

## Antioxidant and phytochemical evaluation of mangrove *Avicennia marina* and seaweed *Acanthophora* sp.

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### ABSTRACT

The present study comparatively evaluates the phytochemical composition and antioxidant potential of the mangrove *Avicennia marina* and the red seaweed *Acanthophora* sp., with the aim of identifying promising natural sources of bioactive compounds. Qualitative phytochemical screening revealed that *A. marina* possesses a broader spectrum of secondary metabolites, including tannins, flavonoids, alkaloids, terpenoids, steroids, and saponins, whereas *Acanthophora* sp. showed the presence of alkaloids, flavonoids, steroids, and saponins. Thin-layer chromatography (TLC) analysis further confirmed the diversity of compounds, with distinct Rf values indicating the presence of both polar and non-polar phytoconstituents in both species. Antioxidant activity was assessed using DPPH radical scavenging and total antioxidant assays. In the DPPH assay, *Acanthophora* sp. exhibited comparatively higher scavenging activity ( $57.2 \pm 2.7\%$  at  $100 \mu\text{g/mL}$ ) than *A. marina* ( $50.1 \pm 2.2\%$ ), indicating superior free radical neutralizing ability. Similarly, total antioxidant capacity results showed significantly higher ascorbic acid equivalents in *Acanthophora* sp. ( $85.5 \pm 2.4$  at  $100 \mu\text{g/mL}$ ) compared to *A. marina* ( $47.7 \pm 2.4$ ), suggesting enhanced electron-donating capacity. Although *A. marina* contains a wider variety of phytochemicals, *Acanthophora* sp. exhibited stronger antioxidant efficiency, likely due to the presence of potent phenolic and marine-derived bioactive compounds. Therefore, these findings indicate that *Acanthophora* sp. is a more promising candidate for antioxidant applications, while *A. marina* remains a valuable source of diverse phytoconstituents. These results highlight the potential of marine algae as effective natural antioxidants for pharmaceutical, nutraceutical, and functional food applications.

**Keywords:** *Avicennia marina*, *Acanthophora* sp., Phytochemical, Antioxidant activity, Marine bioactivity.

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### 1 Introduction

Oxidative stress plays a critical role in the onset and progression of several chronic diseases, including cancer, cardiovascular disorders, diabetes, and inflammatory conditions. The limitations of synthetic antioxidants, particularly their potential toxicity and instability, have led to growing interest in natural, safer alternatives derived from plant and marine sources. In this context, bioactive compounds such as polyphenols, alkaloids, terpenoids, tannins, and saponins have gained considerable attention due to their potent antioxidant and therapeutic properties (Lakshme et al., 2021). Therefore, the exploration of underutilized natural sources rich in such phytochemicals is essential for the development of novel pharmacological and nutraceutical agents.

Mangroves are unique coastal plants that thrive in intertidal regions under extreme environmental

conditions, including high salinity, tidal inundation, and low oxygen availability. These stress conditions stimulate the production of diverse secondary metabolites with significant biological activities (Lakshme et al., 2021). Among mangrove species, *Avicennia marina* is widely distributed in tropical and subtropical regions and is considered a dominant true mangrove species (Triest et al., 2021). It has been traditionally used to treat various ailments, including inflammation, diabetes, skin infections, and gastrointestinal disorders. Phytochemical investigations have revealed that *A. marina* contains a wide array of bioactive compounds, including tannins, fatty acids, alkaloids, polyphenols, saponins, and quinones, which contribute to its pharmacological potential (Ali, 2021; Lang et al., 2023; Mitra et al., 2023). Despite these findings, comparative evaluations of its antioxidant capacity relative to that of marine macroalgae remain insufficient.

Marine seaweeds are another important reservoir of bioactive compounds, particularly because they adapt to fluctuating environmental conditions in coastal ecosystems. They are rich in polysaccharides, phenolics, vitamins, minerals, and polyunsaturated fatty acids, all of which contribute to their biological activities (Cadar et al., 2025; El-Beltagi et al., 2022). Red seaweeds (Rhodophyta), including *Acanthophora* sp., are especially abundant and are known for their nutritional and medicinal value. Although seaweeds are widely utilized in traditional diets and as sources of nutraceutical compounds, the antioxidant potential of *Acanthophora* species has not been extensively explored (Guillén et al., 2022). Given their ability to produce unique metabolites, they represent a promising yet underexploited resource for natural antioxidants.

Despite the recognized pharmacological importance of both mangroves and seaweeds, there is a lack of systematic comparative studies evaluating their phytochemical profiles and antioxidant capacities using standardized *in vitro* assays. Additionally, limited information is available on how differences in environmental stress between mangrove and marine ecosystems influence the composition and efficacy of their bioactive compounds. Therefore, this study aims to comparatively assess the phytochemical constituents and antioxidant activities of *Avicennia marina* and *Acanthophora* sp. using DPPH radical scavenging, total antioxidant capacity, and hydrogen peroxide scavenging assays. It is hypothesized that variations in ecological conditions lead to distinct antioxidant profiles, thereby identifying potential candidates for pharmaceutical, nutraceutical, and functional food applications (Guillén et al., 2022).

## 2 Materials and methods

### 2.1 Collection of samples

Fresh leaves of *Avicennia marina*, Figure 1a, and thalli of *Acanthophora* sp. Figure 2a was collected during the post-monsoon season (January–February 2025) from the Pichavaram mangroves (11.417°N, 79.800°E) and the Rameshwaram coastline (9.290°N, 79.300°E), Tamil Nadu, India, under low- to mid-tidal conditions to ensure the collection of healthy and metabolically active samples. The specimens were taxonomically identified by Dr Pitchiah Sivaperumal using standard morphological keys and further authenticated, with voucher specimens deposited at the Centre for Marine and Aquatic Research, SIMATS, Chennai (Accession Nos SIMATS/CMAR/MG/AM-01 and SIMATS/CMAR/SW/AP-12). The collected materials were thoroughly washed with tap and distilled water to remove debris, salts, and epiphytes, then shade-dried at room temperature (25–28°C) for 2–3 weeks to preserve bioactive compounds, and finally pulverized into a fine powder.

### 2.2. Extraction of crude from mangrove and seaweed

20 g of *Avicennia marina* and *Acanthophora* sp. were extracted with 200 mL of 70% (v/v) ethanol using an orbital shaker at 500 rpm at room temperature for 72 h. The hydroethanolic solvent system was selected to facilitate the extraction of a wide range of phytoconstituents, including phenolics, flavonoids, terpenoids, and alkaloids, of varying polarity. The extracts were filtered through Whatman No. 1 filter paper and concentrated to dryness in a water bath at 60°C.

Yield was calculated on a dry weight basis as percentage yield (w/w) using the formula

$$\text{Extraction} = \frac{\text{weight of dried extract}}{\text{initial sample weight}} \times 100,$$

yielding 12.4% for *A. marina* and 10.6% for *Acanthophora* sp. The crude extracts were stored at 4°C in airtight containers for further analysis.

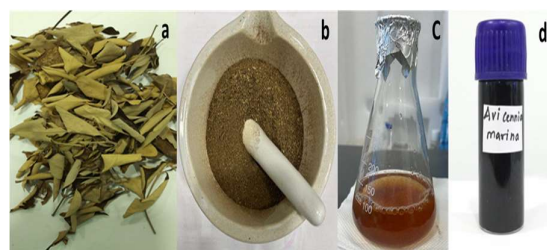


Figure 1 Mangrove sample collection and pre-processing of the samples. (a) *Avicennia marina* plant, (b) powdered sample, (c) Extraction of the sample, and (d) Crude extract

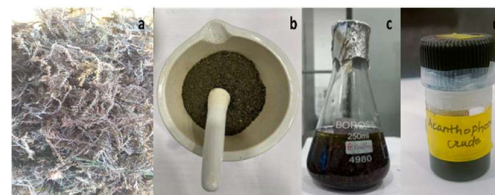


Figure 2 Seaweed sample collection and pre-processing of the samples; (a) *Acanthophora* sp., (b) powdered sample, (c) Extraction of the sample, and (d) Crude extract

### 2.3 Phytochemical activity: *Avicennia marina* and *Acanthophora* sp.

In the current study, a qualitative phytochemical analysis was conducted on ethanolic extracts of the mangrove *Avicennia marina* and the seaweed *Acanthophora* sp. The preliminary screening revealed the presence or absence of various biologically active substances, including tannins, saponins, alkaloids, terpenoids, flavonoids, and steroids, using standard methods (Rengasamy et al., 2020).

### 2.4 TLC analysis of crude extracts of *Avicennia marina* and *Acanthophora* sp.

The preliminary phytochemical characterization of crude extracts from *Avicennia marina* and *Acanthophora* sp. was analyzed by thin-layer chromatography (TLC) (Gomez-Zavaglia et al., 2019). The determination of a compound on a TLC is usually described in terms of its relative mobility or R<sub>f</sub> value. The R<sub>f</sub> value is a unique value for each compound under the same conditions. The retention factor, or R<sub>f</sub>, is defined as the distance travelled by the compound divided by the distance travelled by the solvent. In TLC, the mixtures of compounds were separated based on their differences in solubility and partition coefficient in a binary solvent system. TLC of algae extracts was performed on a silica gel sheet (20×20 cm with 0.2 mm thickness) (Merck, Germany). Further, analytical TLC was performed using a 2×7 cm cut from the above commercially available sheets. The plate was air-dried and heated at 105°C in an oven for 1 h. An aliquot of each crude algal extract was spotted separately using a capillary tube, at a distance of more than 1 cm from the base of the TLC plate, and allowed to dry for a few minutes. Afterward, the plate was developed with the hexane/ethyl acetate solvent system (7:3, v/v) as the mobile phase in a previously saturated glass chamber, using eluting solvents for 15 to 30 min at room temperature (RT). The developed plate was dried under normal air, and the spots were visualized under visible light. The R<sub>f</sub> values of isolated compounds were calculated and analyzed.

$$R_f = \frac{\text{Distance traveled by the streak from the starting point}}{\text{Distance traveled by the solvent from the starting point to the solvent front}}$$

## 2.5 Antioxidant activity of *Avicennia marina* and *Acanthophora* sp.

### 2.5.1 DPPH Assay

Using a slightly modified method, the DPPH radical scavenging activity was measured (Sobuj et al., 2024). In brief, 100 µL of 0.16 mM DPPH solution was mixed with 100 µL of each extract at various dilutions. After the sample was mixed for 1 min and kept in the dark for 30 min, an automated microplate reader was used to measure absorbance at 517 nm. This formula was used to determine the antioxidant capacity:

$$\% \text{ of Inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \times 100$$

The absorbance of the test sample is denoted by a sample, whereas the absorbance of the control is represented by a control. For the different sample concentrations, inhibition values were computed. A positive control was ascorbic acid.

### 2.5.2 Total antioxidant activity

3 ml of the reagent solution was used to prepare a 0.3 ml sample at various concentrations (25, 50, 75, and 100 µg/ml). The reaction mixture was incubated in a water bath at 95°C for 90 minutes. Subsequently, the absorbance of each sample mixture was measured at 695 nm. Ascorbic acid equivalents have been used to express total antioxidant activity.

## 3. Results and Discussion

### 3.1 Phytochemical analysis of *Avicennia marina* and *Acanthophora* sp.

In this present study, mangrove leaves of *A. marina* showed the qualitative presence of saponins, alkaloids, terpenoids, tannins, steroids, and flavonoids (Table 1). Mangroves are rich in highly bioactive compounds with broad-spectrum bioactivities, including insecticides such as steroids, tannins, alkaloids, terpenoids, and saponins (Table 1). Qualitative phytochemical evaluation of *Acanthophora* sp. showed the existence of steroids, alkaloids, saponins, and flavonoids (Table 2). The current study identified bioactive compounds with potential medical applications in crude leaf extracts of seaweeds and mangroves. Analyses of phytochemicals and antioxidants have produced results that appear highly effective. The natural bioactive substances polyphenols, flavonoids, carotenoids, alkaloids, tocopherols, polysaccharides, and peptides found in seaweeds have become ever more abundant (Sivakumar et al., 2020). Furthermore, (Eswaraiah et al., 2020) identified phenols, tannins, and terpenoids in particular mangrove species and demonstrated their antioxidant and antimicrobial potential, while (M & Sudhakar, 2023) highlighted the presence of significant phytochemical and antioxidant potential in two mangrove species. In this study, leaves of the mangrove *A. marina* showed qualitative evidence of saponins, flavonoids, tannins, terpenoids, steroids, and alkaloids. Phytochemical analysis of *Acanthophora* sp. revealed the existence of steroids, flavonoids, alkaloids, and saponins.

Table 1 Phytochemical analysis of mangrove leaves of *Avicennia marina*

Phytochemical test	Present	Absent
Tannins	+	
Saponins	+	
Steroids	+	
Flavonoids	+	
Trepenoids	+	
Alkaloids	+	

Table 2 Phytochemical analysis of seaweed *Acanthophora* sp.

Phytochemical test	Presence	Absence
Tannins		-
Saponins	+	
Steroids	+	
Flavonoids	+	
Trepenoids		-
Alkaloids	+	

**3.2 Phytochemical characterization of *Avicennia marina* and *Acanthophora* sp. using TLC**

Based on the TLC results and typical phytochemical profiles, the observed  $R_f$  values and colors of *Avicennia marina* suggest the presence of various phytochemical compounds. For *Avicennia marina*, spot 1 ( $R_f$  0.24) likely indicates the presence of chlorophyll or related pigments, which are known for their low  $R_f$  values in such solvent systems. Spot 2 ( $R_f$  0.54) could correspond to flavonoids, which generally exhibit moderate mobility. Spot 3 ( $R_f$  0.68) might be due to additional chlorophyll derivatives or other green pigments, including carotenoids. Spot 4 ( $R_f$  0.92) contains more nonpolar compounds, such as terpenoids and steroids, which have higher mobility.

For *Acanthophora* sp., Spot 1 ( $R_f$  0.36) may suggest the presence of tannins or phenolic compounds. Spot 2 ( $R_f$  0.52) could correspond to flavonoids or other pigments similar to those found in *Avicennia marina*. Spot 3 ( $R_f$  0.62) might indicate certain pigments or secondary metabolites like saponins or additional flavonoids. Spot 4 ( $R_f$  0.74) likely represents more non-polar compounds such as lipids (Table 3). In the study by Mitra et al.[15], yellow-coloured spots on TLC sheets were identified as

indicators of bioactive compounds and antioxidants, suggesting the presence of valuable phytochemicals. (Eswaraiyah et al., 2020) supported this observation by associating yellow spots with carotenoids and phenolic compounds, which are known for their antioxidant properties. (Carpena et al.,2022) expanded on this by demonstrating that preliminary phytochemical characterization of various macroalgae, including *Sargassum wightii* (Selvaraj & Ganapathy, 2024), *Sargassum ilicifolium*, *Sargassum longifolium*, *Padina* sp., and *Turbinaria* sp., (Gunathilake et al., 2022) was performed using TLC without standards, revealing diverse pigment compounds through distinct  $R_f$  values under UV and visible light. This approach was also applied to the isolation and partial characterization of bioactive compounds from the brown alga *H. elongata*, underscoring the effectiveness of TLC in identifying and differentiating a wide range of pigments and bioactive substances.

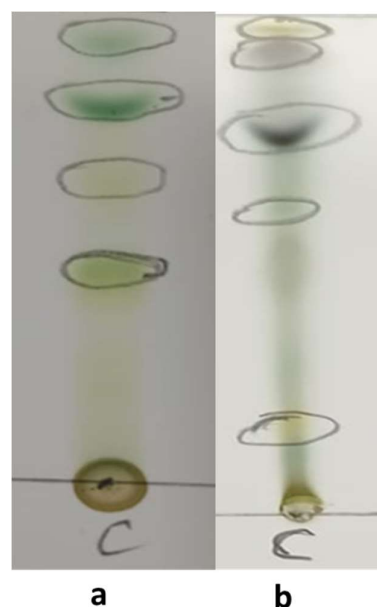


Figure 3 TLC of phytochemical characterization of (a) *Avicennia marina* and (b) *Acanthophora* sp.

Table 3 Thin-layer chromatography analysis of crude extracts

		Mobile phase (Hexane: Ethyl acetate) 7:3 (v/v)		
S.N	CRUDE EXTRACT	SPO T NO	COLO R	$R_f$
1	<i>Avicennia marina</i>	1	Green	0.24
		2	Light yellow	0.54
		3	Dark Green	0.68
		4		0.92

		4	Light Green	0.9 2
2	<i>Acanthophora</i> sp.	1	Light brown	0.3 6
		2	Green	0.5 2
		3	Yellow green	0.6 2
		4	Grey	0.7 4

### 3.3 Antioxidant activity

#### 3.3.1 DPPH assay: *Avicennia marina* and *Acanthophora* sp.

The DPPH assay results for *Avicennia marina*, Figure 4, and *Acanthophora* sp. Figure 5 demonstrates notable antioxidant activity, with *Avicennia marina* showing scavenging activities of  $13.2 \pm 1.5\%$  and  $50.1 \pm 2.2\%$  at 25  $\mu\text{g/ml}$  and 100  $\mu\text{g/ml}$ , respectively, and *Acanthophora* sp. exhibiting  $20.5 \pm 1.8\%$  and  $57.2 \pm 2.7\%$  at the same concentrations. These findings suggest that both species possess significant antioxidant properties, which are attributed to their ability to scavenge the stable DPPH free radical. This process involves the donation of electrons by the antioxidants, thereby neutralizing the radical and preventing further oxidative damage. These results are consistent with previous research demonstrating the effectiveness of DPPH assays for evaluating antioxidant potential. For instance, a study reported that ethanolic extracts of various plant species showed significant DPPH scavenging activity, with ascorbic acid as a standard showing up to 96.25% scavenging activity at 50  $\mu\text{g/ml}$  (Das et al., 2023). In contrast, the current findings reveal a lower scavenging activity for both *Avicennia marina* and *Acanthophora* sp. at similar concentrations, suggesting that while these extracts are effective, they are not as potent as ascorbic acid. Compared with other studies, the antioxidant activity of *Avicennia marina* in this investigation is lower than that reported in some previous studies. For example, a study found that ethanolic extracts of *A. marina* exhibited higher antioxidant activity than ascorbic acid at all tested concentrations (Ramkumar et al., 2017; Sravya et al., 2025). Additionally, another study reported that an 800  $\mu\text{g/ml}$  ethanolic extract of *A. marina* pneumatophore exhibited 69.12% scavenging activity, indicating a stronger antioxidant potential than observed in this study (Sohaib et al., 2022). This discrepancy could be attributed to differences in extraction methods, plant parts used, or experimental conditions. Similarly, the antioxidant activity of *Acanthophora* sp. aligns with findings from other studies highlighting the antioxidant capacity of marine algae. The observed scavenging activities at 25  $\mu\text{g/ml}$  and 100  $\mu\text{g/ml}$  are comparable to those reported for other marine algal extracts, which often exhibit moderate to high antioxidant activity due to the presence of bioactive

compounds such as terpenoids, flavonoids, and polyphenols (Bharath et al., 2020). In summary, while both *Avicennia marina* and *Acanthophora* sp. demonstrate effective antioxidant activity, the extent of their scavenging potential differs from that of standard antioxidants, such as ascorbic acid, and from previous findings. This highlights the importance of further research to explore the underlying mechanisms of antioxidant activity and to optimize extraction and testing conditions to fully understand the antioxidant potential of these marine species.

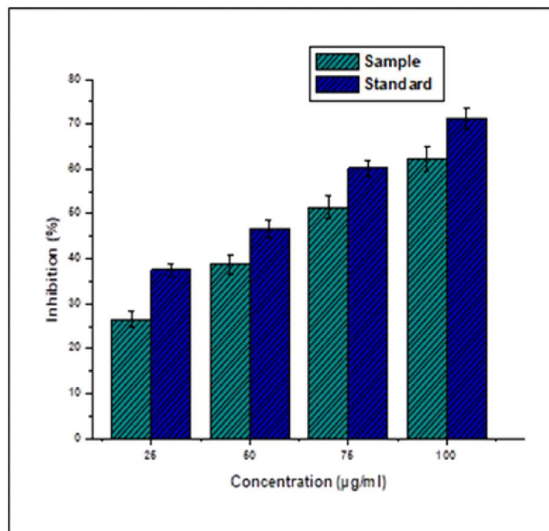


Figure 4 DPPH activity of *Avicennia marina*

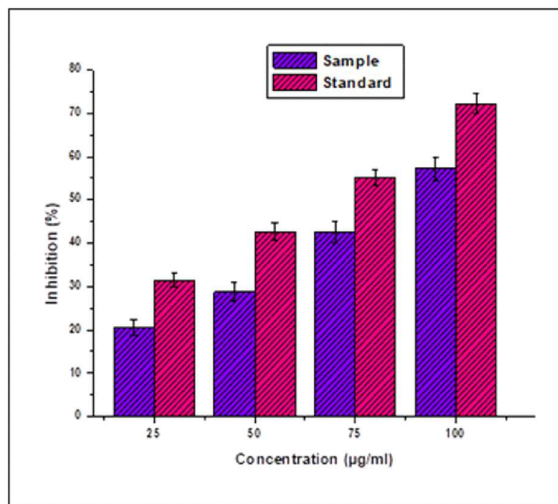


Figure 5 DPPH activity of *Acanthophora* sp.

#### 3.3.2 Total antioxidant assay of *Avicennia marina* and *Acanthophora* sp.

According to the current investigation, the crude *Avicennia marina* extract used in the total antioxidant assay was at 25, 50, 75, and 100  $\mu\text{g/ml}$ . Figure 6 The results showed that these values were

closer to the ascorbic acid equivalent. At the maximum concentration (100 µg/ml), the ascorbic acid equivalent was  $47.7 \pm 2.4$ . At the minimum concentration of crude extract (25 µg/ml), the ascorbic acid was also similarly determined to be  $11.2 \pm 2.3$ . In the current study, the crude *Acanthophora* sp. extract was analyzed at 25 µg/ml to 100 µg/ml in the total antioxidant assay; Figure 7 shows results closer to the ascorbic acid equivalent. At 100 µg/ml, which was the maximum concentration, the ascorbic acid equivalent was  $85.5 \pm 2.4$ . At the minimum concentration of crude extract (25 µg/ml), the ascorbic acid equivalent was determined to be  $51.6 \pm 2.3$  Figure 6. As "free radical scavengers", antioxidants found in seaweed have been shown in recent studies to exhibit strong antioxidant activity in laminarin made from brown seaweed (Agatonovic-Kustrin & Morton, 2018; Michalak et al., 2022). In the total antioxidant assay, concentrations of the extract ranging from 25 µg/mL to 100 µg/mL showed values closely aligned with those of ascorbic acid equivalents. Specifically, at the highest concentration (100 µg/ml), the ascorbic acid equivalent was determined to be  $59.4 \pm 2.6$ .

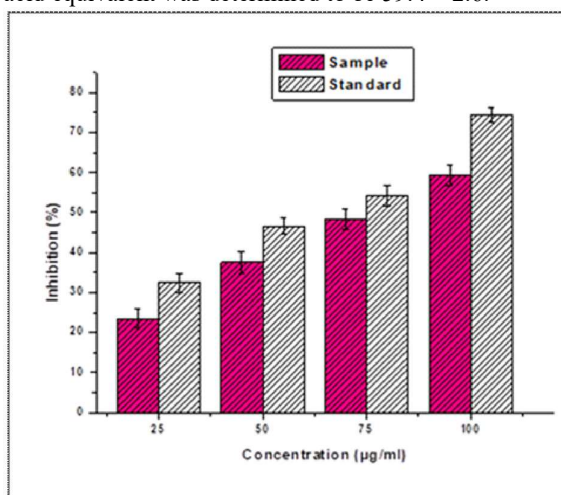


Figure 6 TAA activity of *Avicennia marina*

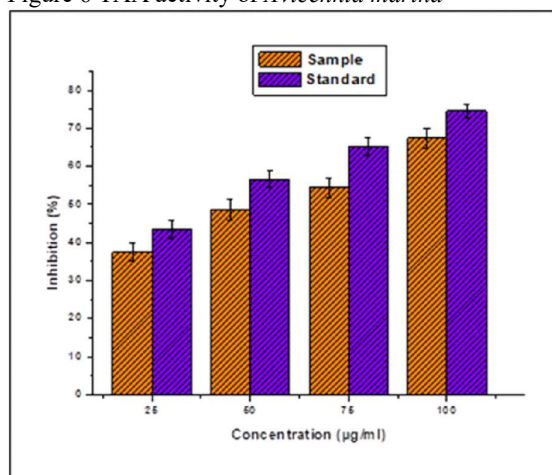


Figure 7 TAA activity of *Acanthophora* sp.

#### 4 Statistical analysis

The obtained experimental data were analyzed using the conventional statistical method. Solvents and samples were compared using analysis of variance (ANOVA). Values were expressed as means  $\pm$  standard deviations. Differences were considered significant at  $p < 0.05$ . Each analysis was conducted in triplicate.

#### 5. Conclusions

This study presents a comparative evaluation of the phytochemical composition and antioxidant potential of *Avicennia marina* and *Acanthophora* sp. The results demonstrated that although *A. marina* contains a wider range of phytochemicals, *Acanthophora* sp. exhibited significantly higher antioxidant activity in both DPPH and total antioxidant assays, indicating stronger free radical scavenging and reducing capacity. These findings suggest that marine-derived bioactive compounds may exhibit greater antioxidant activity than their mangrove-derived counterparts. The study highlights the potential of *Acanthophora* sp. as a promising natural antioxidant source for pharmaceutical and nutraceutical applications, while *A. marina* remains valuable for its diverse phytochemical profile. Further studies focusing on the isolation and characterization of active compounds, along with their biological mechanisms, are recommended to fully explore their therapeutic potential.

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**Competing Interests** The authors have no competing interests to declare that are relevant to the content of this article.

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