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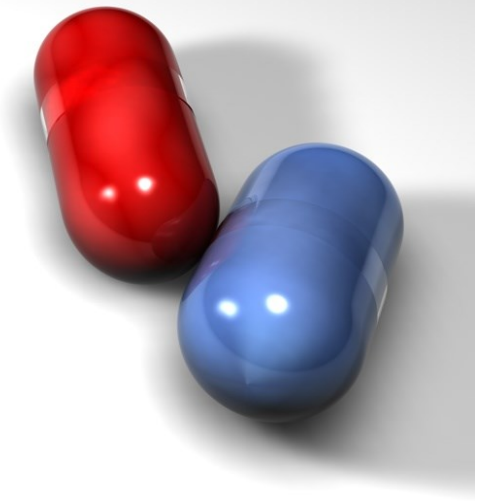

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Letter to the Editor

Pharmacological activities and volatile organic compounds from the endophytic bacteria from the leaves of *Rhizophora apiculata*

Sir,

The endophytic microorganisms with mangrove plants have been reported to be a prolific source of secondary metabolites with promising pharmacological properties. Of these, many compounds exhibit potent biological activities and may be considered as lead compounds for further drug developments (Ancheeva et al. 2018). *Rhizophora apiculata*, a true mangrove plant, is widely distributed in mangrove forests in Vietnam. Phytochemical and biological investigations on the endophytic microorganisms from *R. apiculata* reveal that they can produce secondary metabolites with valuable biological properties such as antibacterial, antifungal, antiviral, anti-cancer activities (Chaeprasert et al., 2010; Klaiklay et al., 2012; Fan et al., 2013; Zhou et al., 2019). Hence, the current study focused on the evaluation of pharmacological activities and volatile organic compounds of ethyl acetate extracts from the endophytic bacteria isolated from the leaves of *R. apiculata*.

Fresh leaves of *R. apiculata* were collected from Phu Loc District, Thua Thien Hue Province, Vietnam. The fresh leaves were surface-sterilized according to the protocol described elsewhere (Dat and Oanh, 2021). The surface-sterilized leaves were crushed in sterile distilled water, and then 100 µL sample solution was spread on nutrient agar (NA, Himedia, India). The plates were incubated at 30°C for 3-5 days. Colonies with different morphological characteristics were identified by 16S rRNA gene sequences (Dat et al., 2021).

From fresh leaves of *R. apiculata*, 10 strains with different morphological features were selected for identification using the 16S rRNA gene sequences. The molecular identification showed that the isolated strains belonged to 5 genera, i.e., *Bacillus*, *Pseudomonas*, *Rossellomorea*, *Vibrio*, and *Staphylococcus* (Supplementary file: Table S1; Figure S1). Interestingly, the majority of the isolated bacteria belonged to the genus *Bacillus*.

The endophytic bacteria were used for producing ethyl acetate extracts and evaluating their biological activities. The extracts of the bacterial strains were obtained by centrifuging the culture solutions at 10,000 rpm for 10 min. Subsequently, the cell-free supernatants were extracted with ethyl acetate (3 times) and evaporated under reduced pressure to obtain the crude extracts.

Biological properties of the bacterial extracts were evaluated. Antimicrobial activity of the extracts was determined by the microdilution (Dat et al., 2021), antioxidant and α-amylase and α-glucosidase inhibitory activities were determined using the protocol described elsewhere (Dat and Oanh, 2021), and xanthine oxidase inhibitory activity was determined using the protocol described elsewhere (Nguyen et al., 2004).

Obtained results showed that 6 extracts exhibited antimicrobial activity against at least one reference microorganism with minimum inhibition concentrations (MICs) from 16 to 256 µg/mL (Table I). Among them, 4 extracts exhibited antimicrobial activity against *S. aureus*, 3 extracts against *E. faecalis*, 4 extracts against *E. coli*, 2 extracts against *P. aegurinosa*, and 3 extracts against *C. albicans*. Several extracts showed antimicrobial activity against multiple reference microorganisms. For example, 4 extracts exhibited antimicrobial activity against 3 reference microorganisms and two extracts against 2 reference microorganisms. Notably, several extracts showed significant antimicrobial activity with MIC values of 32-64 µg/mL.

Antioxidant assays showed that 4 extracts exhibited DPPH radical scavenging activity with IC_{50} values from 33.9 ± 0.7 to 62.2 ± 2.1 µg/mL and 5 extracts exhibited ABTS radical scavenging activity with IC_{50} values from 53.4 ± 2.9 to 79.8 ± 5.4 µg/mL. Interestingly, two extracts, i.e., RAL_NA_4 and RAL_NA_8, exhibited both DPPH and ABTS radical scavenging activities.

α-Amylase and α-glucosidase assays showed that 4 extracts exhibited α-amylase inhibitory activity with IC_{50} values from 63.2 ± 1.4 to 130.1 ± 6.8 µg/mL and two extracts exhibited α-glucosidase inhibitory activity with IC_{50} values from 57.8 ± 1.3 to 175.0 ± 6.8 µg/mL. However, only one extract (RAL_NA_2) exhibited both α-amylase and α-glucosidase inhibitory activities. Regarding the xanthine oxidase, 4 extracts exhibited xanthine oxidase inhibitory activity with IC_{50} values from 56.9 ± 2.8 to 77.2 ± 3.7 µg/mL (Table I).

The volatile organic compounds in the extract of the most promising bioactive strain *Bacillus* sp. RAL_NA_8

Table I

Biological activity of the bacterial extracts

Strain ID	Antimicrobial activity (MIC, µg/mL)				
	S. aureus ATCC 25923	*E. faecalis* ATCC 29212	*E. coli* ATCC 25922	*P. aegurinosa* ATCC 27853	*C. albicans* ATCC 10231
RAL_NA_1	-	-	-	-	-
RAL_NA_2	-	-	64	256	-
RAL_NA_3	-	-	-	-	-
RAL_NA_4	128	-	256	-	64
RAL_NA_5	32	64	-	-	128
RAL_NA_6	-	-	32	-	256
RAL_NA_7	-	-	-	-	-
RAL_NA_8	64	32	-	128	-
RAL_NA_9	256	256	128	-	-
RAL_NA_10	-	-	-	-	-
Ciprofloxacin	1	2	0.5	0.5	-
Fluconazole					2
Strain ID	Antioxidant activity (IC_{50}, µg/mL)		Enzyme inhibitory activity (IC_{50}, µg/mL)		
	DPPH radical scavenging	ABTS radical scavenging	α-Amylase	α-Glucosidase	Xanthine oxidase
RAL_NA_1	>100	>100	>200	>200	77.17 ± 3.71
RAL_NA_2	>100	>100	82.6 ± 3.4	57.8 ± 1.3	>100
RAL_NA_3	>100	79.1 ± 2.9	76.50 ± 2.15	>200	63.5 ± 1.4
RAL_NA_4	62.5 ± 2.1	54.8 ± 1.5	>200	>200	>100
RAL_NA_5	43.8 ± 2.1	>100	63.2 ± 1.4	>200	>100
RAL_NA_6	>100	70.1 ± 2.0	>200	>200	71.5 ± 2.1
RAL_NA_7	>100	>100	>200	175.0 ± 6.8	>100
RAL_NA_8	57.0 ± 0.1	53.4 ± 2.9	130.1 ± 6.8	>200	>100
RAL_NA_9	>100	79.8 ± 5.4	>200	>200	56.9 ± 2.8
RAL_NA_10	33.9 ± 0.7	>100	>200	>200	>100
Ascorbic acid	28.0 ± 0.6	25.0 ± 0.6			
Acarbose	-	-	82.4 ± 1.0	205.4 ± 6.3	-
Allopurinol	-	-	-	-	4.1 ± 0.3

were investigated by the GC–MS analysis using the Agilent 7890B gas chromatograph-assisted Agilent 5977A mass detector (Agilent Technologies, USA). The compounds were identified by comparing the spectra with a stored MS library (W8N08 and NIST08) and the relative percent of individual components was calculated based on GC peak areas. The GC-MS analysis identified 11 volatile compounds in the extract, including major compounds 2,5-furandione, dihydro (62.8%), [(3R,4s,5S)-4-nitrothian-3,5-diyl]-diacetate (11.2%), 1,3-dioxolane, 2,2,4,5-tetramethyl-, trans- (8.4%), 2-(methyl-D3)-cycloheptanone (4.0%), and hyacinthin (3.2%) (Table II; Supplementary file: Figure S2).

The results in the present study are consistent with previous investigations of bioactive natural products from endophytic microorganisms with the mangrove plant *R. apiculata* (Chaeprasert et al., 2010; Klaiklay et al., 2012; Fan et al., 2013; Zhou et al., 2019). For example, Klaiklay et al. (2012) isolated two cytotoxic and antibacterial compounds from the endophytic fungus *Phomopsis* sp. PSU-MA214. The isolated compounds exhibited cytotoxicity against the cancer cell line MCF-7 and KB with IC_{50} of 27-43 µg/mL and antibacterial activity against *S. aureus* and methicillin-resistant *S. aureus* with MICs of 64-128 µg/mL. Zhou et al. (2019) isolated three antimicrobial compounds, fusolanone A-B and fusaric acid, from the endophytic fungus *Fusarium solani* HDN15-410. These compounds exhibited good antimicrobial activity against *Pseudomonas aeruginosa*, *Monilia albican*, *Bacillus subtilis*, and *Vibrio parahaemolyticus* with MICs of 6.25–50 µg/mL.

In conclusion, the endophytic bacteria from the leaves of *R. apiculata* may be a potential source of pharmacological secondary metabolites.

Table II

Volatile organic compounds in the extract of *Bacillus* sp.

Compounds	RT/min	Quantity (%)
Diacetone alcohol	5.671	1.23
2,2,3,4-Tetramethyl-hex-5-en-3-ol	5.939	1.97
2-(Methyl-D3)-cycloheptanone	6.271	4.03
4,6,8-Trimethyl-,9-undecan-5-ol	6.441	1.70
Oxirane, 2,2-dimethyl-3-propyl	8.182	2.74
Hexyl ethyl carbinol	8.496	1.24
1,3-Dioxolane, 2,2,4,5-tetramethyl-, trans-	8.693	8.35
[(3R,4s,5S)-4-nitrothian-3,5-diyl]-diacetate	9.042	11.21
4-Heptanol, 4-ethyl-2,6-dimethyl	9.473	1.48
2,5-Furandione, dihydro	10.181	62.83
Hyacinthin	10.515	3.21

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Supplementary

Table SI

The closest 16S rRNA sequences of the isolated strains obtained in NCBI GenBank

Strain ID	Sequence length (nt)	NBCI closest homologs	Identity (%)
RAL_NA_1	1419	*Staphylococcus argenteus* BG-V-5, OK326042	99.93
RAL_NA_2	1409	*Bacillus cereus* SW9SE, MN068934	100
RAL_NA_3	1379	*Bacillus pseudomycoides* KUBOTAB2, MK855402	99.86
RAL_NA_4	1420	*Bacillus subtilis, AB192294*	100
RAL_NA_5	1449	*Bacillus infantis C4, MF993020*	99.87
RAL_NA_6	1398	*Pseudomonas aeruginosa* ATCC 27853, AF094719	99.93
RAL_NA_7	1379	*Vibrio cholerae TS5-B, LC487859*	99.85
RAL_NA_8	1400	*Bacillus altitudinis 19RS3, MH883312*	99.94
RAL_NA_9	1449	*Bacillus baekryungensis* LS218, FJ937928	99.81
RAL_NA_10	1440	*Rossellomorea vietnamensis 151-6, CP047394*	100

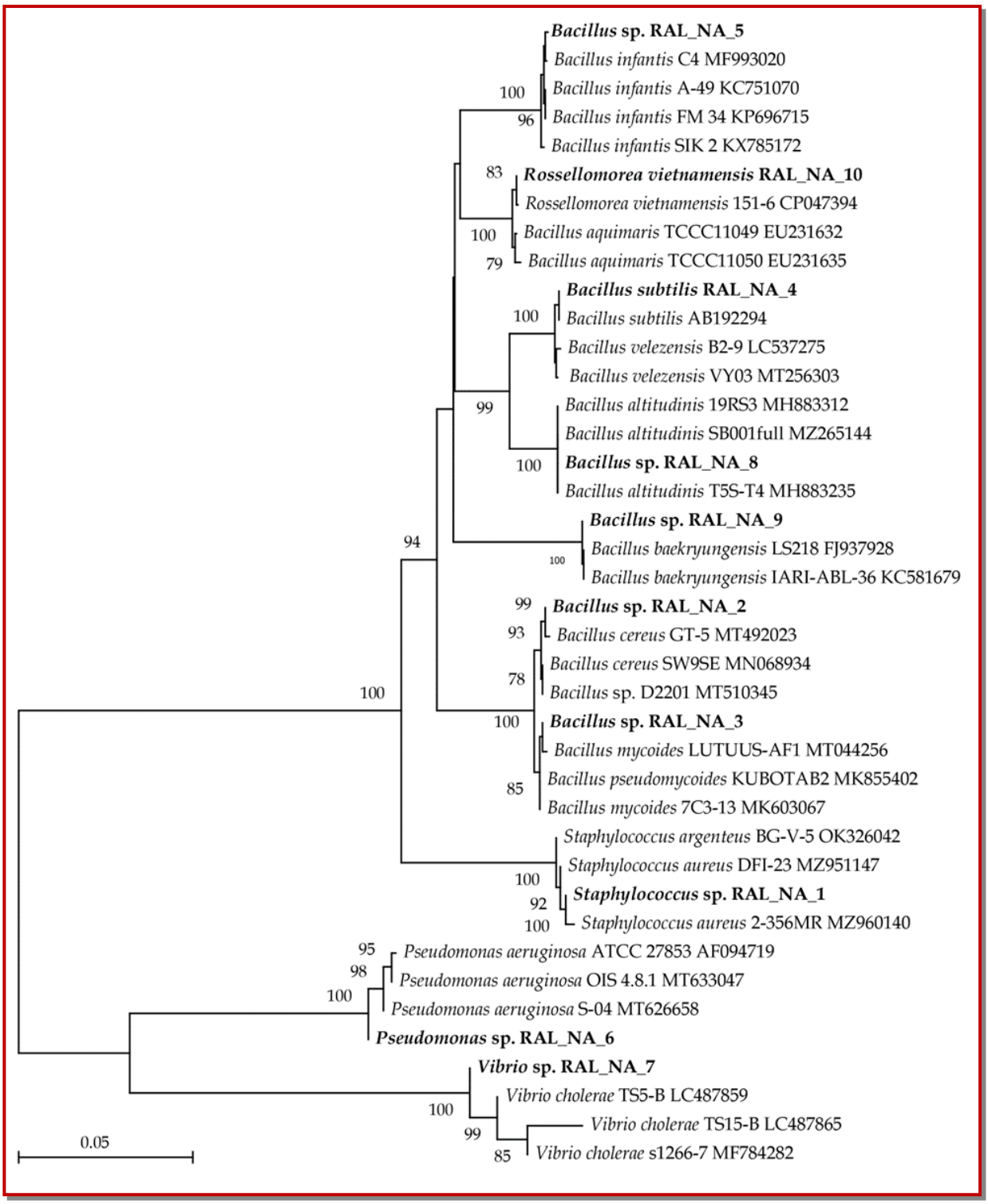

Figure S1: Phylogeny of 16S rRNA gene sequences of the endophytic bacteria from the present study (bold letters) and from NCBI GenBank. Bootstrap support values of branches greater than 75% are given above the corresponding branches

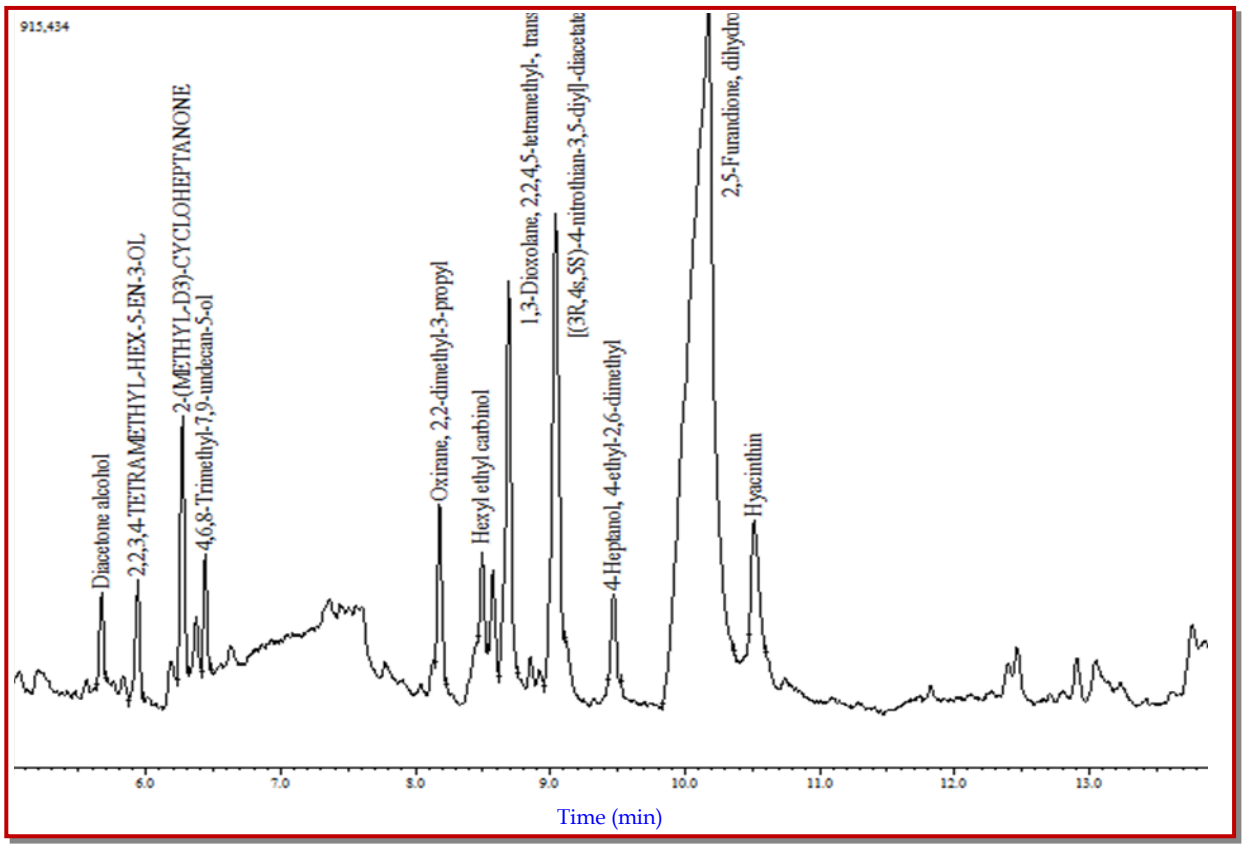

Figure S2: GC-MS chromatogram of the ethyl acetate extract of *Bacillus* sp. RAL_NA_8