Bioactive profile of *Plakortis nigra*, a sea sponge from Mauritius Islands

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**Objective:** To investigate the in vitro antibacterial and antioxidant activities of crude and fractionated extracts of the *Plakortis nigra* (*P. nigra*) sea sponge from *Mauritius* sea waters.

**Methods:** Preliminary qualitative chemical screening of the sponge extracts was conducted by using standard methods while the total phenolic content (TPC) was estimated through the Folin-Ciocalteu method. Antibacterial activity was evaluated against *Staphylococcus aureus*, *Escherichia coli* and *Proteus mirabilis*. The minimum inhibitory concentration was determined by the broth microdilution method. All sponge extracts were assessed for antioxidant activity via the 1,1-diphenyl-2-picrylhydrazyl free radical scavenging in vitro model.

**Results:** Alkaloids, phenols, steroids, terpenoids, tannins and saponins were detected in the sponge extracts and TPC varied from (2.280±0.072) mg to (12.790±0.236) mg gallic acid equivalents per gram extract (*P*<0.05). All the extracts inhibited the growth of at least two bacterial strains whilst the most potent in vitro antibacterial activities were observed in the most polar ethyl acetate and butanol fractions (minimum inhibitory concentration values 0.103–0.211 mg/mL) of *P. nigra*. Each extract scavenged 1,1-diphenyl-2-picrylhydrazyl free radicals while the hexane fraction displayed the highest scavenging ability at (27.50±1.85)% (*P*<0.05). Antioxidant activity was positively correlated with TPC (*R*\(^2\)=0.843). Contrary relationships were also found between antibacterial activity and TPC.

**Conclusions:** The present study validates the antioxidant and antibacterial activity of marine sponge (*P. nigra*) extracts and depicts the sea sponge as a potential source of pharmaceutical leads against infectious and degenerative diseases.

**PEER REVIEW**

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**Comments**
This is an interesting study in which the authors provided novel insight into the potentially important metabolites of a Mauritian sea sponge. Data in this paper appear novel and it is worth publishing.

**ABSTRACT**

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**Conclusions:** The present study validates the antioxidant and antibacterial activity of marine sponge (*P. nigra*) extracts and depicts the sea sponge as a potential source of pharmaceutical leads against infectious and degenerative diseases.

**KEY WORDS**

*Plakortis nigra*, Antibacterial, Antioxidant, DPPH, Phenolics

**1. Introduction**

In the last several decades, research has expanded from land to ocean, which offers immense potential for biological and chemical diversity, in order to find new leads for drug candidates\(^1\). Sponges, principally demosponges, distinguish themselves by producing the greatest panoply of bioactive secondary metabolites from any animal group\(^2,3\), which are of potential pharmaceutical importance\(^4,5\).

Antioxidants are free radical scavengers, which can provide protection to living organisms from damage caused by uncontrolled production of reactive oxygen species (ROS), and have widespread applications in medicine, cosmetics and food industries. There is a need for identifying natural antioxidants due to emerging concerns about safety of synthetic preservatives\(^6\). Avarol, a well-studied...
Microbial infections are still the major cause of mortality all over the world. As the infectious microorganisms evolve and develop resistance to existing pharmaceuticals, the marine sponge may provide novel leads against bacterial, viral, fungal and parasitic diseases. Psammaplin A, responsible for antibacterial activity, has been selected as a promising lead for preclinical assessment[16]. Recent studies have been conducted in the hunt for new antimicrobial substances of sponge origin[8,17-26].

Plakortis nigra (P. nigra) is a demosponge belonging to the Plakinidae family and 25 members of Plakortis species, occurring worldwide, are known to date[27]. The latter are known to produce a wide variety of bioactive compounds (antiviral, antibacterial, antimalarial and anticancer) and have yielded a number of biosynthetically diverse natural products[28-32]. In fact, the Plakortis genus is advertised as one of the most prolific products with respect to new marine natural compounds from 1900 to 2009, accounting for approximately 4% of all Porifera new marine natural products[3].

Mauritius is a small island (1 865 km$^2$) in the Indian Ocean possessing a total exclusive economic zone area of 2.3 million km$^2$. Such a vast maritime territory represents a niche for marine bioprospecting. Literature reveals few studies, describing biological activities of pharmacological, interest in Mauritian sponges[33-38], hence, highlighting the urgent need to put the Mauritian waters on the marine natural products research agenda. In this respect, the present study aimed at evaluating the in vitro antibacterial and antioxidant activities of extracted P. nigra secondary metabolites, so as to ultimately assess the marine sponge’s pharmaceutical potential.

2. Materials and methods

2.1. Sponge collection

Samples (300 g wet weight) of P. nigra were collected from the northern coastal waters of Mauritius (Tron aux Biches, September 2011) via self-contained underwater breathing apparatus diving at a depth of 10-15 m. A fresh sample was deposited at the M auritius Oceanography Institute while a sample of the specimen was sent to the M useum of the University of Amsterdam for taxonomic identification. A voucher specimen was deposited under the accession number ZMA P OR 18310. The freshly collected sponges were cleaned and stored at -70 °C prior to lyophilisation, using a laboratory freeze drier (Labconco), and extraction.

2.2. Extraction

Freeze-dried samples of P. nigra (100 g dry weight) were macerated with methanol/dichloromethane (1:1 v/v) for 96 h in the dark at ambient temperature, and the filtrates were concentrated in vacuo at a maximum temperature of 40 °C, using a rotary evaporator (Heidolph Laborota) to yield the crude extract. Thereafter, distilled water (100 mL) was added to the crude extract and the aqueous suspension was successively partitioned with different solvents in increasing order of polarity: hexane (6×200 mL), ethyl acetate (EtOAc) (5×200 mL) and butan-1-ol (BuOH) (4×200 mL), to afford the corresponding fractions, which were subsequently concentrated in vacuo. M ethanol (MeOH) was added to the concentrated butanol extract, in order to remove any sea salt present, and the extract was ultimately concentrated in vacuo.

2.3. Chemical screening

Standard qualitative chemical screening tests[39] were performed on sponge extracts to detect the presence of major natural chemical groups, such as steroids, terpenes, alkaloids, phenols, tannins, coumarins, anthraquinones, leucoanthocyanins and flavonoids.

The froth test was performed on freeze-dried sponge material to detect any presence of saponins. A total of 0.5 g of freeze-dried sponge material was treated with water for 5 min at 100 °C in a test tube, allowed to cool and shaken vigorously, then the formation of persistent froth (1-2 mL) was observed.

2.4. Determination of total phenolic content (TPC)

The Folin-Ciocalteu procedure[40], with some modifications, was employed to estimate the TPC of the sponge extracts. In capped test tubes, 200 μL aliquots of standard solutions of gallic acid in MeOH (0, 50, 100, 150, 200 and 250 μg/mL), were mixed with 2.0 mL Folin-Ciocalteu reagent (diluted 10-fold with deionised water). After 6 min, 0.8 mL of sodium carbonate (7.5%) was added to neutralise the reaction, and the tubes vortexed. The mixture was allowed to stand for 1 h at room temperature in the dark and absorbance at 760 nm was recorded, against a MeOH blank, using a spectrophotometer (Milton Roy Spectronic 1001 Plus UV-visible). A calibration curve was plotted using the data. Each of the sponge extracts (200 μL) was treated in the same manner. The results were expressed as means of triplicate analyses in milligram gallic acid equivalents (GAE) per gram extract (mg GAE/g).

2.5. 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay

The antioxidant activity of sponge extracts was assessed using an altered DPPH free radical scavenging in vitro model[41]. In capped test tubes, 1 mL of each sponge extract was added to 3 mL of 0.3 mmol/L methanolic solution of DPPH (HiMedia). The tubes were vortexed and allowed to stand for 30 min at room temperature in the dark. The absorbance was measured at 517 nm and a control reading...
was obtained using MeOH. Quercetin served as the positive control. The results were expressed as means of triplicate analyses. The percentage of DPPH radical scavenging activity was calculated with the following equation:

\[ \% \text{ Inhibition} = \left( \frac{A_0 - A_1}{A_0} \right) \times 100 \]

Where \( A_0 \) is the absorbance of the control; \( A_1 \) is the absorbance of test samples.

2.6. Antibacterial assay

Minimum inhibitory concentrations (MICs) of the crude and fractionated extracts were determined using the Mueller-Hinton broth microdilution method as previously described [42]. The reference strains of human pathogens used to test for antibacterial activity included a Gram-positive bacterium, *Staphylococcus aureus* ATCC 25313 (*S. aureus*) and two Gram-negative bacteria, *Escherichia coli* ATCC 25922 (*E. coli*) and *Proteus mirabilis* ATCC 12453 (*P. mirabilis*). Overnight bacterial cultures were standardised with sterile Mueller-Hinton broth (HiMedia) to achieve an absorbance of 0.4-0.6 at 600 nm. Each test sample (100 μL) was 2-fold serially diluted with sterile distilled water (100 μL) in U-bottom sterile 96-well microtitre plates and bacterial suspension (100 μL) added to each well. Chloramphenicol (Sigma) (0.01 mg/mL) was used as a positive control while MeOH was used as a negative control. Following overnight incubation at 37 °C, the MIC, the lowest concentration at which bacterial growth inhibition occurred, was determined by an indicator dye. Ergo, 50 μL of *p*-iodonitrotetrazolium violet (INT) (0.2 mg/mL) was added to wells preceding incubation at 37 °C for 30 min; clear wells indicated growth inhibition. All measurements of MIC values were repeated in triplicates.

2.7. Statistical analysis

The experimental results were expressed as means±SD. All measurements were replicated three times (\( n=3 \)). Statistical analysis was performed using Minitab software version 16.1.0 for Windows at a 5% significance level. One-way analysis of variance (ANOVA, Fisher’s test) was carried out to test for any significant differences among the TPCs of different extracts and the DPPH scavenging activities of different extracts. Significant difference was statistically considered at level of \( P<0.05 \). Linear regression analysis was used to calculate the standard curve of gallic acid and correlations (Microsoft Excel 14.0 software).

3. Results

3.1. Chemical screening

The detected secondary metabolites are summarized in Table 1. Condensed tannins were present in considerable amounts in the hexane and ETOA c fractions of *P. nigra* extracts. Small amounts of coumarins were found only in the crude extract and ETOA c fraction. Globally, the presence of alkaloids, phenols and steroids/terpenes in different proportions were detected in the extracts. Eventually, the *P. nigra* was found to contain saponins.

**Table 1**

| Chemical group | Test sample |
|----------------|-------------|
|                | Crude extract | Hexane fraction | ETOA c fraction | BuOH fraction | Freeze dried |
| Sponge Alkaloids | + | ++ | ++ | + | NT |
| Anthraquinones | - | - | - | - | NT |
| Coumarins | + | - | + | - | NT |
| Leucoanthocyanins | - | - | - | - | NT |
| Flavonols | - | - | - | - | NT |
| Phenols | + | ++ | ++ | + | NT |
| Saponins | NT | NT | NT | NT | ++ |
| Steroids/Terpenes | + | ++ | ++ | - | NT |
| Tannins | + | ++ | ++ | + | NT |

+: Present in trace amount; ++: Present; -: Absent; NT: Not tested.

3.2. TPC

The gallic acid standard curve was established by plotting concentration versus absorbance (\( y=0.0081x, R^2=0.9913 \)), where \( y \) is absorbance and \( x \) is concentration (Figure 1). The sponge extracts contained levels of total phenolics, ranging from 2.28 mg GAE/g extract to 12.79 mg GAE/g extract. The hexane fraction contained the most phenolics with a value of (12.790±0.236) mg GAE/g extract, followed by the crude extract (10.670±0.345) mg GAE/g extract). Meanwhile, the ETOA c and BuOH fractions contained the least phenolics with (4.04±0.29) mg GAE/g extract and (2.280±0.072) mg GAE/g extract respectively.

![Figure 1. Standard curve for gallic acid.](image)

3.3. Antioxidant activity

High antiradical activity (>95%) was recorded for the positive control, quercetin. The least polar hexane fraction displayed the highest free
radical scavenging activity at (27.50±1.85)%, followed by the crude extract (16.60±1.05)%, while the polar EtOAc and BuOH fractions exhibited lower free radical scavenging activity at (12.90±2.92)% and (4.170±0.444)% respectively.

3.4. Antibacterial activity

The EtOAc and BuOH fractions of *P. nigra* provided the lowest MIC values ranging from 0.103 mg/mL to 0.211 mg/mL and performed better than the reference antibiotic chloramphenicol against all pathogens (Table 2).

| Table 2 | MIC of *P. nigra* extracts and positive control against test organisms. |
|---------|-----------------------------------------------------------------------|
| Sample  | E. coli (mg/mL) | S. aureus (mg/mL) | P. mirabilis (mg/mL) |
| Crude extract | 2.460 | 2.460 | ND |
| Hexane fraction | 1.280 | 1.280 | 1.280 |
| EtOAc fraction | 0.103 | 0.206 | 0.206 |
| BuOH fraction | 0.106 | 0.211 | 0.211 |
| Chloramphenicol | 0.625 | 0.313 | 1.250 |

n=3. ND: No antibacterial activity detected at highest concentration of sample; Chloramphenicol: Reference antibiotic used as positive control.

3.5. Correlation between antioxidant activity and TPC

An obvious correlation \((r=0.918, R^2=0.843)\) was noted between *in vitro* antioxidant activity and TPC of the sponge extracts (Figure 2). It revealed that *in vitro* antibacterial activity was strongly positively associated to the TPC.

3.6. Correlation between antibacterial activity and TPC

The investigated correlations between MICs and TPCs of the sponge extracts, when tested against *E. coli* \((r=0.809, R^2=0.654)\), *S. aureus* \((r=0.795, R^2=0.632)\) and *P. mirabilis* \((r=0.987, R^2=0.974)\), revealed that the *in vitro* antibacterial activity was strongly inversely associated to the TPC (Figure 3).

4. Discussion

4.1. Chemical screening

Through preliminary chemical screening, *P. nigra* was revealed as a source of bioactive components, namely alkaloids, coumarins, phenols, tannins, steroids, terpenoids and saponins. Sponges are assumed to produce secondary metabolites to compete for space with other organisms, prevent fouling by other organisms and keep predators away\(^{43}\). Consequently, the flourishing biodiversity of the Mauritian lagoons and the competitive environment of the latter may account for the production of an arsenal of secondary metabolites in the *P. nigra* currently under study. Likewise, a sheet coral was reported to be susceptible to allelochemicals released by its neighbour, the *Plakortis halichondroides*\(^{44}\). The Mauritian *P. nigra* under investigation might have accumulated chemicals in an attempt to maximise its space-capture abilities.

The presence of alkaloids in the probed *P. nigra* was foreseen since perusal of the literature revealed that plakinidines, most cytotoxic alkaloids, have been isolated from sponges of the Plakinidae family in Ireland, Fiji and Vanuatu, while plakinamines A and B, steroidal alkaloids, were isolated from Micronesian *Plakina* sp.\(^{12,30,45}\). The presence of phenols in the *P. nigra* is in keeping with sponges being capable of synthesising phenolics, such as quinones, hydroquinones, and halogenated phenols\(^{45}\). Furthermore, the unique monohydric phenol plakinidone was isolated from a member of the genus, to which the *P. nigra* belongs\(^{46}\). Also, our findings corroborate the lately reported occurrence of phenols and tannins in Mauritian *Stylissa* sp. and *Biemna tubulosa* extracts of different polarities\(^{38}\). Depending on the polarity of a solvent system used during extraction, a mixture of phenolics will be extracted from materials and polar solvents have been reported to extract polyphenolics\(^{47,48}\).
Although the presence of saponins in sponges was once considered as an infrequent occurrence(49), the present study has revealed the *P. nigra* as a source of such metabolites. This sustains recent findings whereby acanthifoliosides, novel steroidal saponins, were obtained from the *Pandaros acanthifolium* sponges belonging to the same class as the *P. nigra*(50). Besides, the presence of steroids or terpenoids in the crude, hexane and ethyl acetate extracts of the *P. nigra* is in line with the preceding claims about the widespread incidence of terpenoids in sponges(45). Alkaloids, steroids, terpenoids, phenols and saponins present in marine sponges, have been associated with various bioactivities, among which antibacterial and antioxidant attributes(2,13,45,49-53).

### 4.2. TPC

In our study, the range of TPC of different *P. nigra* extracts varied (2.28-12.79 mg GAE/g extract). The hexane fraction contained the highest amount of phenolic compounds (12.790±0.236 mg GAE/g extract) and the BuOH fraction contained the least amount of phenolics (2.280±0.072 mg GAE/g extract).

### 4.3. Antioxidant activity

Antioxidants are free-radical scavengers which can provide protection to living organisms from damage caused by uncontrolled production of reactive oxygen species. There is a need for identifying natural antioxidants having less or no side effects since commonly used as synthetic antioxidants like butylated hydroxyanisole and butylated hydroxytoluene (100.00%) when investigated through the DPPH model(8). Likewise, metabolites from the marine sponge *Dendrilla nigras* sp., of the same family as the *P. nigra*, inhibited the growth of *S. aureus*. *Agelas* species from the Pacific and the Caribbean have provided diterpenoids which demonstrated antimicrobial activity against *Bacillus subtilis*, *S. aureus* and *Candida albicans*. Two diterpenoids, exhibiting antimicrobial activity, were obtained from the Okinawan sponge *Agelas nakamurai* while *Luffariella variabilis* has furnished four sesterterpenoid antibiotics(45). Also this study has disclosed a reverse relationship between antibacterial activity and TPC in *P. nigra* extracts. Hence, the fractions with the least TPCs (EtOAc and BuOH), were the most efficient bacterial growth inhibitors. The detected presence of other chemical components in these fractions may indicate a possible synergy. This phenomenon is characterised as the effect of two or more components, applied together, being greater than the effect when each constituent are used separately(59). Chemically defended organisms produce multiple secondary metabolites, opening up the possibility of synergistic or additive effects among various metabolites(60). So, a positive correlation could exist between antibacterial activity and the diverse secondary metabolites detected within sponge extracts in this investigation.

This work constitutes the first report revealing the antibacterial and antioxidant activities of the marine sponge *P. nigra*, collected from the coastline of *Mauritius*. The *P. nigra* was coined as a source of bioactive metabolites, among which saponins are medicinally important metabolites. While displaying moderate antioxidant activity, the investigated sponge was most importantly revealed as a potential source of natural antioxidants. Via this investigation, the *P.
other marine fauna. Little literature appears available for sponge-derived saponins, to which the authors’ declare is rare.

**Innovations and breakthroughs**  
This manuscript provides novel insight into the potentially important metabolites of a Mauritian sea sponge. Extracts show anti-oxidant activity and anti-bacterial activity against both Gram-positive and Gram-negative bacteria. Total phenolic content correlated positively with anti-oxidant potential. The authors conclude that the sponge, *P. nigra*, is potentially rich in novel bioactives that can be exploited commercially.

**Applications**  
Extracts appeared to kill *S. aureus*, one of the most troublesome nosocomial infections in biomedicine. Although this is a preliminary study and significant more investigation is needed. Hexane appeared to be the best solvent for extracting phenols from this sponge.

**Comments**  
We declare that we have no conflict of interest.

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