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Phytochemical Composition and Evaluation of Acute Toxicity, Antioxidant, and Antibacterial Activities of *Spondias mombin* L. and *Myracrodruon urundeuva* Allemão Ethanolic Extracts Against *Vibrio Parahaemolyticus*

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Keywords: Anacardiaceae | antimicrobial agents | bacterial resistance | mass spectrometry | natural products

ABSTRACT

Plant-derived natural products are emerging as promising sources of bioactive compounds against parasites and microorganisms. This study investigated the phytochemical composition, antibacterial and antioxidant activities, and acute toxicity of ethanolic extracts from Spondias mombin L. and Myracrodruon urundeuva Allemão. Extracts were analyzed by ultra-performance liquid chromatography coupled with mass spectrometry (UPLC–MS). Antibacterial activity was tested against Vibrio parahaemolyticus strains, whereas antioxidant activity was assessed using the DPPH assay. Acute toxicity was evaluated with Artemia salina nauplii. The M. urundeuva extract contained hydrolyzable tannins, gallic acid derivatives, anacardic acids, and agathisflavone, along with phenolic acids and dimeric chalcones, including erunvidine B, in the stem bark. Only procyanidins were detected in S. mombin extracts. Both extracts exhibited moderate antibacterial activity and promising antioxidant potential, relevant for neutralizing oxidative bacterial defenses through scavenging of reactive oxygen species (ROS) and reactive nitrogen species (RNS), thereby supporting cellular redox homeostasis. The *M.* urundeuva extract showed the lowest minimum inhibitory concentration (MIC, 0.625–1.25 mg/mL) and, together with S. mombin, demonstrated bactericidal activity surpassing ampicillin. Low acute toxicity was observed for both extracts (LC_{50} > 1000 μg/mL), reinforcing their potential safety. These findings suggest that these plant extracts are promising natural alternatives for controlling V. parahaemolyticus in aquaculture systems.

1 | Introduction

The Gram-negative *Vibrio parahaemolyticus* is responsible for causing acute hepatopancreatic necrosis syndrome (AHPNS), also commonly referred to as early mortality syndrome (EMS), in marine shrimp. Infected shrimp exhibit severe atrophy of the hepatopancreas, loss of appetite, slow growth, and high mortality rates, resulting in substantial economic losses for aquaculture

farmers. Moreover, this pathogen is commonly associated with a range of human illnesses, from mild cases of gastroenteritis to life-threatening conditions such as sepsis and invasive infections [1].

Traditional disease control in shrimp farming has heavily relied on the use of antibiotics and chemical disinfectants to mitigate the impact of AHPNS-causing bacteria. However, these practices are no longer recommended due to the increasing prevalence of antibiotic-resistant bacteria. The interaction between environmental bacteria carrying multidrug resistance genes and pathogenic strains that affect both captive aquatic organisms and humans may also pose risks to public health and the environment [2].

Consequently, there is a growing interest in exploring natural alternatives, such as plant-derived compounds, to enhance shrimp health and mitigate disease outbreaks. These natural products often possess antioxidant properties that can strengthen the immune responses of aquatic organisms. Importantly, plant antioxidants may also play a direct antimicrobial role by neutralizing the oxidative defenses of bacteria, through scavenging of reactive oxygen species (ROS)/reactive nitrogen species (RNS), thereby contributing to the activation of antioxidant enzymes or terminating oxidative chain reactions, which may directly contribute to the killing of pathogens. Nonetheless, the potential toxicological effects of these natural products must be evaluated to ensure their safety for both target species and consumers, as exposure to toxic agents may compromise organism health [3]. In this context, low toxicity in acute toxicity models such as *Artemia salina* is essential for ensuring the safe application of plant-derived compounds in aquaculture systems.

The Brazilian flora is very diverse and includes numerous medicinal plants with great potential for the discovery of new bioactive compounds suitable for antibacterial treatments. This biodiversity represents an underexplored reservoir for discovering novel bioactive compounds with potential antimicrobial applications. Notably, plants from the Anacardiaceae family, such as *Spondias mombin* L. [4] and *Myracrodruon urundeuva* Allemão [5], have yet to be explored for their biological activities against *V. parahaemolyticus*.

S. mombin is a medium-sized deciduous tree characterized by long compound leaves, with various parts of the plant traditionally used for medicinal purposes. For instance, the fruit serves as a diuretic and febrifuge agent, whereas the bark and leaves are used to treat hemorrhoids, gonorrhea, and leucorrhea [6]. Phytochemical studies have identified compounds such as tannins, flavonoids (quercetin, rutin, and catechin), phenolic acids (gallic and ellagic acids), and triterpenes (β-amyrin and oleanolic acid) in different parts of the plant, which may be responsible for its observed biological activities [7].

Similarly, *M. urundeuva*, known as aroeira, is widely distributed across northeastern Brazil and extensively used in traditional medicine for its proven antibacterial [8], antifungal [8], larvicidal [9], and antiviral [10] properties. The bark of this plant is commonly used to treat pain and infections in the genitourinary system, skin, subcutaneous tissues, and digestive system [11]. Chemical analyses have revealed the presence of bioactive constituents, including hydrolyzable tannins, chalcones, flavonoids (luteolin, apigenin, isoquercitrin), and catechins, which contribute to its pharmacological potential, particularly its anti-inflammatory and antimicrobial properties [12].

Despite their traditional use and known antimicrobial properties, the efficacy of S. mombin and M. urundeuva against V. parahaemolyticus has not been thoroughly investigated. Considering the urgent need for alternative disease management strategies in aquaculture, this study aims to investigate the chemical composition and antibacterial effect of ethanolic extracts (EEs) from the stem bark of *S. mombin* and *M. urundeuva* against *V. parahaemolyticus* strains, along with their antioxidant activity and acute toxicity in *A. salina* nauplii. It is anticipated that this study will advance our understanding of plant-based natural products as potential phytochemicals for preventing microbial resistance and oxidative stress in shrimp aquaculture systems.

2 | Results and Discussion

2.1 | Chemical Characterization

The chemical compositions of the EEs from *S. mombin* and *M. urundeuva* are described in Tables 1 and 2, respectively.

S. mombin bark extract was rich in procyanidins. A total of 11 compounds were identified, all belonging to the flavonoid class. These included procyanidins composed of (epi)catechin units and their structural derivatives, such as galloylated, ethyl-linked, or heterodimeric forms. Of these, the (epi)catechin-(epi)catechin B-type proanthocyanidin was the most abundant, accounting for 36.76% relative abundance, likely formed by a C4—C8 or C4—C6 bond between two flavan-3-ols such as catechin or (epi)catechin [13].

Figure 1A shows the base peak chromatogram (BPC) of the *S. mombin* bark extract. The major peaks, which were identified on the basis of the elution order, are listed in Table 1. Although catechin and (epi)catechin monomers have been identified in S. mombin extracts, the presence of their ethyl-linked dimers has not been explicitly reported previously, which may be due to genotypic variation or the limitations of previous analytical methods.

Procyanidins are in fact proanthocyanidins, or condensed tannins, oligomers or polymers of flavan-3-ol units, composed exclusively of (+)-catechin and (−)-epicatechin subunits [14]. These compounds have anti-inflammatory [11] and anticancer activities [15], as well as antioxidant properties [16]. Other flavonoids and constituents such as hydrolysable tannins, derivatives of phenolic acids, alkaloids, and glycosides are common compounds in the chemical composition of *S. mombin* [4, 17].

The analysis of the bark extract from *M. urundeuva* revealed sixteen components, including five hydrolysable tannins, five phenolic acids, and six dimeric chalcones often described as chalcone-related derivatives (Figure 1B). The most abundant compound was identified as a chalcone, designated erunvidine B, representing 26.53% of the total ion intensity, with its isomer (3.18%) and derivative (2.92%) increasing the total relative abundance of related compounds to 32.63%.

TABLE 1 | Chemical compounds identified in the stem bark ethanolic extract from *Spondias mombin* through UPLC–ESI–MS.

Peak	Retention time (min)	$[M-H]^-$ measured mass (*m/z*)	$[M-H]^-$ theoretical mass (*m/z*)	MS/MS (% abundance)	Formula	Mass error (ppm)	Relative abundance (%)	Putative compound	References
1	3.27	745.1414	745.1405	577.1403 (51), 407.0751 (83), 289.0713 (100), 245.0744 (29), 169.0175 (18)	$C_{37}H_{30}O_{17}$	1.2	2.94	(Epi)catechin-(epi)gallocatechin gallate	[14, 22]
2	3.38	865.1982	865.1980	695.1358 (04), 577.1352 (76), 407.0773 (77), 289.0721 (100), 245.0711 (30)	$C_{45}H_{38}O_{18}$	0.2	4.41	B-Type procyanidin trimer	[14, 23]
3	3.50	579.1492	579.1503	289.0739 (100), 245.0811 (16), 205.0506 (07)	$C_{30}H_{28}O_{12}$	−1.9	36.76	(Epi)catechin-(epi)catechin analogue (B-type)	[14, 22]
4	3.73	729.1442	729.1456	577.1358 (29), 559.1218 (07), 407.0767 (30), 289.0715 (59), 169.0129 (100)	$C_{37}H_{30}O_{16}$	−1.9	3.68	Procyanidin dimer monogallate	[11, 24]
5	3.91	881.1578	881.1565	729.1456 (09), 577.1434 (10), 559.1151 (04), 407.0748 (12), 289.0709 (100), 245.088 (26), 169.0104 (21)	$C_{44}H_{34}O_{20}$	1.5	5.51	Procyanidin dimer digallate (B-type)	[14, 24]
6	4.13	441.0824	441.0822	289.0692 (100), 169.0121 (45), 125.0239 (35)	$C_{22}H_{18}O_{10}$	0.5	9.19	(Epi)catechin-gallate	[11, 24]
7	4.44	605.1664	605.1659	315.0871 (37), 289.0701 (100)	$C_{32}H_{30}O_{12}$	0.8	4.41	(Epi)catechin-ethyl-dimer	[14, 24]
8	4.76	647.2137	647.2129	605.1656 (26), 467.1334 (24), 315.0880 (60), 289.0703 (100), 245.0776 (18)	$C_{35}H_{36}O_{12}$	1.2	3.68	(Epi)catechin-ethyl-dimer derivative	—
9	5.17	511.1614	511.1604	289.0738 (31), 169.0145 (100)	$C_{27}H_{28}O_{10}$	2.0	2.21	(Epi)catechin-galloyl derivative I	—
10	5.35	781.2086	781.2097	629.2219 (16), 511.1762 (32), 481.1525 (72), 459.2932 (54), 289.0729 (100), 169.0207 (80)	$C_{24}H_{46}O_{28}$	−1.4	2.57	(Epi)catechin-galloyl derivative II	—
11	5.46	663.1711	663.1714	511.1549 (31), 493.1488 (49), 289.0702 (32) 169.0126 (100)	$C_{34}H_{32}O_{14}$	−0.5	2.57	(Epi)catechin-galloyl derivative III	—
12	6.52	459.2949	459.2958	325.1428 (100), 310.1191 (45), 295.0959 (40), 223.1119 (18), 183.0166 (05)	$C_{24}H_{44}O_8$	−2.0	22.06	Unidentified	—

TABLE 2 | Chemical compounds identified in the stem bark ethanolic extract from *Myracrodruon urundeuva* through UPLC–ESI–MS.

Peak	Retention time (min)	$[M–H]^-$ measured mass (*m/z*)	$[M–H]^-$ Theoretical mass (*m/z*)	MS/MS (% abundance)	Formula	Mass error (ppm)	Relative abundance (%)	Putative compound	References
1	3.15	483.0788	483.0775	439.0889 (07), 331.0775 (17), 271.0485 (46), 169.0164 (100)	$C_{20}H_{20}O_{14}$	2.7	2.12	Gallic acid–galloyl hexoside	[11, 21]
2	3.28	497.1276	497.1295	483.1217 (13), 327.0739 (05), 313.0567 (42), 271.0406 (06), 183.0471 (12), 169.0132 (100)	$C_{22}H_{26}O_{13}$	−3.8	3.18	Gallic acid–galloyl hexoside derivative I	[21]
3	3.40	353.0875	353.0873	191.0537 (100)	$C_{16}H_{18}O_9$	0.6	4.77	Caffeoylquinic acid	[21]
4	3.55	635.0875	635.0884	483.1098 (21), 465.0745 (31), 169.0142 (100)	$C_{27}H_{24}O_{18}$	−1.4	1.86	Tri-galloyl-hexoside	[11]
5	3.67	923.1675	923.1671	771.1532 (11), 191.0543 (100), 169.0140 (66)	$C_{46}H_{36}O_{21}$	0.4	1.33	Galloyl-quinic acid derivative	—
6	3.90	787.1006	787.0994	635.0662 (10), 465.0865 (18), 313.0585 (22), 169.0105 (100)	$C_{34}H_{28}O_{22}$	1.5	1.06	Tetra-galloyl-hexoside	[11, 21]
7	3.98	939.1093	939.1104	787.0925 (04), 769.1088 (08), 617.0864 (06), 465.0776 (10), 169.0119 (85)	$C_{41}H_{32}O_{26}$	−1.2	3.98	Penta-galloyl-hexoside	[11, 25]
8	4.12	467.0979	467.0978	357.0589 (71), 217.0130 (72), 169.0098 (11)	$C_{24}H_{20}O_{10}$	0.2	6.63	Gallic acid derivative I	[11]
9	4.43	629.1298	629.1295	467.0974 (78), 357.0594 (100), 217.0091 (52), 169.0116 (32)	$C_{33}H_{26}O_{13}$	0.5	5.84	Gallic acid derivative II	—
10	4.52	619.1007	619.0994	449.0884 (43), 357.0578 (88), 169.0113 (57)	$C_{20}H_{28}O_{22}$	2.1	4.77	Gallic acid derivative III	[11]
11	4.93	827.1835	827.1823	659.1743 (23), 523.1068 (49), 387.0880 (85), 251.0721 (100), 135.0132 (42)	$C_{42}H_{36}O_{18}$	1.5	2.92	Erunvidine B derivative	—
12	5.10	525.1196	525.1186	415.0838 (28), 389.1030 (40), 135.0094 (65)	$C_{30}H_{22}O_9$	1.9	7.43	Erunvidine A	[11]
13	5.19	525.1189	525.1186	415.0851 (16), 389.1032 (45), 135.0085 (56)	$C_{30}H_{22}O_9$	0.6	13.26	Erunvidine A isomer I	[11]

(Continues)

TABLE 2 | (Continued)

Peak	Retention time (min)	$[M–H]^-$ measured mass (*m/z*)	$[M–H]^-$ Theoretical mass (*m/z*)	MS/MS (% abundance)	Formula	Mass error (ppm)	Relative abundance (%)	Putative compound	References
14	5.40	525.1182	525.1186	415.0826 (18), 389.1019 (40), 371.0897 (70), 135.0083 (56)	$C_{30}H_{22}O_9$	−0.8	11.14	Erunvidine A isomer II	[11]
15	5.45	523.1030	523.1029	387.0851, (53), 371.0902 (23), 251.0674 (52), 135.0087 (36)	$C_{30}H_{20}O_9$	0.2	26.53	Erunvidine B	[11]
16	5.67	523.1039	523.1029	387.0861 (38), 371.0903 (05), 251.0706 (100), 135.0130 (27)	$C_{30}H_{20}O_9$	1.9	3.18	Erunvidine B isomer	[11]

Previous research has reported the presence of proanthocyanidins, gallic and ellagic acids, flavonoids, and ellagitannins [18] in wood extracts, as well as chalcones in the stem bark [19]. In *M. urundeuva*, the presence of the biflavonol agathisflavone is common exclusively in the leaf extract, whereas erunvidine B is predominantly present in the stem bark extract [11]. Although erunvidine A isomers II and III, as well as erunvidine B isomer II, have also been previously reported in extracts of M. urundeuva, these findings confirm their presence in the species and highlight its unique chemical profile, underscoring the potential for further phytochemical and pharmacological research.

Dimeric polyphenolic compounds, often described as dimeric chalcones or flavonoid derivatives, are a class of polyphenols found in some species of the Anacardiaceae family, especially in *M. urundeuva*, where erunvidines were first reported [20]. The chemical profile of the *M. urundeuva* bark extract in the current study is in accordance with that detected for the species and genera and reported in the literature [10, 11, 21].

2.2 | Antibacterial Activity

Both EEs from *S. mombin* and *M. urundeuva* exhibited antibacterial activity against the bacterial strains used in this study, with varying efficacy across the two strains of *V. parahaemolyticus*. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values ranged from 0.625 to 1.25 mg/mL against these strains (Table 3). Notably, among the plant extracts tested, M. urundeuva displayed stronger antibacterial activity, with an MIC of 0.625 mg/mL for each strain, whereas S. mombin demonstrated a higher MIC and MBC of 1.25 mg/mL for both the reference strain (IOC 18950) and the farmed shrimp isolate. These results yielded MBC/MIC ratios of ≤4, which indicates bactericidal activity for both EEs, as defined by Pankey and Sabath [26].

Table 3 also shows that *V. parahaemolyticus* isolated from farmed shrimp exhibited high resistance to ampicillin, with an MIC of 10 000 μg/mL, compared to 625 μg/mL for the reference strain, indicating acquired resistance in shrimp farming systems. Enrofloxacin showed a lower MIC (0.15 μg/mL) and MBC (0.31 μg/mL) for both strains, demonstrating its superior inhibitory effect compared to ampicillin. Oxytetracycline was highly effective against the reference strain (MIC of 0.00060 μg/mL), but the farmed shrimp isolate exhibited a significant increase in MIC and MBC values (1.22 and 2.44 μg/mL, respectively), indicating a relatively larger concentration needed for bactericidal effects.

These findings underscore the importance of monitoring antibiotic resistance in aquaculture to prevent the emergence of resistant bacterial strains. The use of antibiotics in this context poses a potential risk to public health, as it may foster the development of a pool of transferable resistance genes between pathogenic bacteria in the aquatic environment. Indeed, the indiscriminate use of antibiotics in shrimp farming is regarded as the primary cause of the occurrence of antibiotic-resistant bacterial strains [2].

All combinations of EEs tested in this study exhibited bactericidal activity against both *V. parahaemolyticus* strains. Extracts in equal proportions of *S. mombin* and *M. urundeuva* (50:50) or with a higher share of M. urundeuva (25:75) demonstrated synergistic interactions (fractional inhibitory concentration index [FICi] < 1), indicating enhanced antibacterial activity through combined action. Conversely, increasing the proportion of S. mombin to 75% resulted in an indifferent effect ($1 < FICi \leq 2$), suggesting a diminished interactive contribution. These findings, on the basis of the FICi classification by Fratini et al. [27], indicate that formulations favoring M. urundeuva or maintaining a balanced ratio may be more effective in antimicrobial applications. This reinforces the potential use of plant-based antimicrobials as viable alternatives or complements to conventional antibiotics, particularly in the context of increasing resistance among aquaculture-associated pathogens. Although further studies are needed to fully elucidate the mechanisms of action and optimize the therapeutic use of these extracts, the chemical structures of the most abundant compounds identified in the extracts provide a better understanding of the antibacterial

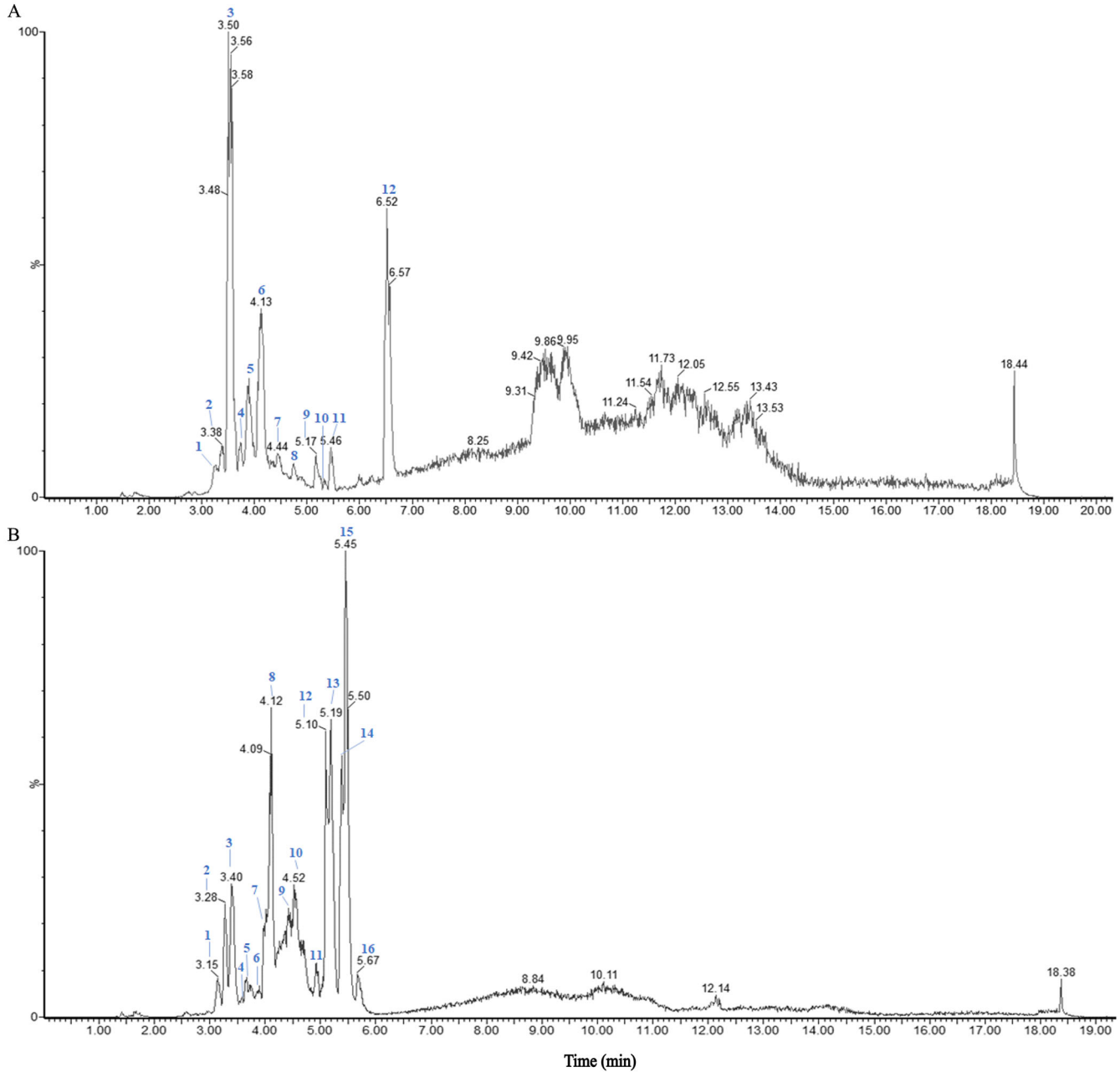

FIGURE 1 | UPLC-QTOF-MS chromatogram (negative-ion mode) of the stem bark ethanolic extract from *Spondias mombin* (A) and *Myracrodruon urundeuva* (B).

mechanism. Phenolic compounds such as B-type procyanidin and erunvidine B (Figure 2) are intimately associated with the antibacterial properties of the combined EEs of *S. mombin* and *M. urundeuva*. The multiple catechol units found in procyanidin B provide strong metal-chelating and radical-scavenging properties. Additionally, these features contribute to damaging bacterial membranes, which facilitates leakage of cellular components. Urundeuvin B with its hydroxyl groups and hydrophobic aromatic rings capable of donating hydrogen atoms disrupts bacterial membranes and impairs their functions. Together, these features may act synergistically to enhance the antibacterial effects of the combined plant extracts, supporting their potential as alternative treatments against antibiotic-resistant bacteria [8, 13, 17].

In future studies, we recommend exploring additional extract ratios (e.g., 10:90 or 20:80) to refine the understanding of dose–response relationships and optimize synergistic effects between plant extracts, as well as investigating mixtures prepared with solvents of varying polarity to isolate and evaluate different classes of bioactive compounds.

Previous studies have indicated that the leaf extract of *M. urundeuva* is a significant source of antimicrobial agents, showing activity against *Streptococcus mutans* biofilm [5] and *Lactobacillus casei* [24], for instance. Additionally, the aqueous extract of *M. urundeuva* has demonstrated potential antifungal activity against strains of *Candida albicans*, *Candida tropicalis*, and *Candida krusei* [28].

TABLE 3 | Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Spondias mombin* and *Myracrodruon urundeuva* ethanolic extracts against *Vibrio parahaemolyticus* (IOC 18950) and *V. Parahaemolyticus* isolated from farmed shrimp hemolymph.

Species/antibiotics/ Percentage combinations	*V. parahaemolyticus*(IOC 18950)			*V. parahaemolyticus*(farmed shrimp)		
	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC
Spondias mombin	1.25	1.25	1	1.25	1.25	1
Myracrodruon urundeuva	0.625	0.625	1	0.625	1.25	2
			Antibiotics			
	MIC (μg/mL)		MBC (μg/mL)	MIC (μg/mL)	MBC(μg/mL)	
Ampicillin	625		625	10000	*	
Enrofloxacin	0.15		0.31	0.15	0.31	
Oxytetracycline	0.00060		0.0012	1.22	2.44	
			Mixture of extracts			
	MIC (mg/mL)	MBC (mg/mL)	FICi** (Interaction type)	MIC (mg/mL)	MBC (mg/mL)	FICi** (Interaction type)
S. mombin(25%) + *M. urundeuva*(75%)	0.625	0.625	0.875 (synergistic)	1.25	1.25	1.750 (Indifferent)
S. mombin(50%) + *M. urundeuva*(50%)	0.625	0.625	0.750 (synergistic)	0.625	1.25	0.750 (synergistic)
S. mombin(75%) + *M. urundeuva*(25%)	0.625	0.625	0.625 (synergistic)	1.25	1.25	1.250 (Indifferent)

[a]Antibiotic-resistant.
[b]FICi: Fractional inhibitory concentration index, values based on [27].

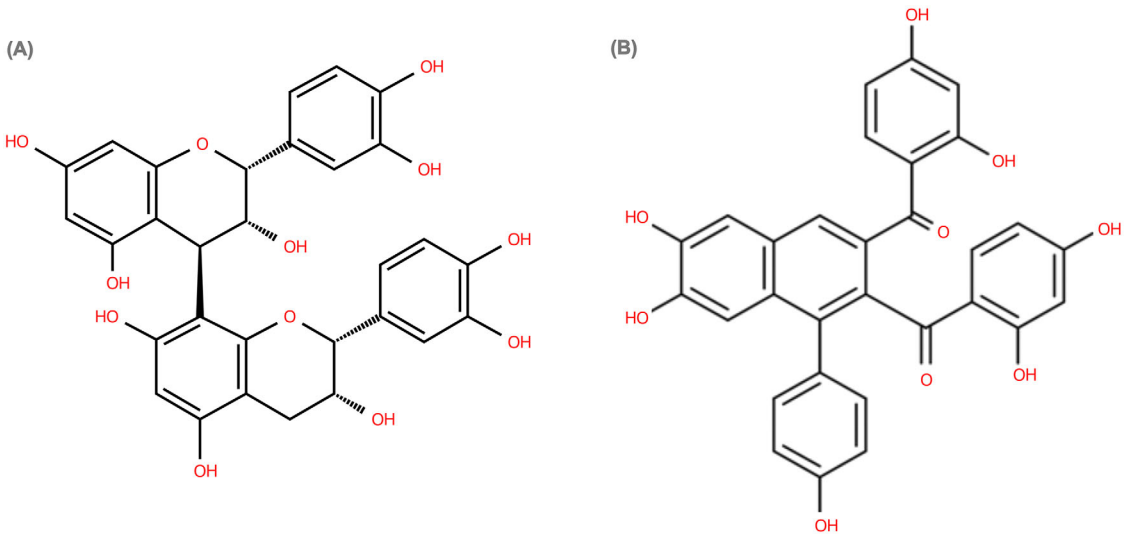

FIGURE 2 | Chemical structure of the main compounds of as B-type procyanidin (A) and erunvidine B (B).

Several extracts of *S. mombin* have been reported to have important antibacterial activity against *Klebsiella pneumoniae*, *Serratia marcescens*, *Salmonella typhi*, *Enterobacter aerogenes*, *and Staphylococcus aureus*, among others [4, 29–31]. Some fractions of the extract may have leishmanicidal [32], antiviral [21, 33], and antifungal [29] activities.

Gallic acid, a phenolic compound identified in the *M. urundeuva* extracts in this study (Table 2), was characterized as a weak antibacterial agent against S. aureus (MIC > 1 mg/mL) by Lima et al. [34], whereas Borges et al. [35] reported moderate activity against *Pseudomonas aeruginosa* (MIC > 0.5 mg/mL). Conversely, a study by Lu et al. [36] showed that gallic acid exhibits

strong antimicrobial activity against *Aeromonas hydrophila* and *Aeromonas sobria*. This compound has also demonstrated potent antifungal activity against the common pathogenic *C. albicans* (31.25 μg/mL) [37]. Gallic acid derivatives have also shown antimicrobial activity against fungi and Gram-positive and Gram-negative bacteria [38].

This observed bactericidal effect of gallic acid against pathogenic bacteria is likely to be attributed to its ability to promote irreversible changes in the properties of the bacterial membrane, such as charge, intra- and extracellular permeability, and physicochemical properties, through changes in hydrophobicity, decrease in negative surface charge, and occurrence of local rupture or formation of pores with consequent leakage of constituents [35]. Furthermore, gallic acid can cause severe shrinkage of bacterial cell volume in a dose-dependent manner, which compromises membrane integrity [36].

Another class of molecules recognized for their antimicrobial properties is tannins, largely due to the presence of o-diphenol groups, which allow them to act as iron chelators, thus depriving microorganisms of this essential element [39]. The presence of hydroxyl groups may also play an important role in the ability to permeate bacterial cell walls [40]. Due to these characteristics, some tannins may have potential anthelmintic, antibacterial, and antiviral activities [41].

Flavonoids constitute a diverse class of polyphenolic compounds that have attracted considerable interest due to their varied biological activities. These compounds have been recognized as potential antimicrobial, antiviral, antifungal, and anthelmintic agents [42]. Notably, the presence of agathisflavone in plant extracts has been shown to exhibit significant antibacterial and antiviral properties [43]. Isolated from the extract of Anacardium occidentale, agathisflavone displays moderate antibacterial activity against several pathogens, including Escherichia coli, P. aeruginosa, S. aureus, Proteus mirabilis, Bacillus subtilis, K. pneumoniae, and Clostridium sporogenes [44]. The antibacterial effects of certain flavonoids are attributed to multiple mechanisms, including their ability to disrupt pathogen binding to host cells, neutralize released toxins, damage the cytoplasmic membrane, thereby reducing its fluidity, and inhibit both nucleic acid synthesis and energy metabolism [42].

Chalcones and their derivatives exhibit a broad spectrum of biological activities, including antibacterial properties [45, 46]. In silico studies have revealed that these molecules exhibit a significant binding affinity for cellular proteins, which supports their potential antibacterial activity [43]. These organic compounds, considered intermediates in the biosynthesis of flavonoids, may act synergistically with antibiotics, resulting in increased antibacterial activity. Their α,β-unsaturated ketone moiety is regarded as a key factor in overcoming resistance mechanisms, thus increasing bacterial susceptibility to antibiotics [46]. The inhibitory effects of chalcones on human pathogenic microorganisms can be correlated with the substitution patterns of their aromatic rings, particularly the presence of a C-4 hydroxyl group, a C-4 oxygenated substituent, or a C-3 isoprenoid side chain [47].

In turn, procyanidins have proven antibacterial action [48]. The antibacterial activity of these compounds is due to their ability to cause damage to the bacterial membrane, thus inducing changes in external structures and thus causing leakage of cytoplasmic components such as proteins, nucleic acids, and K^+ [49].

TABLE 4 | Antioxidant activity of the ethanolic extracts against the DPPH solution (100 μM).

Species	IC_{50} ± SD	AAI ± SD	Antioxidant activity
Trolox[a]	2.75 ± 0.14[a]	14.39 ± 0.78[a]	Very strong
Spondias mombin	1.88 ± 0.17[b]	21.14 ± 2.06[b]	Very strong
Myracrodruon urundeuva	2.16 ± 0.05[b]	18.22 ± 0.39[b]	Very strong

Note: Means (±standard deviations) followed by different letters in the same column indicate significant differences using the Tukey test ($p < 0.05$).
Abbreviation: AAI, antioxidant activity index.
[a]Control. $IC_{50,}$ concentration that provides 50% inhibition of the free radical DPPH (mg/mL).

2.3 | Antioxidant Activity

The antioxidant activity index (AAI) of the EEs was significantly higher than those of the control (Table 4). The differences between the groups were statistically significant ($p < 0.05$).

Plant extracts can be classified according to the degree of antioxidant activity based on the criteria proposed by Scherer and Godoy [50], as follows: low antioxidant activity, when AAI < 0.5; moderate antioxidant activity, with an AAI between 0.5 and 1.0; strong antioxidant activity, with AAI between 1.0 and 2.0; and very strong, when the AAI > 2.0. In this study, all EEs demonstrated very strong antioxidant activity against DPPH free radicals (100 μM), with AAI values well above 2.0. Specifically, *S. mombin* exhibited the highest antioxidant potential (AAI = 21.14), followed by *M. urundeuva* (AAI = 18.22), both significantly higher than the standard antioxidant Trolox (AAI = 14.39) ($p < 0.05$). These results highlight the remarkable free radical scavenging capacity of the tested extracts and support their potential application as natural antioxidant agents in food preservation, pharmaceuticals, or aquaculture health management.

The methanolic extract of *S. mombin* leaves was previously described to have an IC_{50} of 417 ± 10 μg/mL in DPPH solution (65 μM) [31]. In a 300 μM DPPH solution, the IC_{50} was 11.8 ± 0.3 μg/mL [51]. In the present study, the *S. mombin* stem bark extract, composed exclusively of procyanidins, showed an IC_{50} of 1.88 ± 0.17 μg/mL, which may demonstrate the superior potential of the bark extract compared to the leaves. Cabral et al. [17] found that the *S. mombin* leaf extract at a concentration of 60 μg/mL reduced the absorbance in DPPH solution by approximately 70%, suggesting that the phenolic acids present in the extract and fractions are responsible for the radical scavenging activity.

M. urundeuva extracts, obtained with n-butanol, ethanol, and methanol, demonstrated antioxidant activity in DPPH solution, with all fractions showing an IC_{50} of less than 4 μg/mL [52]. Likewise, Vieira et al. [53] reported similar findings with methanolic extract from leaves and stem bark yielding IC_{50} values of 12.9 ± 0.2

TABLE 5 | Lethal concentration (LC_{50}) values of *Spondias mombin* and *Myracrodruon urundeuva* ethanolic extract in *Artemia salina* nauplii.

Species	LC_{50} (μg/mL)
S. mombin	1339.88 ± 32.73[a]
M. urundeuva	1470.60 ± 00.00[a]

Note: Equal lowercase letters in the same column indicate no statistically significant difference ($p > 0.05$). Mean value ± standard deviations.

and 10.9 ± 0.5 μg/mL, respectively, in DPPH solution. These extracts were characterized by a high phenolic content and demonstrated strong free radical scavenging potential.

The EE of *M. urundeuva* leaves, which is rich in phenols, flavonoids, and condensed tannins [54], reported an IC_{50} of 217 ± 0.0 mg/mL, in a DPPH solution (65 μM). In this study, the EE from the *M. urundeuva* stem bark, rich in tannins, phenolic acids, flavonoids, and chalcones, is suggested as the primary contributor to the observed antioxidant potential.

These findings align with previous studies reporting that the major phytochemical constituents of S. mombin and M. urundeuva stem bark extracts, particularly hydrolyzable tannins [55], flavonoids [56], phenolic acids [57], chalcones [58], and procyanidins [16], exhibit pronounced antioxidant activity, supporting their contribution to the high antioxidant potential observed in these extracts.

Understanding the biological relevance of these antioxidant properties requires consideration of their role in mitigating oxidative stress, a physiological condition resulting from an imbalance between the production of ROS and the availability of antioxidant defenses, leading to potential damage to DNA, proteins, and lipids [59]. The efficient and rapid elimination of ROS is essential for maintaining proper cellular function and ensuring the survival of the organism [60]. In response to natural oxidative stress, there is an increase in the concentration of the enzyme superoxide dismutase (SOD), which catalyzes the dismutation of the superoxide anion into less harmful molecules, thereby protecting the host from oxidative damage at cellular and molecular levels [61]. Therefore, the antioxidant capacity of the organism in response to oxidative stress induced by inflammation during infections is of paramount importance for preserving cellular integrity and overall health, directly influencing immune performance and survival. Given these considerations, the investigation of phytoextracts as a dual strategy to target both bacterial pathogens and oxidative stress is crucial, offering a promising approach to combat infections while enhancing antioxidant defenses for overall health management.

2.4 | Acute Toxicity

The estimated median lethal concentration (LC_{50}) of the EEs required to kill 50% of the *A. salina* nauplii was greater than 1000 μg/mL (Table 5). No mortality was observed in the control groups, even after 48 h of incubation. The LC_{50} averages for both extracts did not show a statistically significant difference ($p > 0.05$). EEs were, therefore, classified as nontoxic ($LC_{50} > 1000$ μg/mL) in accordance with Meyer et al. [62].

Although the EE from the stem bark of *S. mombin*, rich in flavones, was found to be nontoxic in this study, other researchers have reported different results. For instance, Clementino et al. [63] found the LC_{50} of the EE from the stem bark of *S. mombin*, rich in polyphenols, to exhibit moderate toxicity against *A. salina* (893.62 μg/mL). Similarly, Luna et al. [64] found the toxic effects of *S. mombin* seed extracts, which were rich in phenols and flavones.

In contrast with our results, previous studies have reported toxicity of *M. urundeuva* extracts. The crude hydroalcoholic extract of the bark of *M. urundeuva* exhibited toxicity against brine shrimp, with LC_{50} between 20 and 50 μg/mL [65]. Likewise, the EE from the leaves and branches of *M. urundeuva* was also toxic (LC_{50} of 39.38 μg/mL) when tested on *A. salina* nauplii in the second larval stage [66].

Tannins, commonly present in the EE of *M. urundeuva*, can also cause acute toxicity in *Phaeodactylum tricornutum*. The presence of these organic compounds can cause changes in the morphotype of these marine microalgae and complete growth inhibition [67]. Furthermore, in mammals, high doses of tannins can cause nausea, a likely result of the binding of proteins in the stomach, and when present in the blood, may lead to constipation, hepatotoxicity, or damage to other organs [41].

3 | Conclusions

The natural compounds described in this study within the EEs of *M. urundeuva* and *S. mombin*, specifically erunvidine B and (epi)-catechin-(epi)-catechin analogue (B-type), are mentioned in the literature as potential antimicrobial agents. Therefore, these organic compounds could be regarded as the primary contributors to the bactericidal activity observed against *V. parahaemolyticus*. Due to the complex composition of these extracts, their activity on microbial physiology probably results from a synergistic interaction among their compounds. This is the first report of the antibacterial activity of the EE of the stem bark of *M. urundeuva* and *S. mombin* against *V. parahaemolyticus*.

The ability of EEs to eliminate free radicals in vitro and thus possibly have the potential to function as antioxidant agents suggests the presence of electron-donating compounds, which can react with free radicals to convert them into more stable products.

Furthermore, our findings also demonstrate that these EEs exhibit no significant toxic effects.

4 | Experimental Section

4.1 | Plant Material

The plant material used for pharmacological analysis was the stem bark of *S. mombin* and *M. urundeuva*. Collection was carried out in the morning of March 2018 in Parnaíba, Piauí, Brazil (03° 05′ 12.5″ S, 041° 47′ 01.2″ W). Herbarium preparation followed the procedures outlined by Fidalgo and Bononi [68], and identification was performed by Ms. Renata Dourado. Species determination was based on specific botanical keys [69],

with comparisons to specimens deposited in the Parnaíba Delta Herbarium (HDELTA) at the Federal University of Delta do Parnaíba (UFDPar). The taxa were classified according to the Angiosperm Phylogeny Group IV [70], and scientific names were validated using the Missouri Botanical Garden database [71]. The genetic accession was registered on the SISGEN platform and approved by the Brazilian Council for Genetic Heritage Management (CGEN) of the Ministry of the Environment, under registration number A00721D. The specimens were archived in the UFDPar collection under registration numbers HDELTA-5465 (*S. mombin*) and HDELTA-5477 (*M. urundeuva*).

4.2 | Preparation of the EE

The collected plant material was dried in an oven with air circulation at 40°C and then powdered using a Croton-type knife mill (TE-650-Tecnal, Piracicaba, Brazil) with four knives and a 10-mesh sieve, at 1730 rpm. The resulting powder was added to an Erlenmeyer flask containing analytical-grade ethyl alcohol (1:4 mass/volume ratio) and manually stirred for 1 min. The extraction was carried out by static maceration for 24 h, at room temperature (~25°C), in the absence of light, and in triplicate. Subsequently, the crude macerate was subjected to vacuum filtration to separate the filtrate from the residual powder. Immediately after, the filtrate was concentrated in a water bath at 40°C. The concentrated extract was transferred to a flask and heated in an oven at 40°C until the complete elimination of the solvent. The obtained EE was stored at 4°C until further analysis.

4.3 | Chemical Characterization

The solid-phase extraction (SPE) technique was used as an initial clean-up step for the EE of each plant (25 mg of EE) to eliminate interfering compounds prior to chemical analysis using a reverse-phase cartridge (Supelco C18-500 mg/6 mL; Sigma-Aldrich).

The adsorbent was activated with 5 mL of methanol (HPLC LiChrosolv) followed by conditioning with 5 mL of ultrapure water. The extract was then solubilized in 0.3 mL of methanol and applied to the cartridge. To eliminate interfering compounds prior to chemical analysis, the cartridge was rinsed with 5 mL of ultrapure water. Finally, analytes of interest were eluted with 5 mL of methanol:water (9:1, v/v). An aliquot of each fraction was solubilized in 1 mL of methanol and filtered through a 0.22 μm polytetrafluoroethylene (PTFE) membrane (Analítica, São Paulo, SP, Brazil) before being injected into the ultra-performance liquid chromatography–mass spectrometry (UPLC–MS) system at a concentration of 1 mg/mL.

The chromatographic separation was carried out on an Acquity UPLC coupled to a quadrupole time-of-flight mass spectrometry (Q/TOF-MS) system (Waters Corporation, Milford, MA, USA). Chromatographic runs were performed on a Waters Acquity UPLC BEH column (150 × 2.1 mm^2; 1.7 μm), at a temperature of 40°C, and mobile phases were composed of acetonitrile-water (containing 0.1% formic acid). Varied ratios of acetonitrile (A) and water containing 0.1% formic acid (B), from 2% B to 95% B in 15 min, were worked as a gradient elution procedure to achieve chromatographic separation. During the whole process, the mobile phase flowed at a rate of 0.4 mL/min and kept each sample injection volume at 5 μL.

Electrospray ionization (ESI) source was set up at both positive and negative ion modes to perform mass spectrometric analysis. The ESI mode was obtained in the 110–1180 Da range with a source temperature of 120°C, 350°C desolvation temperature, 500 L/h desolvation gas flow, 0.5 V extraction cone, and 2.6 kV capillary voltage. Leucine encephalin was used as the lock mass for an accurate mass calibration. The mass spectrometer was operated in the MSE (high-energy mass spectrometry) mode of acquisition. The instrument was controlled by the MassLynx 4.1 software (Waters Corporation, Milford, USA).

The constituents of the extract were experimentally characterized using the molecular formula suggested by MassLynx 4.1 software, based on their accurate masses (error < 10 ppm), MS fragmentation patterns, and previous reports of their occurrence in the same species, genus, and/or botanical family. Additionally, the compounds were compared with public MS databases such as ChemSpider, PubChem, MassBank, Knapsack, and Metlin.

4.4 | Antibacterial Activity

The antibacterial activity of the EE was assessed against bacterial strains of *V. parahaemolyticus* provided by the Oswaldo Cruz Institute (OCI18950) and *V. parahaemolyticus* isolated from the hemolymph of farm-reared *Litopenaeus vannamei* shrimps from northeastern Brazil. The hemolymph from farmed shrimp samples was collected from ventral sinus by a puncture of the first abdominal segment with a 1-mL syringe containing ice-cold anticoagulant solution (2–8°C). Subsequently, the hemolymph was inoculated onto Petri dishes containing thiosulfate-citrate-bile-saccharose agar (TCBS, Difco) and incubated at 37°C for 24 h. *V. parahaemolyticus*-positive colonies were transferred to tryptic soy agar (TSA) plates supplemented with 2% NaCl.

Identification of the pathogen was confirmed using the OMNILOG GEN III system (Biolog Inc., USA) according to the manufacturer's instructions. Presumptive *V. parahaemolyticus* colonies were inoculated in BUG Agar (Biolog Universal Agar; Biolog Inc., USA) medium with 2% NaCl and incubated at 34°C for 24 h. After incubation, a single colony was picked from a plate and transferred into a 10 mL-inoculation fluid (IF-B) (Biolog Inc., USA). The inoculated IF-B was dispensed into a GEN III microplate. The microplate was incubated at 33°C for 24 h. The readings were carried out by the OmniLog Data Collection software using a semiautomated Biolog MicroStation system microplate reader.

The antibacterial activity of each EE was evaluated using the microdilution method in nutrient broth medium, with minor modifications, as follows. A stock solution of each EE (100 mg/mL) with 10% dimethyl sulfoxide (DMSO) and 90% distilled water was previously prepared. The strains were maintained on Mueller Hinton agar (Merck) and incubated at 34°C ± 2°C. The bacterial suspensions were adjusted to 0.5 McFarland with sterile saline solution until the concentration was 1.5×10^8 CFU/mL. Then, the bacterial suspension broth was dispensed into a 96-well microplate for testing with different EEs concentrations

(mg/mL), with a final volume of 100 μL. These concentrations were obtained in the 96-well plate as follows: 180 μL of the nutrient broth with bacteria was pipetted into the well in the first row (A) of the 96-well microplate, and in the other wells (B to H), 100 μL was dispensed on each. Subsequently, 20 μL of the stock solution (100 mg/mL) of EE was pipetted into well A (totaling 200 μL in the well). Then, from well "A" to well "H," the solutions were diluted in series (dilutions by 2) with nutrient broth, pipetting 100 μL from one well to another. At the end, 100 μL of solution from well H was discarded, and the final volume of all wells was maintained at 100 μL.

Oxytetracycline, enrofloxacin, and ampicillin were used as positive controls. Each microplate had three quality control groups: sterility control (wells with nutrient broth without bacterial inoculum); growth control (nutrient broth with bacterial inoculum); and toxicity control of the diluent used (nutrient broth with bacterial inoculum and DMSO solution). All tests were performed in triplicate. The microplates were incubated at 34°C ± 2°C for 24 h under aerobic conditions.

In this study, the 2,3,5-triphenyltetrazolium chloride (TTC) was used as a dye for the detection of microbial growth by adding 20 μL of a 3% solution (w/v) to each well, with an additional incubation of 1 h, under the same temperature. The MIC was defined as the lowest concentration of EE capable of completely inhibiting bacterial growth.

The MBC was determined by pipetting 10 μL of each well, in which bacterial growth was inhibited, onto Petri dishes containing Mueller Hinton agar (2% NaCl) incubated at 34 ± 2°C for 24 h. The MBC is the lowest concentration of each EE that shows no bacterial growth on the agar.

An assay was also carried out to verify the potential synergism of the antibacterial activity of each EE, which was tested in mixtures at different proportions (25%–75%, 50%–0%, and 75%–25%).

The FICi of mixtures of EEs was calculated according to Fratini et al. [27], using the following formula: $FICi = (MIC_{AB}/MIC_A) + (MIC_{BA}/MIC_B)$, where MIC_{AB} is the MIC of plant-extract A tested in combination, MIC_A is the MIC of plant-extract A tested alone, MIC_{BA} is the MIC of plant-extract B tested in combination, and MIC_B is the MIC of plant-extract B tested alone. The natures of the interaction between extracts were defined on the basis of the FICi value as follows: synergistic ($FICi < 1$), commutative (additive) ($FICi = 1$), indifferent ($1 < FICi \leq 2$), and antagonistic ($FICi > 2$).

4.5 | Antioxidant Activity

The antioxidant activity of the two plant EEs was determined against 2,2-diphenyl-1-picrylhydrazyl (DPPH) as free radical scavenger [72]. Aliquots of 30 μL of each EE solution at different concentrations, 15.62, 7.81, 3.91, 1.95, 0.98, 0.49, 0.24, and 0.12 μg/mL, were added to 200 μL ethanolic DPPH solution (100 μM) in 96-well microplates. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as a standard, being analyzed at concentrations of 15.63, 7.81, 3.91, 1.95, and 0.98 μM. The absorbance was read on a spectrophotometer at 517 nm immediately after the addition of the EEs to be tested (time 0) and after incubation at room temperature for 30 min under agitation and in the absence of light conditions. Readings for each sample were performed on the xMark Microplate Absorbance Spectrophotometer, using the Microplate Manager software.

Free radical sequestering activity was calculated by the following equation: $I\ (\%) = [(\text{Abs control} - \text{Abs test})/\text{Abs control}] \times 100$, where Abs control is the absorbance of the control reaction (ethanolic DPPH solution), and Abs test is the absorbance in the presence of the tested EE at different concentrations. The inhibitory concentration (mg extract/mL) at which 50% DPPH radicals were scavenged (IC_{50}) was obtained by interpolation from linear regression analysis. Tests were carried out in triplicate.

4.6 | Acute Toxicity

The acute toxicity assay was performed according to Meyer et al. [62] using freshly hatched *A. salina* nauplii (INVE Aquaculture, Belgium). Dried cysts were incubated in natural seawater (salinity 35 g/L; dissolved oxygen 5.3 mg/L; pH 8.2; and temperature 27.5°C) previously filtered through a PTFE membrane filter with a 0.22 μm pore size, under light conditions and continuous aeration. After 24 h, the *A. salina* nauplii were separated from the non-hatched cysts using a Pasteur pipette. All water used in the experiment was also prefiltered in a 0.22 μm pore size filter.

Toxicity assays were performed in 96-well microplates with 200 μL of EE solution in seawater at different concentrations (2000, 1000, 500, 250, 125, 62.5, 31.2, and 15.6 μg/mL), and 10 nauplii to each well, in order to determine dose–response relationship. Each horizontal row of the 96-well plate corresponded to the EE tested concentrations, and vertical column contained samples and controls. The assay had two control groups: the first control group consisted of only nauplii and filtered seawater, and the second contained nauplii and filtered seawater with 1% DMSO. EEs were diluted in seawater with 1% DMSO. Counts of live and dead larvae were performed using a stereoscopic microscope after 24 h of incubation, under normal photoperiod conditions and ambient temperature (27–30°C). Larvae were considered dead when no movement was detected after 30 s of observation. As no significant mortality was observed after 24 h of incubation, mortality rates were recorded after 48 h of exposure. EEs were considered toxic if the lethal concentration (LC_{50}) value is less than 1000 μg/mL, whereas nontoxic if it is greater than 1000 μg/mL [62].

4.7 | Statistical Analyses

The Statistical Package for the Social Sciences (SPSS) version 20.0 was used for statistical analysis. After confirming normality of data distribution using the Shapiro–Wilk tests and data homogeneity through boxplots, significant differences between the AAI of the EEs and the control (Trolox) were assessed using one-way analysis of variance (ANOVA), followed by Tukey's test ($\alpha < 0.05$). The average lethal concentration (LC_{50}) was determined by the Probit regression analysis method with 95% confidence limits. A Student's *t*-test for independent samples ($\alpha < 0.05$) was used to

evaluate the differences between the LC_{50} means of the EEs. All data were expressed as mean values ± standard deviations.

Author Contributions

All authors have contributed to the intellectual content of this article following these requirements: (1) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (2) drafting or revising the article for intellectual content; and (3) final approval of the published article.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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