Chemical Constituents and Bacterial Activity of Essential Oils of Five Wax Apples (*Syzygium samarangense*) from Dong Thap Province, Vietnam

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Summary

In the present study, essential oils from the leaves of five varieties of wax apple trees, ('An Phuoc', 'Hoa An', 'Hong Dao', 'Sua', and 'Xanh Duong') collected in Dong Thap Province, Vietnam were isolated using hydrodistillation, and their constituents were for the first time identified via gas chromatography - mass spectrometry. A total of 74 compounds from essential oils were identified. These compounds were classified into four clusters by hierarchical clustering analysis. The main constituents of the essential oils isolated from the leaves of five varieties of wax apple trees were o-cymene, α-cubebene, epizonarene, β-gurjunene, and α-selinene. The antibacterial activity of the essential oils isolated from the leaves of five varieties of wax apples were evaluated for the first time. The results showed that the essential oils could inhibit the growth of four tested microorganisms: *Bacillus cereus, Escherichia coli, Salmonella enteritidis* and *Staphylococcus aureus*.

Key words

wax apple, *Syzygium samarangense*, essential oil, antibacterial, Dong Thap Province

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Introduction

Syzygium genus (Myrtaceae) is a large genus with over 1,200 species. The *Syzygium* species are grown in subtropical and tropical regions of Africa and Madagascar, Asia and throughout Oceania and the Pacific region, especially in Southeast Asia and Australia (Srivastava and Chandra, 2013). Many previous studied showed the medicinal properties from species belonging to genus, including antibacterial, anticancerous activities, and also that they are nutritious and high antioxidant food (Peter et al., 2011; Khandaker and Boyce, 2016).

Wax apple (*Syzygium samarangense* /Blume/ Merr. & L.M.Perry) is originated from the Philippines and cultivated in temperate regions of Asia, especially in Southeast Asia. The wax apple fruit is used for food and contains phenols, flavonoids, and other antioxidant components, while the leaves contain many constituents, including glycosides, proanthocyanidins, anthocyanidins, ellagitannins, flavanones, flavonol, triterpenoids, chalcones, and volatile terpenoids. The extracts from flowers, seed, and leaves of this plant are active against many bacteria and fungi, including *Pseudomonas aeruginosa, Klebsiella pneumonia, Cryptococcus neoformans Staphylococcus aureus, Mycobacterium smegmatis* and *Candida albicans* (Peter et al., 2011; Khandaker and Boyce, 2016).

In Vietnam, wax apple is cultivated from northern to southern regions and the plant has a variety of qualities of fruits and different plant forms belonging to the environmental conditions of geographic regions. There are many wax apple varieties in Vietnam, including 'Sua', 'Xanh Duong', 'An Phuoc', 'Hong Dao', 'Dieu Do', 'Dao Hue', 'Huyet', 'Hoa An', etc. Varieties 'An Phuoc', 'Xanh Duong', 'Sua', 'Hong Dao' and 'Hoa An' are cultivated in a broad range of geographic regions of Dong Thap Province. To date, the chemical composition, antimicrobial and antioxidant activities of the compounds which are extracted from flowers, leaves and fruits of the wax apples have been shown by many recent studies (Reddy and Jose, 2011; Peter et al., 2011; Prasanna et al., 2015; Khandaker and Boyce, 2016). However, in Vietnam, the number of studies about this plant are limited and the bioactivity of this species has not been researched.

In this study, we first identified the chemical composition and antibacterial activity of essential oils from leaves of five popular varieties of wax apples ('An Phuoc', 'Xanh Duong', 'Sua', 'Hong Dao', 'Hoa An') in Cao Lanh City, Dong Thap Province, Vietnam, which will give more information to improve economic value of this plant.

Materials and methods

Materials

Plant material

Leaves of five wax apple trees were collected from Dong Thap Province (Figure 1) and the collected sites are presented in Table 1.

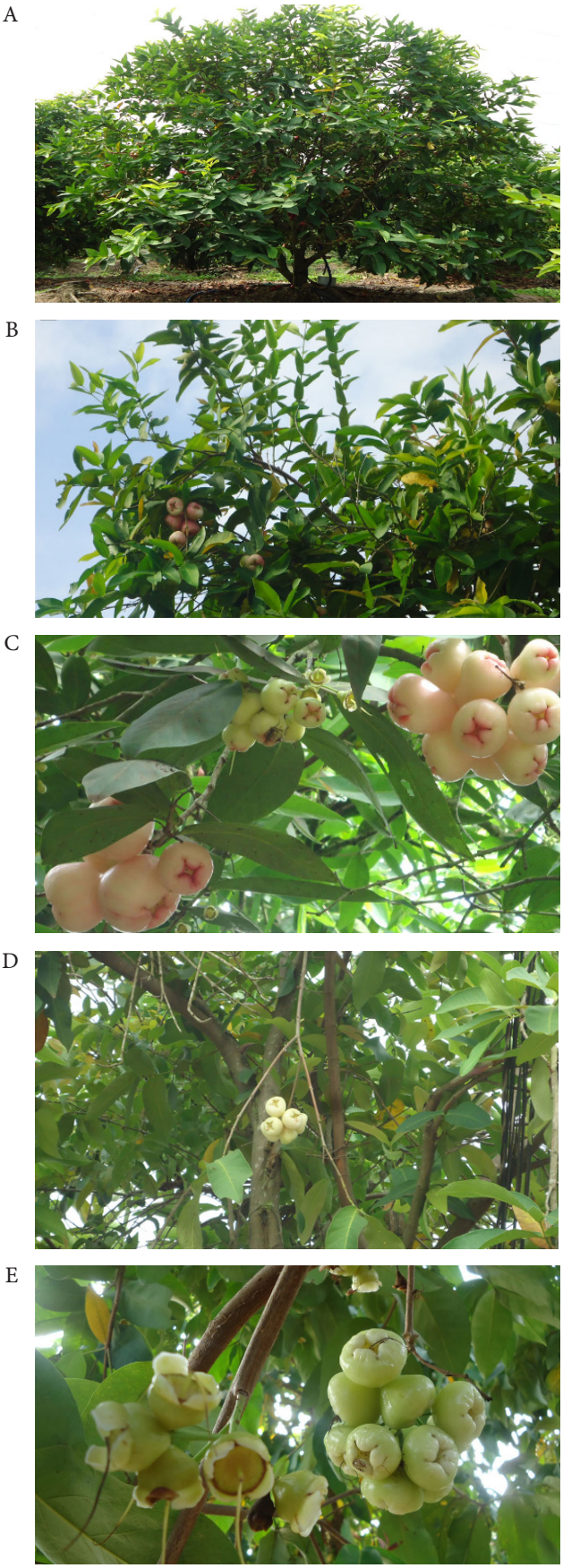

Figure 1. Five studied wax apple varieties in habitat. A - 'An Phuoc', B - 'Hong Dao', C - 'Hoa An', D - 'Sua', E - 'Xanh Duong'

Table 1. Detailed information of collected sites of five varieties of wax apple in Cao Lanh City, Dong Thap Province

Code of Samples	Names	Collected sites
AP	An Phuoc	Ward 1
HD	Hong Dao	Hoa Thuan ward
HA	Hoa An	Hoa An Commune
SU	Sua	Ward 11
XD	Xanh Duong	Tan Thuan Tay Commune

Bacterial strains

To determine the antibacterial activity of essential oils from leaves of five varieties of wax apple trees four bacterial strains were used: two Gram-positive (*Bacillus cereus* ATCC 11774, *Staphylococcus aureus* ATCC 25923), and two Gram-negative (*Escherichia coli* ATCC 25922, *Salmonella enteritidis* ATCC 13976). These bacterial strains were obtained from the microbiology collection, Department of Biotechnology, Institute of Food and Biotechnology, Industrial University of Ho Chi Minh City, Vietnam. All bacterial strains were maintained at -20°C in 20% glycerol solution and inoculated into Luria-Bertani (LB) broth at 37°C for 24 h to be re-activated again before using.

Methods

Distillation of the essential oils

Five hundred gram of the fresh leaves of five varieties of wax apple trees were pulverized and introduced into a 1.5 L flask; subsequently, distilled water was added until samples were completely submerged. Hydro-distillation was performed in a Clevenger type apparatus at 100°C for three hours with normal pressure. The essential oils were collected in the receiver arm of the apparatus and transferred into clean and dark bottles. The essential oils were dried by Na_2SO_4 and stored at 4°C (Olivia et al., 2014).

Gas chromatography/mass spectrometry (GC/MS) analysis

The chemical constituents of essential oils from the leaves of five varieties of wax apple trees were identified via the gas chromatography-mass spectrometry analysis on an Aglient GC 7890B-MS 5975C. An HP-5MS capillary column (30 m x 250 µm) coated with a 0.25 µm film was used for separation. Helium was used at the pressure of 13.209 psi as the carrier gas. The injected volume was 0.2 µL for each sample. The column temperature program was as follows: start at 50°C, then increase linearly to 320°C at 8°C min^{-1}. The retention indices of compounds were calculated using C8-C30 Alkanes Calibration Standard obtained from Sigma Co.

Antibacterial activities

The antibacterial assay was conducted using the method detailed described by Bauer et al. (1996). The bacteria were inoculated in LB Broth until a turbidity of 0.5 McFarland standards was reached. Subsequently, 100 µL bacterial suspensions was spread on sterile Mueller Hinton plate and a sterile 6 mm diameter discs were put on the inoculated surface. The sample of 20 µL was added onto each disc and the plates were maintained at 4°C for 2 h to allow extract diffusion into the medium. The plates were kept at 37°C for 24h and the antibacterial activity of sample was determined via the inhibition zone diameter of tested bacteria. Sterilized distilled water was used as negative control and Gentamycin antibiotic discs (supplied by Nam Khoa BioTek, Vietnam) were used as positive control.

Data analysis

Agglomerative Hierarchical Clustering (AHC) was performed to classify the essential oils isolated from the leaves of five varieties of wax apple trees into clusters according to their similarities in the concentration of compounds identified by GC/MS. Principal Component Analysis (PCA) was performed on the concentrations of compounds using covariance matrix to identify the main constituents of each of the five essential oils to reveal the differences among them. The analyses were conducted using XLSTAT Sensory (Addinsoft, Boston, USA). The experiments of antibacterial activities were repeated in triplicate. Excel 2010 was utilized to calculate the average and standard deviation of measurements. The results obtained from experiments were expressed in the form of a mean ± standard deviation (SD).

Results and discussion

Essential oil composition

Table 2 presents the chemical compositions of essential oils from the leaves of five varieties of wax apple trees ('An Phuoc', 'Hoa An', 'Hong Dao', 'Sua', and 'Xanh Duong'). A total of 74 chemical constituents have been identified. Figure 2 showes the results derived from AHC and PCA. The chemical compositions of essential oils was divided into four clusters: Cluster I contained 'Xanh Duong' with the high presence of α-selinene, α-eudesmol, caryophyllene, and caryophyllene oxide; Cluster II contained 'Hoa An' with α-cubebene and γ-terpinene; Cluster III contained 'An Phuoc' with the high presence of α-pinene, o-cymene, and caryophyllene; and Cluster IV contained 'Hong Dao' and 'Sua' with caryophyllene, β-gurjunene, β-elemene, pizonarene, γ-terpinene, prehnitene, 4-carene, β-cutjunene, of which the last four constituents were found in both 'Hong Dao' and 'Sua'.

As aforesaid in the Introduction, chemical composition from the leaves of wax apple have been shown by recent studies (Reddy and Jose, 2011; Prasanna et al., 2015). However, chemical constituents of this species is the still unknown in Vietnam. The concentrations of chemical compositions of plant essential oils are found to vary depending on the geographical regions where they are cultivated (Hassiotis et al., 2010; Devkota et al., 2013). The chemical constituents from the leaf oils of the five varieties of wax apple trees were quite similar to those from previous studies. However, there was a significant difference in their concentrations as compared to those in previous studies. According to Reddy and Jose (2011), the essential oil of wax apple leaf collected from Thrissur district of Kerala, South India contained β-selinene (11.61%),

Table 2. Chemical compositions of the essential oils from the leaves of five varieties of wax apples from Dong Thap Province

Compounds	RT	Code	The amount (%)				
			AP	HA	HD	SU	XD
α-thujene	9.49	C1	3.95	3.87	-	4.9	-
α-pinene	9.59	C2	14.02	4.25	0.63	0.11	-
β-phellandrene	9.94	C3	-	0.78	-	-	-
β-pinene	10.04	C4	-	-	-	2.27	-
β-myrcene	10.05	C5	-	-	0.7	1.21	-
α-phellandrene	10.28	C6	-	2.72	5.5	-	-
Sabinen	10.31	C7	-	1.87	0.17	-	-
p-menthatriene	10.46	C8	-	-	4.91	-	-
m-xylene	10.48	C9	-	-	-	4.18	-
β-cymene	10.49	C10	-	5.61	-	-	-
o-cymene	10.50	C11	13.47	2.11	-	-	-
Prehnitene	10.55	C12	-	-	4.53	3.44	-
γ-terpinene	10.96	C13	7.42	8.74	7.65	6.49	0.77
terpinolene	11.09	C14	-	-	-	2.26	-
4-carene	11.33	C15	6.44	3.77	4.52	1.83	-
α-terpinene	11.35	C16	-	0.88	-	2.30	-
3-menthene	11.56	C17	-	0.95	-	-	-
3-carene	11.71	C18	1.58	-	-	-	-
Terpinene-4-ol	11.95	C19	3.73	3.62	0.84	-	1.41
Methyl citronellate	12.27	C20	-	-	-	1.98	-
9-octadecen-1-ol, (Z)-	12.39	C21	-	0.43	-	-	-
2-tridecyne	12.46	C22	0.86	-	-	-	-
8-tetradecyn-ol acetate	14.49	C23	-	-	0.42	-	-
Dehydroterpin monoacetate	12.53	C24	-	1.80	-	-	-
3-methylenecycloheptene	12.77	C25	0.52	-	-	-	-
2-pentenoic acid, 2-methyl-, (E) -	12.79	C26	-	-	-	0.89	-
1H-imidazole, 2-ethyl-4,5-dihydro-4-methyl-	13.04	C27	2.74	-	-	-	-
γ-elemene	13.05	C28	-	-	4.75	-	-
1,6,10-dodecatrien-3-ol, 3,7,11- trimethyl -	13.06	C29	-	-	-	2.47	-
4-hexen-1-ol, trifluoroacetate	13.18	C30	0.75	-	-	-	-
Copaene	13.30	C31	2.64	-	-	-	0.99

Compounds	RT	Code	The amount (%)				
			AP	HA	HD	SU	XD
β-elemene	13.49	C32	-	-	8.24	-	0.63
α-cubebene	13.58	C33	-	21.49	-	-	-
Longifolene	13.61	C34		-	9.37	-	-
Caryophyllene	13.65	C35	13.27	-	-	9.32	19.82
α-cadinene	14.02	C36	2.97	-	-	-	-
α-selinene	14.08	C37	-	-	-	-	20.11
γ-muurolene	14.10	C38	-	5.62	-	-	-
Epizonarene	14.13	C39	-	-	13.10	-	-
Humulene	14.15	C40	-	-	-	2.33	1.15
α-gurjunene	14.19	C41	3.24	2.60	-	-	5.66
α-copapene	14.32	C42	5.09	-	-	-	-
δ-cadinene	14.41	C43	-	-	-	1.96	4.79
Elemol	14.63	C44	-	2.14	-	6.18	-
Caryophyllene oxide	14.68	C45	-	-	-	-	15.02
γ-neoclovene	14.70	C46	-	1.92	-	-	-
γ-cadinene	14.72	C47	-	-	4.34	-	3.72
β-vatirenene	14.79	C48	-	-	1.49	-	-
Estran-3-one, 17-(acetyloxy)-2-methyl-, (2α, 5α, 17β)	14.83	C49	-	3.29	-	-	-
β-gurjunene	14.88	C50	-	-	4.93	10.73	-
Aromandendrene	15.03	C51	-	1.80	4.87	-	-
γ-eudesmol	15.06	C52	-		-	3.00	-
Cubenol	15.09	C53	-	2.81	-	-	-
α-eudesmol	15.11	C54	-	-	-	-	19.65
Bicyclo [4.4.0] dec-1-ene, 2- isopropyl-5-methyl-9-methylene-	15.14	C55	5.61	-	-	-	-
γ-maaliene	15.20	C56	-	-	-	4.76	-
Trans-farnesol	15.25	C57	-	-	-	-	2.97
α-cadinol	15.29	C58	4.62	-	-	-	-
α-muurolene	15.31	C59	-	1.81	-	-	-
Patchoulene	15.34	C60		-	4.07	-	-
Farnesal	15.56	C61	-	5.33	-	2.99	-
2-butenoic acid, 3-(phenylamino)-, ethyl ester	15.61	C62	-	-	3.66	-	-
Methyl farnesate	15.67	C63	-	-	-	1.9	-

Compounds	RT	Code	The amount (%)				
			AP	HA	HD	SU	XD
caparratriene	15.69	C64	-	0.92	-	-	-
γ-gurjunene	15.70	C65	0.17	-	3.27	-	-
Phthalic acid, ethyl octadecyl ester	15.79	C66	-	-	1.22	-	-
Alloaromadendrene	15.81	C67	0.24	-	-	4.92	-
Isoaromadendrene epoxide	15.91	C68	-	2.24	-	-	-
Cyperene	15.94	C69	-	-	1.74	-	-
γ-sitosterol	15.99	C70	0,24	-	-	-	-
Lanosterol	16.10	C71	-	0.86	-	-	-
Longifolenaldehyde	16.14	C72	-	-	-	1.39	-
Isoaromadendrene epoxide	16.25	C73	0.81	0.89	-	-	-
Phytol	17.24	C74	0.31	0.53	1.26	1.67	-
TOTAL			94.45	95.65	96.18	85.48	96.69

Note: AP-"An Phuoc", HA-"Hoa An", HD-"Hong Dao", SU-"Sua", XD-"Xanh Duong"

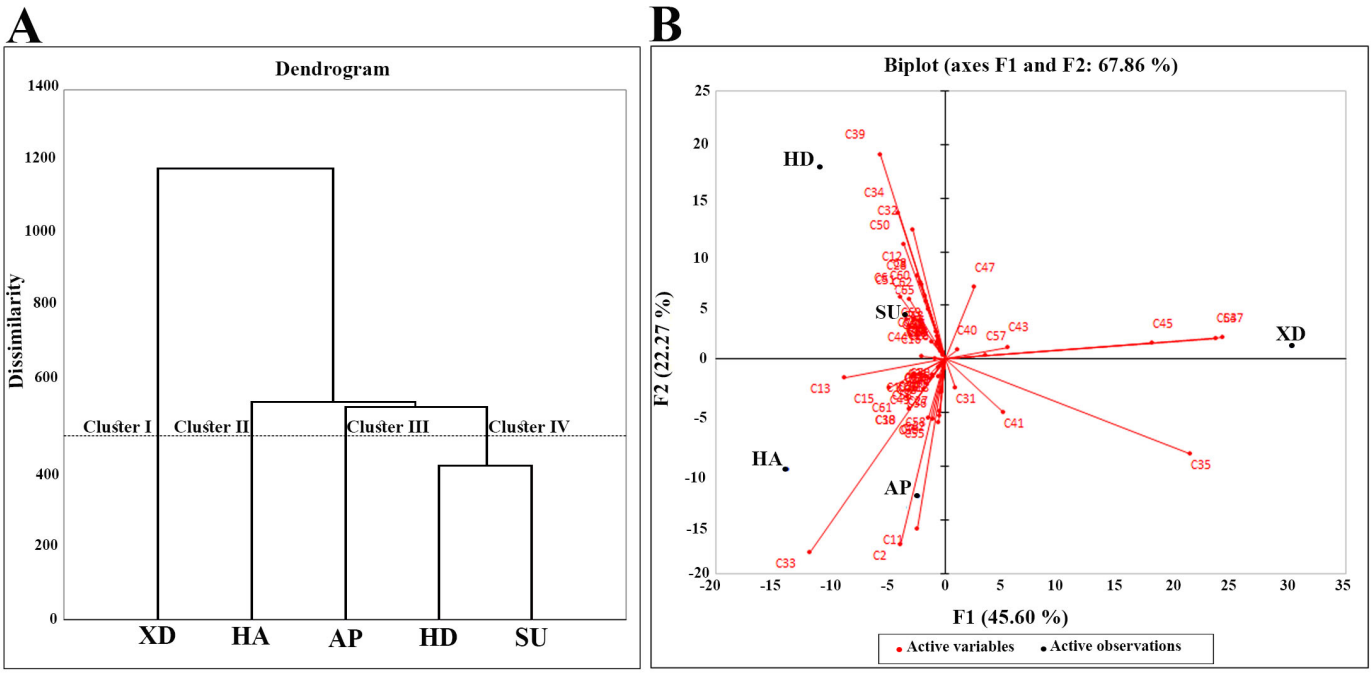

Figure 2. Comparison of chemical composition of essential oils of five varieties of wax apples. A – the AHC tree diagram presents the four clusters of the essential oils: Cluster I (XD), Cluster II (HA), Cluster III (AP), Cluster IV (HD and SU); B – PCA biplot presents the main compound constituents of the five varieties of wax apples: 'An Phuoc' (AP), 'Hong Dao' (HD), 'Hoa An' (HA), 'Sua' (SU), 'Xanh Duong' (XD)

α-selinene (11.40%), γ-terpinene (10.68%), β-aryophyllene (10.20%), β-gurjunene (9.48%), α-thujene (7.40%) and terpinene-4-ol (6.80%). In this study, γ-terpinene was found in 'An Phuoc', 'Hoa An', 'Hong Dao', 'Sua' and 'Xanh Duong' in concentrations of 7.42%, 8.74%, 7.65%, 6.49%, and 0.77%, respectively. 'An Phuoc', 'Hoa An', 'Hong Dao', and 'Xanh Duong' contained terpinene-4-ol in concentrations of 3.62%, 3.73%, 0.84%, 1.41%, respectively. Furthermore, α-thujene was found in 'An Phuoc' (3.95%), 'Hoa An' wax apple (3.87%) and 'Sua' (4.9%). The essential oil of 'Sua' and 'Hong Dao' wax apple leaf had β-gurjunene with percentage of 10.37% and 4.93%, respectively, while α-selinene (20.11%) was only found in 'Xanh Duong'. In this study, β-Selinene and β-aryophyllene were not identified in the essential oils of five varieties of wax apple trees. Prasanna et al. (2015) suggested that viridiflorol (15.05%), α-cubebene (7.71%), β-pinene (11.64%), α-pinene (9.61%), and α-terpineol (5.19%) were the main constituents of the essential oil of wax apple leaf which collected from Hyderabad, India. 'An Phuoc', 'Hoa An', 'Hong Dao', and 'Sua' had α-pinene in concentrations of 14.02%, 4.25%, 0.63%, and 0.11%, respectively. α-Cubebene (21.49%) and β-pinene (2.27%) were only found in 'Hoa An' and 'Sua', respectively. Viridiflorol and α-terpineol were not present in five studied varieties.

The bioactivities of some main compounds identified in the essential oils isolated from the five varieties in this study have been documented in previous studies. Fidyt et al. (2016) showed that caryophyllene, one of the main constituents of the essential oils of 'An Phuoc', 'Sua', and 'Xanh Duong', not only regulates the growth and proliferation of several types of cancer cells but also reinforces the efficacy of classical chemotherapeutics. Antioxidant and antiproliferative properties of cymene and γ-terpinene, two compound identified in the essential oils of five varieties of wax apple trees in this study, against a panel of human cancer cell lines were also investigated by Fitsiou et al. (2016). Anticancer effect of α-humulene, a compound identified in the essential oils of 'Sua' and 'Xanh Duong', toward a broad spectrum of cancer cell lines, such as MCF-7, PC3, A-549, DLD-1, M4BEU, and CT26 cells, has been reported (Legault et al., 2003). Bombarda et al. (2001) suggested that aromandendrene, a constituent of the essential oils of 'Hoa An' and 'Hong Dao', had a strong antioxidant activity. Recently, Alakanse et al. (2018) demonstrated that α-selinene from *Syzygium aqueum*, the main constituent of the essential oil of 'Xanh Duong', was effective against aromatase p450 in breast carcinoma of postmenopausal women. The presence of these bioactive compounds suggests the further application of these essential oils in medicine and pharmaceutical industry.

Antibacterial activity

Data stated in Table 3 and Figure 3 showed that the essential oils from the leaves of five varieties of wax apples were evaluated by the diameter of the growth of inhibition zone of tested bacteria: *S. aureus, B. cereus, S. enteritidis* and *E. coli*. Accordingly, the essential oil from the leaves of 'Sua' was able to resist against four studied bacteria, while the essential oils from 'An Phuoc', 'Hoa An', 'Hong Dao', and 'Xanh Duong' could inhibit the growth of two tested bacteria. The essential oil of 'An Phuoc' exhibited strong antibacterial activity against *E. coli* (20.2 ± 0.7 mm). The best antibacterial activity of 'Hoa An' essential oil was shown with zones of inhibition for *S. aureus* (17.2±1.2 mm).

Table 3. The inhibition zone of essential oils from the leaves of five varieties of wax apples from Dong Thap Province

Tested bacteria	Growth inhibition zone (mm)				
	AP	HA	HD	SU	XD
S. aureus	-	17.2±1.2	11.2±1.0	8.0±0.4	9.2±0.3
B. cereus	9.0±0.5	-	14.5±0.5	11.3±1.2	16.3±1.0
S. enteritidis	-	-	-	7.3±0.6	-
E. coli	20.2±0.7	17.0±1.5	-	8.7±0.8	-

Note: AP-"An Phuoc", HA-"Hoa An", HD-"Hong Dao", SU-"Sua", XD-"Xanh Duong"

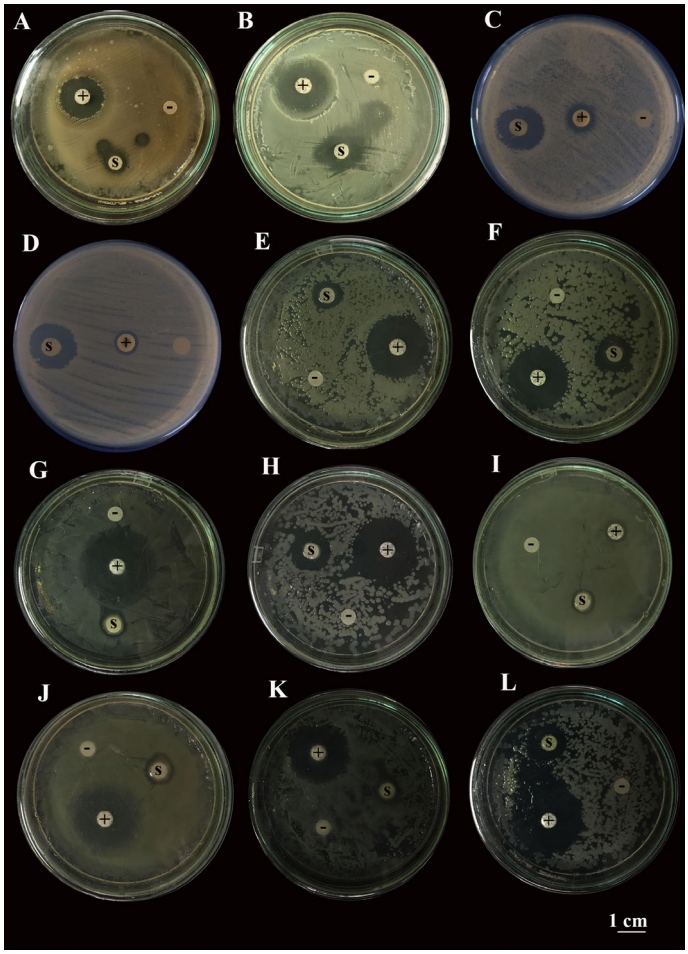

Figure 3. Antibacterial activity of essential oils from five wax apples against test bacteria. A – *B. cereus* (An Phuoc wax apple), B – *E. coli* (An Phuoc wax apple), C – *S. aureus* (Hoa An wax apple). D – *E. coli* (Hoa An wax apple), E – *S. aureus* (Hong Dao wax apple), F – *B. cereus* (Hong Dao wax apple), G – *S. aureus* (Sua wax apple), H – *B. cereus* (Sua wax apple), I – *S. enteritidis* (Sua wax apple), J – *E. coli* (Sua wax apple), K – *S. aureus* (Xanh Duong wax apple), L – *B. cereus* (Xanh Duong wax apple). (-) Negative control with sterilized distilled water, (+) Positive control with discs containing gentamicin, (S) Sample of essential oil from the leaves.

The essential oil of 'Hong Dao', 'Sua', and 'Xanh Duong' could strongly inhibit the growth of *B. cereus* (14.5 ± 0.5 mm,11.3±1.2 mm, 16.3 ±1.0 mm, respectively).

Some certain previous studies showed the antimicrobial activity of essential oils from the wax apples. For instance, Reddy and Jose (2011) demonstrated that the essential oils from the leaves of wax apple collected from Thrissur district of Kerala, South India could inhibit the growth of various pathogenic bacteria, such as *Bacillus cereus, Enterobacter faecalis, Salmonella typhi, Staphylococcus aureus, Escherichia coli, Proteus vulgaris, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Serratia marcescen*. In another study, Prasanna et al. (2015) proved the essential oil from the leaves of wax apple collected from Hyderabad, India was able to resist against *Escherichia coli* and *Candida rugose*. Aromadendrene, β-pinene, terpinen-4-ol, γ-terpinene, and cymene have antibacterial properties. For instance, aromandendrene isolated from essential oil of some species of *Eucalyptus genus* showed antibacterial effect against many pathogenic bacteria such as *Staphylococcus aureus*, *Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa* and *Acinetobacter* (Bombarda et al., 2001; Mulyaningsih et al., 2010 and 2011). Carson and Riley (1995) suggested that terpinen-4-ol, γ-terpinene, and cymene in essential oil of *Melaleuca alternifolia* were active against *Bacillus subtilis, Bacteroides fragilis, Candida albicans, Clostridium perfringens, Enterococcus faecalis, Escherichia coli, Lactobacillus acidophilus, Moraxella catarrhalis, Mycobacterium smegmatis, Pseudomonas aeruginosa, Serratia marcescens* and *Staphylococcus aureus*. Furthermore, Leite et al. (2007) also proved that β-pinene could inhibit the growth of Gram positive bacteria that belonge to *Streptococcus* and *Staphylococcus* genuses including *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyrogenes* and *Streptococcus pneumonia*.

Conclusion

The present study identified a total of 74 compounds of the essential oils from the leaves of five varieties of wax apple trees: 'An Phuoc', 'Hoa An', 'Hong Dao', 'Sua', and 'Xanh Duong' collected in Dong Thap Province, Vietnam. The results suggested that the concentrations of the main constituents of the essential oils of the five studied varieties were a variety and significantly different from what was reported in previous studies. The main constitutes of essential oils were: o-cymene (13.47% - 'An Phuoc'), α-cubebene (21.49% - 'Hoa An'), epizonarene (13.10% - 'Hong Dao'), β-gurjunene (10.73% - 'Sua'), and α-selinene (20.11% - 'Xanh Duong'). The essential oils from the leaves of five varieties of wax apple trees in this study showed the antibacterial effect against four tested microorganisms: *B. cereus, E. coli, S. enteritidis* and *S. aureus*.

References

Alakanse O.S., Sulaiman F.A., Arannilewa A.J., Anjorin F.F., Malachi O.I., Ayo I.E., Oladejo A.A. et al. (2018). α-selinene from *Syzygium aqueum* against aromatase p450 in breast carcinoma of postmenopausal women: in silico study. J. Biomed Pharm Sci. 2: 1-7.

Bauer A.W., Kirby W.M., Sherris J.C., Turck M. (1996). Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. 45: 493-496.

Bombarda I., Raharivelomanana P., Ramanoelina P.A.R., Faure R., Bianchini J.P. (2001). Spectrometric identifications of sesquiterpene alcohols from niaouli (*Melaleuca quinquenervia*) essential oil. Analytica Chimica Acta 447: 113-123.

Carson C.F., Riley T.V. (1995). Antimicrobial activity of the major components of the essential oiI of *Melaleuca alternifolia*. Journal of Applied Bacteriology 78: 264-269.

Devkota A., Stefano D.A., Stefano C., Gabriella I., Pramod K.J. (2013). Chemical composition of essential oils of *Centella asiatica* (L.) Urban from different habitats of Nepal. International journal of pharmaceutical & Biological archives 4: 300-304.

Fidyt K., Fiedorowicz A., Leon SD., Szumny A. (2016). βcaryophyllene and β-caryophyllene oxide natural compounds of anticancer and analgesic properties. Cancer Medicine 5: 3007-3017.

Fitsiou E., Anestopoulos I., Chlichlia K., Galanis I.K., Panayiotidis M.I., Pappa A. (2016). Antioxidant and antiproliferative properties of the essential oils of Satureja thymbra and Satureja parnassica and their major constituents. Anticancer research 36: 5757-5764.

Hassiotis C.N., Lazari D.M., Vlachonasios K.E. (2010). The effects of habitat type and diurnal harvest on essential oil yield and composition of *Lavandula angustifolia* Mill. Fresenius Environmental Bulletin 19: 1491-1498.

Khandaker M.M., Boyce A.N. (2016). Growth, distribution and physiochemical properties of wax apple (*Syzygium samarangense*): A review. Austrailan Journal of Crop Science: 1640-1647. doi: 10.21475/ajcs.2016.10.12.PNE306.

Legault J., Dahl W., Debiton E., Pichette A., Madelmont J.C. (2003). Antitumor activity of Balsam Fir oil: production of reactive oxygen species incluced by α-Humulene as possioble mechanism of action. Plana. Med. 69: 402-407.

Leite A.M, Lima E.O, Souza E.L, Diniz M.F.F.M., Trajano V.N. (2007). Inhibitory effect of β-pinene, α-pinene and eugenol on the growth of potential infectious endocarditis causing Gram-positive bacteria. Rev. Bras. Cienc. Farm. 43: 121-126.

Mulyaningsiha S., Sporera F., Zimmermannb S., Reichlinga J., Winka M. (2010). Synergistic properties of the terpenoids aromadendrene and 1,8-cineole from the essential oil of *Eucalyptus globulus* again stantibiotic-susceptible and antibiotic-resistant pathogens. Phytomedicine 17: 1061-1066.

Mulyaningsih S., Sporer F., Reichling J., Wink M. (2011). Antibacterial activity of essential oils from *Eucalyptus* and of selected components against multidrug-resistant bacterial pathogens, Pharmaceutical Biology 49: 893-899.

Olivia B.C., Claudio H.S.D.M., Luiz, E.C.B., Ines S.R., Vieira R.F., Humberto R.B. (2014). Essential Oil Constituents and Yields from Leaves of *Blepharocalyx salicifolius* (Kunt) O. Berg and *Myracrodruon urundeuva* (Allemão) Collected during Daytime. International Journal of Forestry Research. https://doi.org/10.1155/2014/982576.

Peter T.D., Padmavathi1 R., Jasmin S., Sarala A. (2011). *Syzygium samarangense*: a review on morphology, phytochemistry & pharmacological aspects. Asian Journal of Biochemical and Pharmaceutical Research 41: 155-163.

Prasanna A.R.L, Venkata R.K., Bhakshu M.D.L., Veeranjaneya R.L., Narasimha R.B. (2015). Investigation of chemical and pharmacological properties of essential oils from two *Syzygium* species of Andhra Pradesh, India. Int. J. Pharm. Pharm. Sci. 7: 375-380.

Reddy L.J., Jose B. (2011). Chemical composition and antibacterial activity of the volatile oil from the leaf of *Syzygium samarangense* (Blume) Merr. & L.M. Perry. Asian Journal of Biochemical and Pharmaceutical Research 3: 263- 269.

Srivastava S., Chandra D. (2013). Pharmacological potentials of *Syzygium cumini*: a review. Journal of the Science of Food and Agriculture 93: 2084-2093.

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