



Phytochemical composition and antibacterial activity of ethanol extract of *Amorphophallus lanceolatus* tuber (Araceae)

DOI: [10.7904/2068-4738-X\(20\)-5](https://doi.org/10.7904/2068-4738-X(20)-5)Hong-Thien VAN^{1#}, Ngoc-Buu TRAN^{1#}, Tran-Tien TRINH¹, Nhu-Truc Thi VO¹, Van-Son LE², Tan-Viet PHAM¹, Gia-Buu TRAN¹, Tan-Quoc Pham LE^{1*}¹Institute of Biotechnology and Food-technology, Industrial University of Ho Chi Minh City, 12 Nguyen Van Bao Street, Go Vap District, Ho Chi Minh City, VIETNAM²Binh Chau-Phuoc Buu Nature Reserve, Bung Rieng ward, Xuyen Moc District, Ba Ria-Vung Tau Province, VIETNAM*Corresponding author: lephamtanquoc@iuh.edu.vn

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Abstract. *Amorphophallus lanceolatus* is a rare member belonged to Araceae family and is recorded as an endemic species of Binh Chau-Phuoc Buu Nature Reserve (Vietnam). The phytochemical composition and antimicrobial activity of *A. lanceolatus* have not been studied yet. In this study, we determined the phytochemical composition of ethanol extract of *A. lanceolatus* tuber using liquid chromatography-mass spectrometry (LC-MS) and evaluated the antibacterial activity of ethanol extract via disk diffusion test. We identified 12 compounds in ethanol extract of *A. lanceolatus* tuber, such as polyethylene glycol-diglycidyl ether, lycopersin, heptacosane, n-heneicosylcyclopentane, homalomenol F, maltitol, pyrinuron, octadecane, 2 (1 H) naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl), 2-methylfluoranthene, quinic acid, 1H-imidazole. Moreover, we proved that ethanol extract of *A. lanceolatus* tuber could inhibit the growth of *B. cereus*, *E. coli*, *P. aeruginosa*, *S. enteritidis*, *S. typhimurium*, and *S. aureus*.

Keyword: antibacterial activity, *A. lanceolatus*, chemical composition, ethanol extract, LC-MS.

Introduction

Amorphophallus genus, including of 200 species in over the world and 30 species in Vietnam, is belonged to Araceae family [PHAM-HOANG, 2000; BOYCE *et al.*, 2012; NGUYEN, 2017; TRAN *et al.*, 2017].

Several studies indicated the potential application of species of this genus in food industry and medicine. In previous study, Singh and Wadhwa suggest that root of *A. paeoniifolius* consists of high amount of starch, carbohydrate, energy, and several essential minerals [SINGH and WADHWA, 2014].

On the other hand, the methanol extract of *A. paeoniifolius* also exhibits analgesic activity, antihelmintic activity, as well as antimicrobial activity against several Gram-positive and negative bacteria.

Recently, Tran and collab. have reported that there are 6 species of *Amorphophallus* genus, such as *A. konjac*, *A. corrugatus*, *A. krausei*, *A. yunnanensis*, *A. yuloensis*, and *A. paeoniifolius*, those are distributed in

Northern region of Vietnam and consist of glucomannan in their starches [TRAN *et al.*, 2017].

Amorphophallus lanceolatus is described for the first time by Serebryanyi (1995) with specimens collected in Xuyen Moc District, Ba Ria-Vung Tau Province, Vietnam.

At the beginning, this species is classified as a member of *Pseudodracontium* genus and is named as *P. lanceolatum*. However, Hettterscheid and Claudel suggest that all specific characteristics of species belonged to *Pseudodracontium* genus are similar with those of *Amorphophallus* genus and molecular evidences also show the close genetic relationship of *Pseudodracontium* genus and *Amorphophallus* genus.

Therefore, they delete *Pseudodracontium* genus and re-classify all member of *Pseudodracontium* genus into *Amorphophallus* genus. As the consequence, scientific name of *P. lanceolatum* is changed into *P. lanceolatus* [HETTERSCHIED and CLAUDEL, 2012].

To date, *A. lanceolatus* has only been found in Xuyen Moc District, Ba Ria–Vung Tau Province, Vietnam, and there are only a few numbers of studies about classification of this species [SEREBRYANYI, 1995; PHAM–HOANG, 2000; HETTERSCHIED and CLAUDEL, 2012; NGUYEN, 2017].

In this study, we determine the chemical composition and antibacterial activity of ethanol extract of *A. lanceolatus* tuber, and provide the more information for further application of this species.

Material and methods

Materials

Plant material

Specimens of *A. lanceolatus* were collected from Binh Chau–Phuoc Buu Nature Reserve, Bung Rieng ward, Xuyen Moc District, Ba Ria–Vung Tau Province, location of about 10°32'51"N; 107°31'35"E, July 4, 2018, 48 m in elevation (Figure 1).

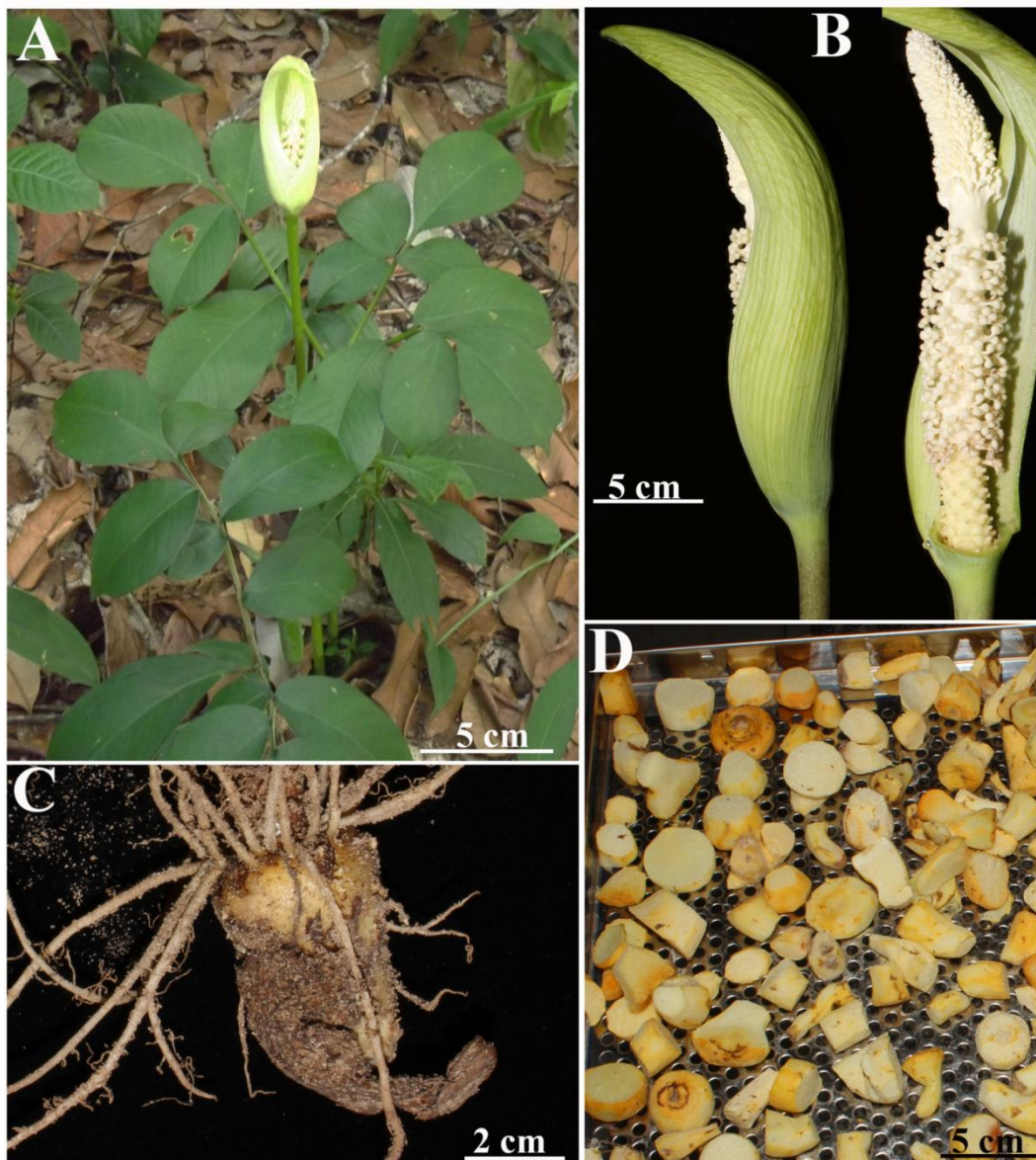


Figure 1. *Amorphophallus lanceolatus*. A. Habitat. B. Spathe and spadix. C. Tuber. D. Dried tubers

Bacterial strains

Six bacterial strains were used to determine the antibacterial activity. Two Gram–positive bacteria, including *Bacillus*

cereus (ATCC 11774), *Staphylococcus aureus* (ATCC 25923), and four Gram–negative bacteria, including *Escherichia coli* (ATCC 25922), *Pseudomonas*



aeruginosa (ATCC 27853), *Salmonella enteritidis* (ATCC 13976), and *Salmonella typhimurium* (ATCC 13311) were obtained from the microbiology collection, Department of Biotechnology, Institute of Food and Biotechnology, Industrial University of Ho Chi Minh city, Viet Nam.

All bacterial strains were maintained at $-20\text{ }^{\circ}\text{C}$ in 20 % glycerol solution and inoculated into Luria–Bertani broth at $37\text{ }^{\circ}\text{C}$ for 24 h to be re-activated again before using in further experiments.

Methods

Extraction procedure

Fresh tubers of *A. lanceolatus* were washed and peeled to remove the outer part of tubers. The peeled tubers were subsequently sliced and moderate dried at $50\text{--}55\text{ }^{\circ}\text{C}$ until the mass of samples remained unchanged. The dried tubers were ground using an electric grinder into fine powder and stored at $4\text{ }^{\circ}\text{C}$. 50 g of the dried powder of tubers of *A. lanceolatus* were soaked with 450 mL of 98% ethanol for 5 weeks. The extract was filtrated using Whatman number 1 filter paper.

The filtrate was eventually concentrated in reduced pressure at $60\text{ }^{\circ}\text{C}$ until the residue remained $\frac{1}{2}$ volume of the initial filtrate [BUTNARIU, and BOSTAN, 2011, ALTEMIMI *et al.*, 2017]. The ethanol in concentrated extract was eliminated by sublimation drying. The obtained residue was stored at $4\text{ }^{\circ}\text{C}$ until further use.

Liquid chromatography mass spectrometry (LC–MS)

The LC–MS analysis of extract was performed at the Central Laboratory for Analysis, University of Science, Vietnam National University of Ho Chi Minh City with given procedure. In brief, aliquot of ethanol extract was injected to HPLC Agilent 1200 infinity liquid chromatography system (Agilent Technologies, CA, USA) coupled with MicroTOF–QII mass spectrometer (Bruker Daltonics, Germany).

The chromatographic separation was carried out using an ACE3–C₁₈ analytical column (4.6 x150 mm, 3.5 μm) at $40\text{ }^{\circ}\text{C}$ as a stationary phase. The mobile phase consisted of deionized

water with 0.1 % formic acid as solvent A and acetonitril with 0.1 % formic acid as solvent B. The flow rate was kept at 0.3 mL/min. Gradient elution program for the chromatographic separation was presented in Table 1.

Table 1.

Gradient elution program for the chromatographic separation

Time (min)	Solvent A'	Solvent B'
0	90	10
15	0	100
30	0	100
31	90	10
40	90	10

('): presented as the percentage of volume of mobile phase

The mass spectrometer was operated with electrospray ionization source (ESI) at positive mode and mass spectra data were recorded on mode for a mass range $50\text{--}2000\text{ m/z}$. Data analysis was performed using Data Analysis software (Bruker, Germany) [BUTNARIU, *et al.*, 2016].

Antibacterial activities

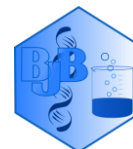
The antibacterial assay of ethanol extract of *A. lanceolatus* tuber was performed using Bauer method [BAUER *et al.*, 1996]. The bacteria were cultured in LB Broth until reached a turbidity of 0.5 McFarland standard. The bacterial suspensions (100 μL) were then spread on sterile Mueller Hinton plate and a sterile 6 mm diameter discs were placed on the inoculated surface. 20 μL of sample were added onto each disc and the plates were maintained at $4\text{ }^{\circ}\text{C}$ for 2 hours to allow diffusion into the medium.

The plates were incubated at $37\text{ }^{\circ}\text{C}$ for 24h and the antibacterial activity of sample was determined via the diameter of the zone of inhibition of tested bacteria.

Sterilize distilled water was used as negative control and Gentamycin antibiotic discs (Nam Khoa BioTek, Viet Nam) were used as positive control [IANCULOV, *et al.*, 2004, BUTU, *et al.*, 2014].

Data analysis

Experimental results were analyzed by the one-way analysis of variance (ANOVA) method and significant differences among the means from triplicate analyses at ($p < 0.05$) were



determined by Fisher's least significant difference (LSD) procedure using Statgraphics software (Centurion XV).

The values obtained were expressed in the form of a mean±standard deviation (SD).

Results and discussion

Phytochemical analysis

In this study, mass spectra of compounds which identified in the ethanol extract of *A. lanceolatus* tuber were compared with previous studies about

chemical composition of species of *Amorphophallus* genus.

As presented in Table 2 and Figure 2, we found 12 compounds in ethanol extract of *A. lanceolatus* tuber, such as polyethylene glycol–diglycidyl ether, lycopersin, heptacosane, n–heneicosylcyclopentane, homalomenol F, maltitol, pyrinuron, octadecane, 2 (1*H*) naphthalenone, 3,5,6,7,8,8*a*–hexahydro–4,8*a*–dimethyl–6–(1–methylethenyl), 2–methylfluoranthene, quinic acid, 1*H*–imidazole.

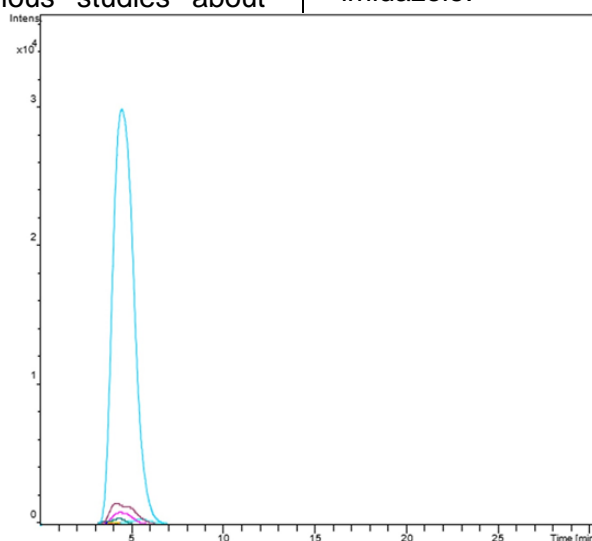


Figure 2. Chromatogram (LC–MS) of the ethanol extract of *A. lanceolatus* tuber obtained by maceration.

Quinic acid is a cyclohexanecarboxylic acid which is used as an astringent and material for synthesis of pharmaceuticals such as Tamiflu [BARCO *et al.*, 1997, BUTNARIU, *et al.*, 2015].

Karkacier and Artik report that the fruits are ripe enough; they have 91–92 % total dry matter and 62–67% total soluble solids, which consist of 34–42 % sucrose, 10–12 % fructose, and 7–10 % glucose [KARKACIER and ARTIK 1995, SAMFIRA, *et al.*, 2015].

Table 2.

Phytochemical composition of ethanol extract of *A. lanceolatus* tuber.

Compounds	Retention time (min)	Molecular Weight	References
2–Methylfluoranthene	3.6	217	[SABATE <i>et al.</i> , 1999]
Polyethylene glycol–diglycidyl ether	3.7	526	[ZURATUL <i>et al.</i> , 2013]
n–Heneicosylcyclopentane	3.8	364	[AKIRA and HIKARU, 2002]
Homalomenol F	3.8	353	[HU <i>et al.</i> , 2008]
Maltitol	3.8	344	[DOBREVA <i>et al.</i> , 2013]
Octadecane	3.8	254	[ZHOU <i>et al.</i> , 2016]
1 <i>H</i> –imidazole	3.8	182	[SYBRIN <i>et al.</i> , 2018]
Lycopersin	3.9	381	[VENUGOPAL <i>et al.</i> , 2014]
Pyrinuron	3.9	272	[LAM, 1985]
2(1 <i>H</i>) naphthalenone, 3,5,6,7,8,8 <i>a</i> –hexahydro–4,8 <i>a</i> –dimethyl–6–(1–methylethenyl)	3.9	218	[JEYARAMAN and ARUMUGAM, 2015]
Quinic acid	3.9	192	[SERBAN and MICHAEL, 2012]
Heptacosane	4.2	380	[KHAN <i>et al.</i> , 2007; ZHANG <i>et al.</i> , 2013]

Maltitol is a polyol and can be used as sugar substitute because of its high

sweetness [DOBREVA *et al.*, 2013, RASHED and BUTNARIU, 2014].

1H-imidazole is used as an antifungal drug and antibiotics to combat the infection of anaerobic bacterial and parasitic infection [MITAL, 2009].

Antibacterial activity

Antibacterial activity of ethanol extract of *A. lanceolatus* tuber was evaluated by the diameter of the zone of

inhibition of tested bacteria (Table 3 and Figure 3). The results showed that ethanol extract of *A. lanceolatus* tuber could inhibit the growth of six tested bacteria, such as *B. cereus*, (B) *E. coli*, (C) *P. aeruginosa*, (D) *S. enterritidis*, (E) *S. typhi*, (F) *S. aureus*.

Table 3.

The inhibition zone of ethanol extract of *A. lanceolatus* tuber against six tested bacteria.
(-): No inhibition

Tested bacteria	Growth inhibition zone (mm)			
	Original extract	2-fold diluted extract	4-fold diluted extract	6-fold diluted extract
<i>B. cereus</i>	-	8.0 ± 1.0 ^a	8.0 ± 0.0 ^a	7.3 ± 0.6 ^a
<i>E. coli</i>	-	7.0 ± 0.5	-	-
<i>P. aeruginosa</i>	-	6.2 ± 0.3	-	-
<i>S. enterritidis</i>	-	7.2 ± 0.3 ^b	6.2 ± 0.3 ^a	-
<i>S. typhimurium</i>	-	-	-	8.8 ± 0.3
<i>S. aureus</i>	-	12.2 ± 0.3 ^b	10.0 ± 1.0 ^a	9.2 ± 0.3 ^a

Various lowercase letters in the same row denote significant difference (p < 5%) between extraction solvents

However, diameters of the zones of inhibition were various in different bacterial strains and dilution factors.

As shown in Table 3, there was no inhibitory effect of ethanol extract on any tested bacterial strains whereas diluted extract showed antibacterial activity

against all tested bacterial strains. One possible explanation for differing results is diffusion ability of extract.

The original extract has so high concentration of compounds that it cannot be diffused on agar plate; thus, it does not show its antibacterial activity.

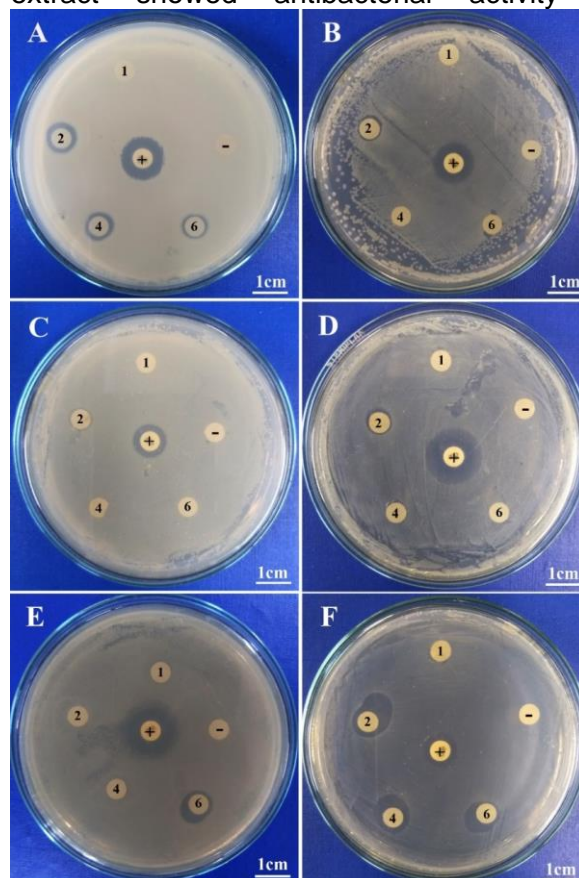


Figure 3. Antibacterial activity of ethanol extract of *A. lanceolatus* tuber against tested bacteria. (A) *B. cereus*, (B) *E. coli*, (C) *P. aeruginosa*, (D) *S. enterritidis*, (E) *S. typhi*, (F) *S. aureus*. The paper discs numbered as 1, 2, 4, and 6 were loaded with original extract, two-fold four-fold, and six-fold diluted extracts, respectively. (-) Negative control with sterilized distilled water, (+) Positive control with discs containing gentamicin.

Among 6 tested bacteria, ethanol extract exhibited the highest antibacterial

activity against *S. aureus* (Figure 3F), with 2-fold diluted extract (12.0±6.0 mm)



following by 4-fold (10.0 ± 3.0 mm) and 6-fold diluted extracts (9.0 ± 4.0).

In the same manner, 2-fold diluted extract showed the highest inhibitory effect against *S. enteritidis* (Figure 3D) with diameter of inhibition zone about 7.0 ± 1.0 mm, following by 4-fold diluted extract (6.0 ± 0.5 mm); whereas 6-fold diluted extract did not inhibited the growth of *S. enteritidis*.

On the other hand, only 2-fold diluted extract had antibacterial activity against *E. coli* (Figure 3B) and *P. aeruginosa* (Figure 3C) with diameter of inhibition zone about 7.0 ± 1.0 mm versus 6.0 ± 1.0 mm, respectively.

Note that, *S. typhimurium* was inhibited by 6-fold diluted extract but was not inhibited by original extract or the other diluted extracts. In contrast, all of diluted extracts have antibacterial activity against *B. cereus*.

Some previous studies also suggested anti-bacterial activities of ethanol and methanol extract of rhizomes of species belonged to *Amorphophallus* genus.

Khan and collab. proved that amblyone, a triterpenoid isolated from of *A. campanulatus*, could inhibit the growth of four Gram positive bacterial strains (*Bacillus subtilis*, *Bacillus megaterium*, *Staphylococcus aureus*, *Streptococcus β -haemolyticus*) and 6 Gram negative bacterial strains (*Escherichia coli*, *Shigella dysenteriae*, *Shigella sonnei*, *Shigella flexneri*, *Pseudomonas aeruginosa*, and *Salmonella typhi*) [KHAN et al., 2007, PETRACHE, et al., 2014].

Moreover, anti-bacterial activities of various extracts of *A. paeoniifolius* against *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsilla*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* were also reported [MUTHUKUMARAN et al., 2016].

Chidanand and collab. indicated that both acetone and ethanol extract of *A. konkanensis* tuber exhibited a strong antibacterial activity against *B. subtilis*, *M. aureus*, *P. aeruginosa*, *E. coli*, *S. typhi*, and *K. pneumoniae* [CHIDANAND et al., 2015].

Conclusions

In this study, we identified 12 compounds, such as (1) polyethylene glycol-diglycidyl ether (PEG-DGE), (2) lycopersin ($C_{20}H_{14}O_8$), (3) heptacosane ($C_{27}H_{56}$), (4) n-heneicosylcyclopentane, (5) homalomenol F, (6) maltitol, (7) pyrinuron ($C_{13}H_{12}N_4O_3$), (8) octadecane ($C_{18}H_{38}$), (9) 2 (1H) naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6- (1-methylethenyl), (10) 2-methylfluoranthene, (11) quinic acid ($C_7H_{12}O_6$), (12) 1H-imidazole in ethanol extract of *A. lanceolatus* tuber.

We also proved the antimicrobial activity of extract in 6 bacterial strains, including *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enteritidis* and *Salmonella typhimurium*.

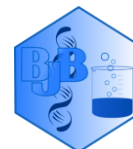
Therefore, this study provides the information for further application of *A. lanceolatus*.

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