oxyradical scavenging capacity in scleractinian corals, such as *Madracis pharensis*, when exposed to PAHs (Nardi *et al.*, 2024). Similarly, short-term exposure to oil has been associated with elevated oxidative stress in *Porites lobata* (Downs *et al.,* 2006). The lack of significant effects observed here could be due to several factors. First, low-sulphur fuel oil, known to float on water, may have rapidly dispersed to the coastline due to ocean currents. Additionally, the natural resilience of coral species in the impacted area could have facilitated recovery over the three-year period since the oil spill. It is possible that studies conducted closer to the time of exposure would have revealed more pronounced impacts on coral bioactivity. This suggests that, over time, corals may possess adaptive or mitigative responses to initial oil-induced stress.

Conclusions

This preliminary study aimed to evaluate the residual effect of the Wakashio oil spill on the antimicrobial and antioxidant properties of selected hard coral species three years after the incident, while addressing the knowledge gap regarding their pharmaceutical potential in the WIO. The findings indicate that *A. selago, P. damicornis*, *M. alcicornis*, and *P. rus* exhibit significant antioxidant activity, with *A. selago*, *M. alcicornis*, and *P. rus* also demonstrating antimicrobial effects against *Pseudomonas aeruginosa*. Polar solvents were more effective in extracting these bioactive compounds. Hard corals are, therefore, effective marine resources for the pharmaceutical industry. No significant impact of the oil spill on coral bioactivity was detected. However, further research is required to explore additional coral species, their secondary metabolites, and a broader range of biological and environmental factors. This comprehensive approach will deepen our understanding of the effects of oil spills on coral bioactivity, supporting both environmental conservation and pharmaceutical developments.

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