Chemical composition, antibacterial and antioxidant activities of acetone extract from the branches and leaves of *Jasminum annamense* subsp. *annamense* (Oleaceae)

Hong Thien Van¹, Nguyen Hung Ngo², Nguyen Thuan Nguyen¹, Nguyen An Nguyen¹, Tan Viet Pham¹, Hanh Thi Dieu Nguyen¹, Gia Linh Thieu¹, Thi Hoai Thuong Dang⁴, Quoc Hung Nguyen⁵, Thanh Tho Le³, Van Son Le¹, Sao Mai Dam*¹

¹Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City, No. 12 Nguyen Van Bao Street, Go Vap District, Ho Chi Minh City, Vietnam, ²Faculty of Fundamental Science, Industrial University of Ho Chi Minh City, No. 12 Nguyen Van Bao Street, Go Vap District, Ho Chi Minh City, Vietnam, ³Center of Analytical Services and Experimentation HCMC, Vietnam, No. 2 Nguyen Van Thu, Dakao Ward, District 1, Ho Chi Minh City, Vietnam, ⁴Binh Chau-Phuoc Buu Nature Reserve, Bung Rieng ward, Xuyen Moc District, Ba Ria-Vung Tau Province, Vietnam

# ABSTRACT

*Jasminum annamense* subsp. *annamense* is a rare subspecies of *Jasminum annamense* belonging to the Oleaceae family. The aims of this study were to address the chemical profiles, antibacterial and antioxidant activities of acetone extract isolated from branches and leaves of *Jasminum annamense* subsp. *annamense* for the first time. The chemical constituents of acetone extracts of studied samples were investigated by gas chromatography-mass spectrometry. There were a total of 24 components identified from the leaf extract, including lup-(29)-en-3-one (27.93%), levodopa (19.68%), trans-cinnamic acid (7.58%), linoleic acid (6.35%) as the major compounds. Meanwhile, 26 components were reported from the branch extracts which are sorbitol (25.74%), lupeol (13.3%), cis-vaccenic acid (6.97%), glycerin (6.35%) and n-hexadecanoic acid (5.86%) were the main components. The two acetone extracts of *J. annamense* subsp. *annamense* exhibited antibacterial effect against *Bacillus cereus*, *Escherichia coli*, *Salmonella enteritidis* and *Staphylococcus aureus* based on disk diffusion assay. In addition, leaf and branch extracts of the studied species also display no antioxidant activity in the ABTS assay with IC₅₀ values of 511.75±5.39 and 664.46±3.73 µg/ml, respectively. This is the first report on the chemical and biological properties of *J. annamense* subsp. *annamense* and provides a promising perspective for developing good sources of antioxidant and antimicrobial compounds against both Gram positive and negative bacteria.

# KEYWORDS:
*Jasminum annamense* subsp. *annamense*, phytochemical, antimicrobial, antioxidant activity

# INTRODUCTION

*Jasminum* L. is also known as “Jasmine” and consists of 200 species widely distributed in Africa, Asia, Australia and Mediterranean regions (Dam et al., 2020). Almost Jasmine plants have been used in traditional medicine to treat many diseases. For instance, the plant was commonly used for venereal diseases and removing intestinal worms. Flower extract was used to cure vesicles, ulcers, boils, eye disorders and skin diseases. Leaf extract was active against breast tumors, stomachitis, aphthous, toothache, throat, ulceration in the mouth, and gums (Jaya et al., 2019). Furthermore, many Jasmine plants have been reported to possess biological activities, including antioxidant, antimicrobial, anti-diabetic, anti-inflammatory and cytotoxic properties (Jaya et al., 2019; Li et al., 2020). In Vietnam, 37 species, 6 subspecies and 1 variety of *Jasminum* genus have been recorded by previous reports (Pham, 2000; Bui, 2016).

*Jasminum annamense* Wernham is commonly known as “Lài trung bò” in Vietnamese and has two subspecies, including *J. annamense* subsp. *annamense* and *J. annamense* subsp. *glabrescens*. It is a rare species and distributed in several regions of Thailand, Laos and Vietnam. In Vietnam, this plant is found in Dak Lak, Thu’a Thien Hue, Lam Dong, Khanh Hoa and Ba Ria-
Van et al.

Vung Tau Provinces (Dam et al., 2020; Bui, 2016). To date, only one of our previous works reported the chemical components and biological properties of this species. Accordingly, Dam et al. showed 7 compounds from ethanol extracts isolated from J. annamense subsp. annamense leaves and stems using liquid chromatography/mass spectrometry analysis. Furthermore, these extracts also possessed antibacterial effects against Bacillus cereus and Salmonella typhimurium (Dam et al., 2020). Consequently, the information on the chemical composition and biological activities of acetone extracts of J. annamense subsp. annamense are limited. The present study, therefore, firstly investigated the chemical components, antibacterial and antioxidant properties of acetone extracts obtained from leaves and branches of J. annamense subsp. annamense.

MATERIALS AND METHODS

Plant Material

Specimens of J. annamense subsp. annamense were collected from Binh Chau-Phuoc Buu Nature Reserve, Ba Ria-Vung Tau Province, Vietnam (Figure 1). The location of collection is about 10°32'47"N; 107°28'57"E and the area was around 43 m above the mean sea level.

Bacterial Strains

The antibacterial activity of the acetone extracts from the leaves and the branches of J. annamense subsp. annamense was tested against four bacterial strains, including two Gram-negative bacteria (Escherichia coli (ATCC 25922), Salmonella enteritidis (ATCC 13976)) and two Gram-positive bacteria (Bacillus cereus (ATCC 11774), Staphylococcus aureus (ATCC 25923)). The strains were preserved in 20% glycerol solution at 20°C and activated by cultivation in Luria-Bertani broth at 37°C for 24h and the antibacterial activity of the acetone extracts of J. annamense subsp. annamense were measured.

Extraction Procedures

The J. annamense subsp. annamense leaves and the branches were moderately dried at 50°C until their weights were unchanged. The dried specimens were ground into powder using the electric grinder. Subsequently, 50g of the dried powder were macerated in 250 ml of 99% acetone solution at room temperature for 72 hours. The Whatman paper was used to filter the studied extracts. The process was repeated twice. The filtrate was concentrated under reduced pressure at 60°C to obtain the brown extract, subsequently subjected to sublimation drying to completely remove the remaining acetone (Bobinaitė et al., 2013).

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The chemical composition of the obtained acetone extracts of studies species was analyzed on Gas Chromatography-Mass Spectrometry (GC-MS) system equipped with an Agilent 7890A GC coupled with a 5975C VL MSD Triple-Axis selective Detector equipped with a ZB-5MS capillary non-polar column (30.0μm length x 0.25mm i.d. x 0.25μm film thickness). Helium was the carrier gas used at a constant pressure of 13.209 psi. The injection temperature was 250°C; the injection volume was 0.1 μl; the split ratio was 10:1. The oven temperature was programmed to proceed from 60°C to 240°C at the rate of 3°C/min. The constituents were identified on the basis of a comparison between their mass spectra with the internal library (NIST 2017 library and the Wiley 8th edition libraries). The equation proposed by van den Dool and Kratz was utilized to calculate the retention indices (arithmetic indices) of the oil components relative to the homologous series of C9-C17 n-alkanes.

Antibacterial Activity Assay

The method described by the CLSI (Clinical and Laboratory Standards Institute) was used to study the antibacterial activity (Clinical and Laboratory Standards Institute, 2010). The tested bacterial strains were inoculated into LB Broth for growing until the turbidity of 0.5 McFarland standards. A bacterial culture of 100 μL of bacterial culture was spread on a sterile Mueller Hinton plate. The sterile 6 mm diameter discs were placed on the surface of the inoculated Mueller Hinton plate. Each disc was then added with 10 μl of the essential oil. The plate was incubated at 37°C for 24h and the antibacterial activity of the sample was determined via the measurement of the inhibition zone diameter of the tested bacteria. Sterilized distilled water was used as a negative control whereas Gentamicin antibiotic discs (supplied by Nam Khoa BioTek, Vietnam) were used as a positive control.

Determination of Antioxidant Activity of Extract

The ABTS radical scavenging properties of the acetone extracts from J. annamense subsp. annamense leaves and the branches were identified using Maeng et al. (2017) work. Firstly, 7 mM ABTS was added to 2.45 mM K2SO4 of the sample. The mixture was slightly shaken and placed in the dark for 18 hours at 37°C (solution A). 0.1 mL studied extract was mixed in 3 mL solution A. This mixture was diluted to 5 ml of acetone and slightly shaken and placed in the dark for 15 minutes. The UV-vis spectrophotometer (UVS 2800, Labone, USA) and UVWin6 Software v6.0.0 were used to record the absorbance of the solution at 734 nm. The reference standard was ascorbic acid. Ascorbic acid...
standard curve (0-15 ppm) was constructed with the equation 
\[ y = -0.0278x + 0.421, \quad R^2 = 0.9990, \]
where \( y \) is the absorbance at 734 nm and \( x \) is the sample concentration (µg/mL). The sample concentration was calculated from the standard curve equation and the results were expressed as µg/mL ascorbic acid.

Data Analysis

The antibacterial assay was conducted in triplicate. The one-way analysis of variance (ANOVA) and Fisher's least significant difference (LSD) procedure (Statgraphics software (Centurion XV)) were used to analyze the experimental results as well as significant differences among the means from triplicate analyses at \( p<0.05 \). The results were presented as mean ± standard deviation (SD).

RESULTS AND DISCUSSION

Phytochemical Composition of Acetone Extracts from *J. annamense* subsp. *annamense*

The chemical profiles of acetone extracts from *J. annamense* subsp. *annamense* leaves and branches were shown in Figure 2 and Table 1 & 2. A total of 24 components were identified from the leaf extract of the studied plant, including lup-20(29)-en-3-one (27.93%), levodopa (19.68%), trans-cinnamic acid (7.58%), linolenic acid (6.35%) as the major compounds. In addition, 26 components were detected in the branch extracts which the extract was characterized by the predominance of sorbitol (25.74%), lupeol (13.3%), cis-vaccenic acid (6.97%), glycerin (6.35%) and n-hexadecanoic acid (5.86%). Among them, 7 components, including levodopa, 4-((1E)-3-hydroxy-1-propenyl)-2-methoxyphenol, n-hexadecanoic acid, phytol, stigmasterol, β-sitosterol and lupeol were identified in both leaf and branch extracts.

Previous reports showed the chemical compositions of other *Jasminum* species. For example, the essential oil from the *J. officinale* flowers from Singapore has been reported to contain 3,7,11,15-tetramethyl-2-hexadecen-1-ol (12.31%) and benzyl acetate (11.50%) as the major compounds (Muttiah et al., 2019). The major constituents of *J. grandiflorum* essential oils were mainly composed of phytol (25.77%), 3,7,11-trimethyldodeca-1,6,10-trien-3-ol (12.54%) and 3,7,11,15-tetramethyl-1-Hexadecen-3-ol (12.42%) (Wei et al., 2015). Furthermore, the chemical profiles of the leaf and flower essential oils of *J. pubescens* grown in Egypt have been investigated. As a result, the leaf oil of this species were mainly composed of nonanal (21.2%), β-caryophyllene (10.1%) and phenylacetaldehyde (6.8%) while trans-Nerolidol (27.6%), (Z)-jasmine (8.4%) and phenylacetaldehyde (5.7%) were the main compounds in the flower oil (Tenraz et al., 2009). In addition, the essential oil obtained from *J. sambac* flowers grown in Pakistan was characterized by the predominance of benzyl benzoate (15.63%), jasmine (9.90%) and linalool (8.58%) (Mahmood et al., 2017). Recently, Sultana et al. (2018) provided five new compounds isolated from the methanol extract of *J. auriculatum* collected from India, including (1) (Z)-n-dotriacont-6-enyl piperate [(Z)-n-dotriacontenyl piperate; (2) (Z)-4-pentadecanoyloxyferulic acid; (3) (Z)-n-triacont-8-enio acid [(Z)-8-dehydromellisic acid; (4) (Z)-dodec-6-enyl - O-β-D-glucopyranosyl - (6′→1′)-O-β-D-glucopyranosyl-(6′→1′)-O-β-D-glucopyranoside ([Z]-6-lauroleyl β-D-tetraglucoside; (5) (Z)-dodec-6-enyl - O-α-D-glucopyranosyl-(6′→1′)-O-α-D-glucopyranosyl-(6′→1′)-O-α-D-glucopyranosyl-(6′→1′)-O-α-D-glucopyranoside ([Z]-6-lauroleyl β-D-tetraglucoside (Sultana et al., 2018).

The biological properties of some constituents of acetone extracts from *J. annamense* subsp. *annamense* leaves and branches have been suggested by previous studies. For instance, n-hexadecanoic acid has been reported as the inflammatory agent (Van et al., 2021). Moreover, the cytotoxic, antioxidant, antimicrobial, anxiolytic, anticonvulsant, immune-modulating, antinociceptive and anti-inflammatory properties of phytol were also provided by previous studies (Santos et al., 2013;
Adnan et al., 2019). Stigmastanol and β-sitosterol possessed many pharmacological activities, including anti-osteoarthritis, anti-hypercholesterolemic, cytotoxic, anti-tumor, antioxidant, antimicrobial, antinutagenic and hypoglycemic effects (Kisangau et al., 2009; Babu & Jayaraman, 2020). Furthermore, lupeol, another component of acetone extracts of leaves and branches from studied plant has been reported to possess the antiprotozoal, anti-inflammatory, antitumor, anti-diabetic, antimicrobial, nephroprotective activities (Gallo & Sarachine, 2009; Sharma et al., 2020). Levodopa (3,4-dihydroxy-L-phenylalanine) is commonly known as L-DOPA. This constituent has been widely used worldwide to treat Parkinson disease (Mollov et al., 2005; Lewitt & Fahn, 2016). Another compound, 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol, has been known as antimicrobial, antitumor, antimicrobial, antioxidant, anti-inflammatory and analgesic agents (Ashwathanarayana & Naika 2017; Sultana et al., 2018; Haron et al., 2019).

Lup-20(29)-en-3-one, the most abundant component in the leaf extract of J. annamense subsp. asnnamense, possessed cytotoxicity and trypanocidal activity on mammalian cells (Bossolani et al., 2017). Another major compound in the leaf extract, trans-cinnamic acid, is a natural aromatic carboxylic acid. This compound displayed antioxidant, antimicrobial (Abd El-Raouf et al., 2015), anticancer (Wang et al., 2019), neuroprotective, anti-inflammatory and antidiabetic properties (Guo et al., 2019). In addition, sorbitol is the highest constituent found in the branch extract of the studied plant. It is also known as a systematic name D-glucitol and has been reported as the bulking agent, humectant, sequestrant, stabilizer, sweetener and thickener (Grembecka, 2015). Glycerin, another main component in the branch extract, is also known as glycerol. This compound is a polyol which naturally presents in the structure of triglycerides. There are several applications in various fields, including pharmaceutical, food and cosmetic industries for this compound where it is used as a thickener, sweetener or anti-freezer, humectant and lubricant, etc. (Garcia et al., 2010).

### Antibacterial Activities of Acetone Extracts from J. annamense subsp. annamense

The acetone extracts from J. annamense subsp. annamense leaves and branches showed antibacterial activity against four bacterial strains using agar diffusion method (Table 3). As a result, the leaf extract was found to be effective against S. enteritidis, S. aureus, B. cereus and E. coli with the diameter of inhibition zone about 12.2 ± 0.8 mm, 12.8 ± 1.0 mm, 9.5 ± 1.3 mm and 8.2 ± 0.3 mm, respectively. Meanwhile, the branch extract displayed activity against S. enteritidis (9.0 ± 0.9 mm), B. cereus (8.7 ± 0.8 mm), E. coli (8.5 ± 0.5 mm) and S. aureus (8.3 ± 0.3 mm).

The antibacterial activity of acetone extracts from J. annamense subsp. annamense leaves and branches may be attributed...
to the chemical components present in the extracts. For example, n-hexadecanoic acid was found to be effective against *E. coli* with a minimum inhibition quantity (MIQ) of 1 µg (Van et al., 2021). For instance, β-sitosterol and stigmasterol have been reported to possess antibacterial effects against *S. aureus*, *E. coli*, *P. aeruginosa* and *S. typhimurium* (Edilu et al., 2015). Furthermore, *trans-cinnamic* had an inhibitory effect on a large number of pathogenic bacteria, including 8 Gram-positive and 20 Gram-negative bacterial strains with MIC values ranging 500 to 2500 µg/mL (Yilmaz et al., 2018). Furthermore, phytol strongly displayed activity against *E. coli* (MIC = 62.5 µg/mL), *P. aeruginosa* (MIC = 19 µg/mL) (Pejin et al., 2015), *Enterococcus faecalis* (MIC < 62.5 µg/mL) (B. Pejin, 2014). In addition, lupeol was found to be effective against *Enterococcus faecalis* (MIC = 65 µg/mL), *P. aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213 (MIC = 250 µg/mL) (Shai et al., 2008), *S. aureus* ATCC 25923, *S. typhimurium* ATCC 0232, *Vibrio cholera*, *E. coli* ATCC 35218, *Shigella* spp. batch 0.57 (MIC > 250 µg/mL) (Mathabe et al., 2008).

A large number of extracts obtained from different *Jasminum* species have been reported to possess antibacterial properties. For example, the methanol extracts of *J. abyssinicum* leaves and flowers were found to be effective against *S. aureus*, *Streptococcus pyogenes*, *S. pneumoniae*, *Neisseria gonorrhoea*, *E. coli*, *Bacillus cereus*, *Shigella dysenteriae*, *S. flexineri* and *S. typhimurium* (Geyid et al., 2005). Also, *Bacillus* sp., *E. coli*, *Staphylococcus* sp., *Klebsiella pneumoniae*, *Lactobacillus* sp., *Yersinia* sp. and *Enterococcus* sp. were inhibited by the methanol extracts of *J. angustifolium* flowers (Ramya et al., 2010). Moreover, the leaf ethanol extract isolated from *J. auriculatum* had an inhibitory effect on *B. subtilis*, *S. aureus*, *P. aeruginosa*, *E. coli* and *Micrococcus luteus* (Arun et al., 2016). Many pathogenic bacteria, including *E. coli*, *P. aeruginosa*, *S. aureus*, *B. cereus*, *S. epidermidis*, *V. cholorae*, *P. mirabilis*, *S. flexneri*, *S. enterica* and *K. pneumonia* have been reported to be inhibited by the leaf methanol extract of *J. syringifolium* (Kumar et al., 2014). The different extracts obtained from *J. nervosum* such as ethyl acetate, petroleum ether, methanol, ethanol and water displayed activity against *E. coli*, *P. aeruginosa*, *B. subtilis* and *S. aureus* (Ngan et al., 2008). In addition, the ethanol extracts of *J. grandiflorum* leaves were active against the various bacterial strains, including *E. faecalis*, *H. alvei*, *P. aeruginosa*, *P. vulgaris*, *P. shigelloides*, *S. epidermidis*, *S. aureus*, *S. saprophyticus*, *S. pyogenes*, *S. typhi*, *S. flexneri*, *S. sonnie*, *S. boydi* and *S. dysenteriae* (Ngan et al., 2008).

### Table 3: Inhibition zone of acetone extracts from *J. annamense* subsp. *annamense* leaves and branches

| Tested bacteria | Leaf extract | Branch extract | Gentamycin |
|-----------------|--------------|----------------|------------|
| *B. cereus*     | 9.5±1.3<sup>a</sup> | 8.7±0.8<sup>a</sup> | 18.2±0.8<sup>b</sup> |
| *E. coli*       | 8.2±0.3<sup>a</sup>  | 8.5±0.5<sup>a</sup> | 14.2±0.3<sup>b</sup> |
| *S. enteritidis*| 12.8±1.0<sup>a</sup> | 9.0±0.9<sup>a</sup> | 13.2±0.3<sup>b</sup> |
| *S. aureus*     | 12.2±0.8<sup>a</sup> | 8.3±0.3<sup>a</sup> | 16.8±0.8<sup>b</sup> |

Data are means of triplicates±standard deviations. a, b Different superscript lower-case letters in the same row denote significant differences (p<0.05).

### Figure 3: Radical scavenging activity of the acetone extract from *J. annamense* subsp. *annamense*. a. Leaf, b. Branch, c. Ascorbic acid
et al., 2010). In another study, the leaf ethanol extract of *J. auriculatum* possessed DPPH scavenging effect with an IC₅₀ value of 33.39 µg/mL and total phenolic content of 8.47 mg GAE/g (Arun et al., 2016). In addition, the antioxidant properties of the hydromethanolic and boiling water extracts of *J. grandiflorum* flower buds using different scavenging assays have been reported. As a result, the antioxidant activities of hydromethanolic extract evaluated by hydroxyl peroxide, nitric oxide, superoxide and DPPH had IC₅₀ values of 403.31, 225.51, 1354.30 and 189.93 µg/mL, respectively whereas 397.09, 38.27, 327.89 and 150.57 were as shown by the value of four assays towards the same extracts (Arun et al., 2016).

**CONCLUSION**

In this study, the chemical compositions, antibacterial and antioxidant properties of acetone extract from branches and leaves *Jasminum annamense* subsp. *annamense* were investigated for the first time. As a result, 24 compounds were reported from the leaf extract such as lup-20(29)-en-3-one (27.93%), levodopa (25.74%), lupeol (13.3%), cis-vaccenic acid (6.97%), glycerin (6.35%) and n-hexadecanoic acid (5.86%). The two studied extracts were found to be effective against *HeLa* cells.

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