BIODIVERSITAS
Volume 24, Number 5, May 2023
Pages: 2723-2732

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d240526

Phytochemical analysis of ethanolic *Psidium guajava* leaves extract using GC-MS and LC-MS

RISA NURSANTY[1,2], KHAIRUL NAIM BIN MD PADZIL[1], NUR ISTI'ANAH BINTI RAMLI[1], NOR AINY MAHYUDIN[1], AHMAD HANIFF BIN JAAFAR[1], YAYA RUKAYADI[1,3,♥]

[1]Department of Food Science, Faculty of Food Sciences and Technology, Universiti Putra Malaysia. 43400 UPM Serdang, Selangor, Malaysia
[2]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala. Jl. Syech Abdurrauf No. 3, Kopelma Darussalam, Syiah Kuala, Banda Aceh 23111, Aceh, Indonesia
[3]Natural Medicines and Products Research Laboratory (NaturMeds), Institute of Bioscience, University Putra Malaysia. 43400 UPM Serdang, Selangor, Malaysia. Tel.: +60-3-9769-3045, Fax.: +60-3-9769-2101, ♥email: yaya_rukayadi@upm.edu.my

Manuscript received: 4 April 2023. Revision accepted: 19 May 2023.

Abstract. *Nursanty R, Padzil KNBM, Ramli NIB, Mahyudin NA, Jaafar AHB, Rukayadi Y. 2023. Phytochemical analysis of ethanolic* Psidium guajava *leaves extract using GC-MS and LC-MS. Biodiversitas 24: 2723-2732. Psidium guajava* L. is well known as guava and has been used traditionally as a medicinal plant for a variety of ailments all over the world, especially in infusions and decoctions for oral and topical use. Every part of this plant has been used for treating stomachache and diarrhea in many countries. Other reported uses include dermatitis, sores, epilepsy, and wounds. Medicinal plants have an important role in complementary and alternative medicine because they produce various natural compounds with high therapeutic properties. Many researchers report that the major chemical composition of this plant includes tannins, phenols, flavonoids, saponins, carbohydrates, alkaloids, sterols, terpenoids, and phenolic compounds. The aim of this study is to identify the volatile and non-volatile compounds in ethanolic *P. guajava* leaves extract. This study aimed to identify phytochemical compounds in *P. guajava* leaves extract. The main volatile compounds identified using GC-MS were pyrogallol, α-copaene, caryophyllene, aromadendrene, α-humulene, alloaromadendrene, γ-muurolene, β-selinene, α-selinene, α-muurolene, β-bisabolene, β-bisabolene, (-) globulol, caryophyllene oxide, α-muurolol, and epiglobulol. Based on LC-MS analysis, a number of non-volatile compounds such as flavonoids; apigenin 7-O-β-D-glucuronopyranoside, quercetin, luteolin 7-O-glucuronide, naringenin 4′-O-glucopyranoside, and myrecitin along with phenolic derivatives such as quinic acid, catechin, and epigallocatechin (4β,8)-gallocatechin were tentatively identified.

Keywords: GC-MS, LC-MS, leaves, phytochemical, *Psidium guajava*

INTRODUCTION

Medicinal plants are a source of phytochemical active compounds due to their diversity. These plants represent more than 25,000 species of plants used in the world for pharmaceutical, cosmeceutical, food industry and agriculture. In traditional medicine, crude extracts of different parts of medicinal plants, including the seed, fruit, flower, leaves, root, and twigs, were largely used to treat certain pathogen-related diseases (Devi et al. 2018). Ethnopharmacological phytochemical research is thought to be an effective approach for discovering novel chemical entities with potential as natural antimicrobial (Devi et al. 2018; Daoutidou et al. 2021).

Recently, the use of plant-derived natural products as treatment strategies has gained prominence, and this is mainly due to the fight against the secondary effects of synthetic medicine. Medicinal plants possess an important role in the treatment of complementary strategies and alternative forms of medicine because they produce a large variety of natural compounds with high therapeutic properties. Several plant species have been tested for their antimicrobial activity, but the vast majority of these plants have not been adequately investigated (Brusotti et al. 2014; Devi et al. 2018).

Psidium guajava L. belongs to the Myrtaceae family. *Psidium* is a genus with nearly 150 species that include both tree and shrub forms. The plant is well known by its common name, guava or apple guava. The word guava occurs from Spanish *guaya*, which is a variant of the Arawakan (West Indies) language *guayabo* (Sandoval and Rodríguez 2013; Harper 2020). *Psidium guajava* is a native plant from central and latin America. Currently, it is widespread in tropical and subtropical countries (Kamath et al. 2008; Morais-Braga et al. 2016). The extensively *P. guajava*-producing countries are India, China, Thailand, Pakistan, Mexico, Indonesia, Brazil, Bangladesh, the Philippines, and Nigeria. The major producers of commercial *P. guajava* cultivars are India, regularly producing more than 17,000,000 metric tons (Jegede 2019).

The trunk, leaves, fruits, and seeds of this plant have all been used to treat health problems. Traditionally, *P. guajava* has been used for the treatment of any disease and has been investigated in different countries. Kamath et al. (2008) report that in Malaysia and the Philippines, this plant was used to treat dermatitis, diarrhea, epilepsy, hysteria, sores, menstrual disorders, and wounds. *Psidium guajava* was also used for antidiarrhea and odor absorption by Thai people, according to Nguanchoo et al. (2019). In Vietnam (Lee et al. 2019), Indonesia (Hidayat et al. 2010; Haruna et al. 2022), and Brunei Darussalam, this plant is commonly used to treat diarrhea or stomachaches (Chai

2000). *Psidium guajava* is widely used in Mexico to treat diarrhea, dysentery, respiratory problems, wounds, ulcers, rheumatism, toothaches, and fever. A water-leaf extract is used to lower blood glucose levels in diabetics. This hot tea was very popular among Veracruz residents (Guti´errez et al. 2008; Morais-Braga et al. 2016).

Several studies reported (Rajan et al. 2015; Katewaraphorn et al. 2016; Mendez et al. 2016; Wang et al. 2018) that the leaves of *P. guajava* potential as antimicrobial. Meanwhile, Barbalho et al. (2012) reported that *P. guajava* has metabolite compounds, i.e., flavonoids, tannins, phenolic, sesquiterpene alcohols, triterpenoid acids, and essential oils. It is well known that phytochemical compounds are the critical points of the general mechanism of antimicrobial activity. However, information about the phytochemical compounds of these plants is still lacking.

Gas Chromatography-Mass Spectrometry (GC-MS) and Liquid Chromatography-Mass Spectrometry (LC-MS) have become popular techniques for the identification of volatile and non-volatile compounds in plant species. Numerous previous investigations discovered phytochemical constituents of plants using the GC-MS and LC-MS method. As an example, secondary metabolite of *Luvunga sarmentosa* (Blume) Kurz root from Kalimantan, Indonesia (Syarpin et al. 2023), *Scolymus maculatus* L. phytochemical profile extracted from methanol, ethyl acetate, and n-hexane solvents (Abu-Lafi et al. 2019), phytochemical composition of *Myriactis nepalensis* Less. (Compositae) essential oil (Fu et al. 2022), and quinic acid derivatives of methanolic *Carissa spinarum* L. (Apocynaceae) root extract (Liu et al. 2021). The aim of this study is to identify volatile and non-volatile compounds in the ethanolic *P. guajava* leaves extract that might have contributed to its antimicrobial activity using GC-MS and LC-MS.

MATERIALS AND METHODS

Sample preparation and extraction

Psidium guajava leaves were collected from Taman Pertanian, Universiti Putra Malaysia, Serdang, Selangor, Malaysia (Figure 1) (QGIS.org, %Y 2023). The leaves were washed under running tap water. Next, the leaves samples were oven-dried at 40°C for 24 hours and kept in sealed plastic bags at room temperature away from light before further processing. The *P. guajava* leaves extraction method was referred to Rukayadi et al. (2008). Dried *P. guajava* leaves were ground into a coarse powder by using a dry blender (Panasonic Corporation, Osaka, Japan), then extracted by soaking in 99.8% ethanol (ratio 1:4) for 24 hours at room temperature. Next, the extract was vacuum-filtered through Whatman filter paper No. 2 (Whatman International Ltd., Middlesex, England). The filtrate was then concentrated by using a rotary vacuum evaporator (BUCHI Rotavapor R-200 Switzerland) at 40°C.

Determination of phytochemical compounds in *Psidium guajava* using GC-MS

The GC-MS analysis of ethanolic extracts of *P. guajava* leaves was performed using a QP2010 Ultra gas chromatograph-mass spectrometer (Shimadzu Corporation, Kyoto, Japan) equipped with a BP-5MS column (30.0 m long, 0.25 mm ID, and film thickness 0.25 µm). Helium was used as the carrier gas, with a flow rate of 0.8 mL/min. The oven temperature was programmed as 50°C to 300°C, at an increase rate at 3°C min and hold for 10 min. The injection temperature and the ion-source temperature were 250°C and 200°C, respectively. The peaks were analyzed by comparing their retention times and mass fragment patterns with standard spectra available in the Shimadzu GC-MS National Institute of Standards and Technology (NIST)/Wiley library.

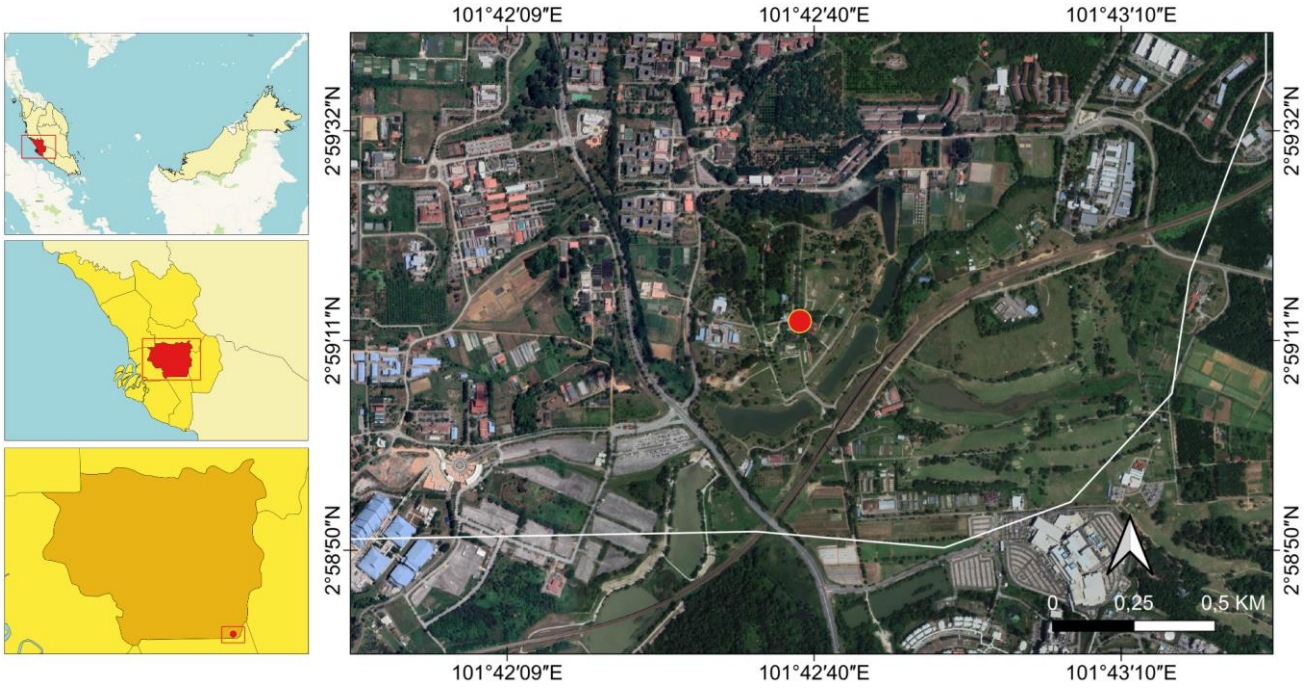

Figure 1. Location of plant collection at Taman Pertanian, Universiti Putra Malaysia, Serdang, Selangor, Malaysia

Determination of phytochemical compounds in *Psidium guajava* using LC-MS

UHPLC was performed on ACQUITY UPLC I-Class system and coupled to a Vion IMS QTOF hybrid mass spectrometer (Waters Corp), consisting of a binary pump, a vacuum degasser, an auto- sampler and a column oven. Ethanolic compounds were chromatographically separated using a column ACQUITY UPLC HSS T3 (100 mm × 2.1 mm × 1.8 µm) also from Waters Corp, maintained at 40°C. A linear binary gradient was carried out using water containing 0.1% formic acid as mobile phase A and acetonitrile as mobile phase B. The mobile phase composition was varied as follows: 0-0.5 min (1% B), then 16.00 min (35% B) followed at 18.00 min (100% B), and 20.00 min (1% B). The injection volume was 1 µL, and the flow rate was set to 0.6 mL/min.

A Vion IMS QTOF hybrid mass spectrometer (Waters Corp) equipped with a Lock Spray ion source. The ion source was operated in positive and negative ionization mode (ESI). Mass spectra were acquired in both positive and negative modes over the mass range m/z 50-1500 at 0.1 s/scan. MS parameters were: capillary voltage, 1.50 kV; reference capillary voltage, 3.00 kV; source temperature, 120°C; desolvation gas temperature, 550°C; desolvation gas flow, 800 L/h, and cone gas flow, 50 L/h. Nitrogen (>99.5%) was employed as desolvation and cone gas.

RESULTS AND DISCUSSION

Determination of phytochemical compounds in *Psidium guajava* using GC-MS

As shown in Figure 1, the GC-MS spectrum confirmed the presence of various components with varying retention times. Individual compounds were identified by comparing of their mass-spectral database with Wiley229, and National Institute of Standards and Technology (NIST11) libraries, ensuring the registered spectra with more than 80% of Similarity Index (SI), and by comparing them with the values published in the literature. Results of the GC-MS analysis showed that a variety of constituents was presented in the active ethanolic of *P. guajava* leaves extract. The active principles with their formula, molecular weight (g/mol), Retention Time (RT), Retention Index (RI), Similarity Index (SI), compound and their biological activity are presented in Table 1.

There were fifteen major compounds identified from the ethanolic *P. guajava* leaves extract chromatogram i.e., pyrogallol, α-copaene, caryophyllene, aromadendrene, α-humulene**,** alloaromadendrene, γ-muurolene, β-selinene, α-selinene, α-muurolene**,** β-bisabolene, (-) globulol, caryophyllene oxide, α-muurolol, and epiglobulol. Six major compounds were identified in the extract accounting for more than 4% including β-selinene (7.89%) (Peak No 12), caryophyllene (7.72%) (Peak No 6), α-selinene (6.78) (Peak No 13), (-) globulol (5.88) (Peak No 22), α- copaene (4.80%) (Peak No 5), and aromadendrene (4.39%) (Peak No 7). Meanwhile, the remaining compounds were less than 3% i.e., pyrogallol, α-humulene**,** alloaromadendrene, γ-muurolene, α-muurolene**,** β-bisabolene, caryophyllene oxide, α-muurolol, and epiglobulol.

Interestingly, based on previous research, all of the major compounds in *P. guajava* leaves extract were thought to have antimicrobial activity. These phytochemical compounds were characterized as listed in Table 1. Based on the GC-MS analysis, β-selinene was the major component in the *P. guajava* leaves extract. Silva et al. (2018) and Soliman et al. (2016) also found β-selinene in *P. guajava* leaves. The β-selinene showed antimicrobial activity against certain strains of Gram-positive and Gram-negative bacteria. Meanwhile, many studies reported that caryophyllene from *P. guajava* extract has antimicrobial activity (Thenmonzhi and Rajan 2015; Ashraf et al. 2016; Soliman et al. 2016; Devi et al. 2018). Furthermore, (-) globulol, α-copaene, and aromadendrene also have antimicrobial activity (Satyal 2015; Thenmonzhi and Rajan 2015; Ashraf et al. 2016). The percentage of compound content in *P. guajava* varies between studies. According to Ashraf et al. (2016) changes in variety, climatic conditions, genotype, and harvesting time may account for the current reported difference in the phytochemical profile. The concentration of β-selinene in this study was 7.89%. Otherwise, Ogunwande et al. (2003) and Silva et al. (2018) only discovered 3.7% and 1.2% β-selinene in *P. guajava* leaves, respectively.

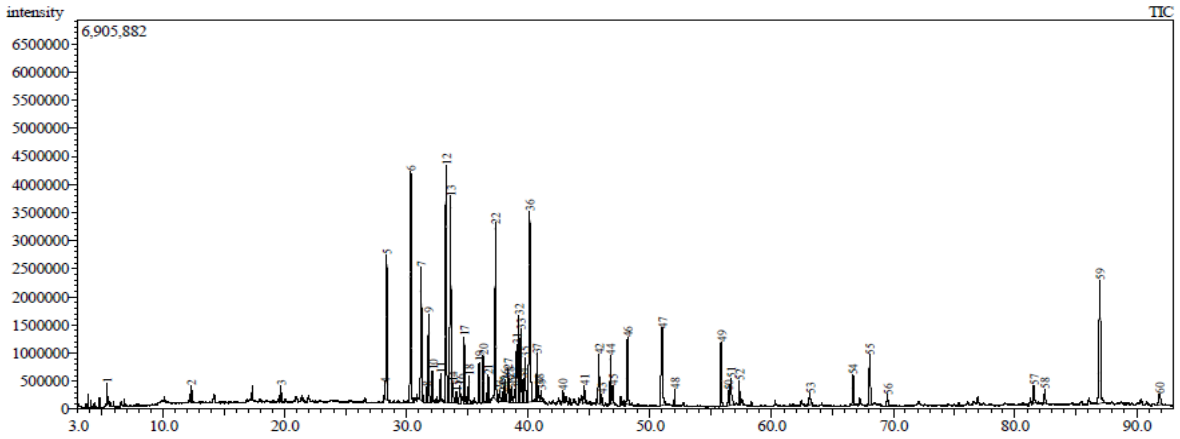

Figure 1. Chromatogram of ethanolic *Psidium guajava* leaves extract by GC-MS

Table 1. Identification of phytochemical compounds in ethanolic *Psidium guajava* leaves extract by using GC-MS

Name of compounds	Formula	MW (g/mol)	Mass fragments *m/z*	Peak (%)	RT (min)	(RI)	(SI)	Type	Activity	References
Eucalyptol	$C_{10}H_{18}O$	154.2493	43.10, 69.10, 81.15, 108.10, 139.00	0.54	12.32	1059	95	Monoterpenes	Anti-inflammatory, Antioxidant	Seol and Kim 2016
α-Terpineol	$C_{10}H_{18}O$	154.2493	43.15, 59.10, 81.10, 93.10, 121.10	0.52	19.69	1191	94	Monoterpenes	Antimicrobial, antioxidant	Sales et al. 2020
Pyrogallol	$C_6H_6O_3$	126.1100	58.15, 80.05, 97.05, 108.05,126.05	0.50	28.21	1342	84	Phenol	Anticancer	Ashraf et al. (2016)
α-Copaene	$C_{15}H_{24}$	204.3562	41.20, 69.10, 81.10, 103.10, 161.15	4.80	28.38	1375	95	Sesquiterpene	Antimicrobial	Satyal (2015); Thenmozhi and Rajah (2015); Ashraf et al. (2016); Soliman et al. (2016); Borah et al. (2019)
Caryophyllene	$C_{15}H_{24}$	204.3511	41.15, 69.10, 93.05,105.05, 133.10	7.72	30.35	1424	97	Sesquiterpene	Antimicrobial	Thenmozhi and Rajah (2015); Ashraf et al. (2016); Soliman et al. (2016); Devi et al. (2018); Naseer (2018); Xu et al. (2018)
Aromadendrene	$C_{15}H_{24}$	204.1878	41.15, 79.10, 105.10, 133.10, 161.15	4.39	31.20	1438	96	Sesquiterpenes	Antimicrobial	Ashraf et al. (2016); Xu et al. (2018)
Cadina-3,5-diene	$C_{15}H_{24}$	204.3562	81.10, 105.05, 119.10, 133.10, 161.05	0.47	31.68	1482	86	Sesquiterpenes	Anticholinesterasic	Lima et al. 2011
α- Humulene	$C_{15}H_{24}$	204.3516	67.10, 80.10, 93.05, 107.10, 147.10	2.94	31.83	1454	97	Sesquiterpenes	Antimicrobial	Satyal (2015); Thenmozhi and Rajan (2015); Soliman et al. (2016)
Alloaromadendrene	$C_{15}H_{24}$	204.3562	41.15, 79.10, 91.05, 119.10, 161.15	1.00	32.14	1464	96	Sesquiterpenes	Antimicrobial	Satyal (2015)
γ-Muurolene	$C_{15}H_{24}$	204.3511	79.05, 105.10, 119.10, 133.10, 161.15,	0.91	32.79	1478	94	Sesquiterpenes	Antimicrobial	Ashraf et al. (2016); Soliman et al. (2016)
β-Selinene	$C_{15}H_{24}$	204.3511	41.20, 79.05, 105.05, 121.10, 147.10	7.89	33.26	1492	96	Sesquiterpenes	Antibacterial	Soliman et al. (2016)
α-Selinene	$C_{15}H_{24}$	204.3511	81.10, 107.10, 133.10, 189.15, 204.15	6.78	33.63	1501	96	Sesquiterpenes	Antimicrobial	El-Ahmad et al. (2013); Soliman et al. (2016)
α-Muurolene	$C_{15}H_{24}$	204.3511	79.03, 93.10, 105.05, 119.10, 161.15	0.65	33.80	1497	91	Sesquiterpenes	Antibacterial	Satyal (2015); Soliman et al. (2016)
β-Bisabolene	$C_{15}H_{24}$	204.3511	69.10, 79.10, 93.10, 107.05, 161.15	0.38	34.10	1508	92	Sesquiterpenes	Antimicrobial	Satyal (2015)
γ-Cadine	$C_{15}H_{24}$	204.3510	79.05, 105.05, 119.10, 133.15, 161.15	0.58	34.94	1512	92	Monoterpenes	Antioxidant	Damasceno et al. (2017)
trans-Calamenene	$C_{15}H_{24}$	202.3352	91.05, 119.10, 144.10, 159.10, 202.15	2.19	34.75	1527	95	Sesquiterpenes	Antioxidant	Gooré et al. (2017)
Naphthalene 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)	$C_{15}H_{24}$	204.3511	41.15, 91.10, 106.05, 161.10, 204.15	0.93	34.14	1536	96	Sesquiterpenes	Antibacterial	Zhang et al. (2017)
Cyclohexane, 1,5-dimethyl-2,3-divinyl-	$C_{15}H_{20}$	164.2900	41.15, 79.10, 107.10, 135.15, 164.15	1.35	35.98	1143	83	Alkene	Insecticide	García et al. (2014)

Nerolidol	$C_{15}H_{26}O$	222.3663	41.15, 69.10, 81.10, 93.10, 107.10	1.58	36.30	1561	90	Sesquiterpenes	Antimicrobial, antiparasitic, antioxidant, antiinflammatory, antiulcer, insecticide and anticancer	Chan et al. (2016)
Caryophyllene alcohol	$C_{15}H_{26}O$	222.3663	41.15, 69.10, 95.10, 111.00, 123.05	0.90	36.72	1575	96	Sesquiterpenes	Antimicrobial, antivirus, antiparasitic, and insecticide	Gupta and Variyar (2016)
(-)-Globulol	$C_{15}H_{26}O$	222.3663	41.15, 43.15, 69.10, 93.10, 121.10	5.88	37.30	1530	91	Sesquiterpenes	Antimicrobial	Thenmozhi and Rajan (2015)
Viridiflorol	$C_{15}H_{26}O$	222.3663	41.15, 43.15, 69.10, 93.05, 109.10	0.34	37.62	1594	89	Sesquiterpenes	Antiinflammatory, antioxidant and anti-mycobacterial	Trevizan et al. (2016)
Unknow compound	-	-	55.10, 80.10, 93.10, 105.05, 138.15	0.29	37.90	1604	-	-	-	-
Unknow compound	-	-	55.15, 59.10, 82.10, 149.15, 164.15	0.44	37.98	1607	-	-	-	-
Copaborneol	$C_{15}H_{26}O$	222.3663	43.15, 69.10, 81.05, 95.10, 161.10	0.72	38.09	1613	90	Sesquiterpenes	Antiinflammatory	Graf and Stappen 2022
(1R,3Z,7Z,11R)-1,5,5,8-tetramethyl-12-oxabicyclo [9.1.0] dodeca-3,7-diene	$C_{15}H_{24}O$	220.3505	41.15, 43.15, 67.10, 96.10, 138.10	1.07	38.32	1592	95	Sesquiterpenes	Antimicrobial	Satyal (2015); Thenmozhi and Rajan (2015); Soliman et al. (2016)
1-Naphthalenol, decahydro-1,4a-dimethyl-7-(1-methylethylidene)-,	$C_{15}H_{26}O$	222.3663	43.15, 67.05, 81.10, 95.10, 135.15	0.78	38.46	1647	84	Sesquiterpenes	Antioxidant and antimicrobial	Ampadu et al. (2022)
Dihydroisocaryophyllene epoxide	$C_{15}H_{26}O$	222.3714	41.15, 67.10, 93.05, 109.10, 133.10	0.51	38.63	1565	78	Sesquiterpenes	Antibacterial	Fu et al. (2022)
6,9,12,15-Docosatetraenoic acid methyl ester	$C_{23}H_{38}O$	346.5466	41.20, 59.10, 81.10, 107.15, 149.10	0.43	38.84	250	78	Fatty acid	Antioxidant	Khan et al. (2019)
Epicubenol	C15H26O	222.3663	41.15, 79.05, 97.10, 119.10, 159.10	1.91	39.05	1631	80	Sesquiterpenes	Antioxidant	Damasceno et al. (2017)
Caryophyllene oxide	C23H24O	220.3505	41.15, 55.10, 67.10, 82.10, 109. 10,	2.89	39.23	1587	86	Sesquiterpenes	Antifungal	Paniandy et al. (2000); Ashraf et al. (2016)
Bicycol [7.2.0] undecan-3-ol<11,11-dimethyl-,4,8-bis(methylene)	$C_{15}H_{24}O$	220.3505	41.15, 55.10, 69.10, 91.05, 136.10	2.41	39.39	1636	95	Sesquiterpenes	Anticancer	Legault and Pichette (2007)
Cadinol	$C_{15}H_{26}O$	222.3663	43.15, 95.10, 121.10, 161.15, 204.20	0.75	39.58	1580	89	Sesquiterpenes	Phytotoxic activity	Mancini et al. (2013)
α-Muurolol	$C_{15}H_{26}O$	222.3663	43.15, 81.10, 105.10, 119.10, 161.15	1.47	39.74	1651	96	Sesquiterpenes	Antimicrobial	Satyal (2015)
Intermedeol	$C_{15}H_{26}O$	222.3663	43.15, 67.10, 81.10, 96.10, 135.15	6.31	40.13	1668	91	Sesquiterpenes	Antitumor	Wu (1998)
Androstan-17-one, 3-ethyl-3-hydroxy-(5 alpha)	$C_{21}H_{34}O_2$	318.4934	41.15, 69.10, 79.05, 107.10, 121.05	1.58	40.71	2251	81	Steroid	Testosterone hydroxylase inducer	Arunmathi and Malarvili (2017)
Doconexent	$C_{22}H_{32}O_2$	328.4883	41.15, 69.10, 91.05, 105.10, 183.15	0.49	40.86	2612	71	Fatty acid	Antimicrobial and antioxidant	Abu-Lafi et al. (2019)
(-)-Isolongifolol	$C_{15}H_{26}O$	222.3663	41.15, 55.10, 81.10, 93.05, 107.05	0.33	41.07	1635	80	Sesquiterpenes	Antioxidant	Jaradat et al. (2018).
β-Dihydroionone	$C_{13}H_{22}O$	194.3175	41.15, 43.15, 69.10, 93.10, 123.10	0.42	42.85	1438	83	Sesquiterpenes	Antimicrobial and insecticide	Paparella et al. (2021)

2-Hydroxy-2,4,4-trimethyl-3-[(1E)-3-methyl-1,3-butadienyl] cyclohexanone	$C_{14}H_{22}O_2$	222.3233	43.15, 69.10, 95,10, 121.05, 139.15	0.56	44.61	1671	79	Phenol	Antibacterial and anticancer	Oirere et al. (2015)
Epiglobulol	$C_{15}H_{26}O$	222.3663	41.15, 55.10, 81.10, 109.10, 123.10	1.64	45.08	1630	79	Sesquiterpenes	Antiinflammatory	Ashraf et al. (2016)
Humulane-1,6-dien-3-ol	$C_{15}H_{26}O$	2223663	41.15, 43.10, 67.10, 109.05, 152.15	0.33	46.04	1757	80	Sesquiterpenes	Antioxidant	Chen-xing et al. (2014)
Neophytadine	$C_{20}H_{38}$	278.5156	41.15, 43.20, 68.10, 95.10, 123.10	1.61	46.74	1827	96	Alkene	Antiinflammatory	Swamy et al. (2017)
Cholestane, 4,5-epoxy-, (4. alpha.,5. alpha.)	$C_{27}H_{46}O$	386.6670	41.00, 43.00, 69.00, 93.00, 109.00	0.58	46.94	2421	81	Steroid	Antimicrobial	Costa et al. (2023)
4,4,8-Trimethyltricyclo [6.3.1.0(1,5)] dodecane-2,9-diol	$C_{15}H_{26}O_2$	238.3657	41.15, 81.10, 107.05, 121.10, 164.15	2.19	48.14	1840	92	Sesquiterpenes	Antioxidant	Carranza et al. (2019)
Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4241	41.15, 43.15, 57.10, 73.05, 129.10	2.6	51.02	1977	95	Fatty acid	Antiinflammatory	Aparna et al. (2012)
Ethyl-palmitate	$C_{18}H_{36}O_2$	284.4772	41.15, 43.15, 57.18, 88.05, 101.10	0.54	52.02	1993	94	Ethyl ester	Antiinflammatory	Saed et al. (2012)
Phytol	$C_{20}H_{40}O$	296.5310	40.15, 41.15, 71.10, 95.10, 123.10	2.13	55.87	2045	96	Diterpene	Antiinflammatory	Carvalho et al. (2020)
Linoelaidic acid	$C_{18}H_{32}O_2$	280.4455	41.15, 67.10, 95.10, 110.15, 124.10	0.49	56.46	2094	91	Fatty acid	Antimicrobial and antioxidant	Gidik, (2021)
7-Tetradecenal (Z)	$C_{14}H_{26}O$	210.3556	41.15, 55.15, 69.10, 97.10, 98.10	0.89	56.65	1609	90	Fatty aldehyde	Antibacterial, antioxidant, and antiinflammatory	Palariya et al. (2019)
Octadecanoic acid	$C_{18}H_{36}O_2$	284.4772	41.15, 73.05, 95.10, 109.10, 129.10	0.82	57.36	2167	84	Fatty acid	Antimicrobial, antiinflammatory and antioxidant	Siswadi and Saragih (2020)
Unknow compound	-	-	77.05, 105.05, 152.10, 181.00, 257.10	0.37	63.14	2360	-	-	-	-
Unknow compound	-	-	91.05, 105.05, 178.10, 249.15, 292.25	0.98	66.68	2488	-	-	-	-
Unknow compound	-	-	91.10, 138.00, 166.00, 193.05, 270.10	1.66	68.06	2541	-	-	-	-
Unknow compound	-	-	91.10, 141.10, 169.10, 249.25, 292.20	0.36	69.52	2596	-	-	-	-
Heptacosanol	$C_{27}H_{56}O$	396.7329	43.20, 57.10, 83.10, 97.10, 111.10	0.59	81.55	2948	94	Fatty alcohols.	Antioxidant and antimicrobial	Rhetso et al. (2020)
α-tochopherol	$C_{29}H_{50}O_2$	430.7061	43.20, 109.10, 137.10, 165.10, 430.35	0.47	82.44	3149	91	Methylated phenols	Antioxidant	Brigelius-Flohe (2006)
Clionasterol	$C_{29}H_{50}O$	414.3861	43.20, 57.15, 81.10, 95.10, 119.05	4.10	86.96	3790	93	Triterphenoid	Antioxidant	Liyanage et al. (2020)
β-Longipinene	$C_{15}H_{24}$	204.3511	69.10, 79.05, 91.05, 105.10, 270.95	0.35	91.87	1475	80	Sesquiterpenes	Antibacterial	Noriega et al. (2019)

Note: MW: Molecular Weight, RT: Retention Time, RI: Retention Index, SI: Similarity Index

Table 2. Identification of phytochemical compounds in *P. guajava* leaves extract by using LC-MS

Compound name	RT (min)	Formula	MW (g/mol)	*m/z* [M+H]+/ [M-H]-	Ion (+/-)	Type	Activity	References
Quinic acid	0.61	$C_7H_{12}O_6$	192.1665	191.0554	[M-H]-	Phenolic	Antimicrobial	Liu et al. (2020)
Catechin	4.85	$C_{30}H_{26}O_{12}$	578.1398	577.1325	[M-H]-	Phenolic	Antibacterial	Kiehne and Engeldhardt (2016)
Apigenin-7-O-β-D-glucorono pyranoside	12.33	$C_{22}H_{20}O_{10}$	444.1056	443.0985	[M-H]-	Flavonoids	Antibacterial	Salehi et al. (2019)
Quercetin	12.62	$C_{15}H_{10}O_7$	302.2357	301.03	[M-H]-	Flavonoids	Antibacterial	Chen et al. (2010)
Luteolin-7-O-glucuronide	7.87	$C_{21}H_{18}O_{12}$	462.0798	461.07	[M-H]	Flavonoids	Antibacterial	Naseer et al. (2018)
Epigallocatechin (4β,8)-gallocatechin	13.93	$C_{30}H_{26}O_{14}$	610.5190	611.13	[M+H]	Phenolic	Antimicrobial	Chen et al. (2010)
Naringenin4′-O-glucopyranoside	11.84	$C_{15}H_{10}O_5$	272.0688	273.0761	[M+H]	Flavonoids	Antimicrobial	Chen et al. (2010)
Myricetin	8.67	$C_{15}H_{10}O_8$	318.2351	319.09	[M+H]	Flavonoids	Antibacterial	Chang et al. (2013)

Determination of phytochemical compounds in *Psidium guajava* using LC-MS

Based on the LCMS (Liquid Chromatography-Mass Spectrometry) analysis, eight compounds were discovered to have antimicrobial activity. There were quinic acid, catechin, apigenin-7-O-β-D-glucuronopyranoside, quercetin, luteolin-7-O-glucuronide, epigallocatechin (4β,8)-gallocatechin, naringenin4′-O-glucopyranoside, and myrecitin. The phytochemical analysis for these eight compounds is shown in Table 2. The negative ion mode detection results obtained 5 compounds that have antimicrobial activity, namely quinic acid, catechin, apigenin 7-O-β-D-glucorono pyranoside, quercetin, and luteolin-7-O-glucuronide. Meanwhile, there were three compounds detected using the positive ion mode, namely epigallocatechin (4β,8)-gallocatechin, naringenin4′-O-glucopyranoside, and myricetin.

Many researchers reported the same compounds, which have antimicrobial activity. Liu et al. (2020) report 17 quinic acid derivatives that were screened by LCMS as potential ligands for the superoxide dismutase (SOD) enzyme from *Carissa spinarum* L. *Carissa spinarum* species is one of the plants that contain quinic acid, which has antimicrobial activity (Berhanuu et al. 2020). According to Wu and Brown (2021), catechin has a number of promising bactericidal effects on bacteria for both Gram-positive and Gram-negative bacteria. Furthermore, these molecules have been shown to inhibit the activity of virulence factors, particularly toxins, and reduce the pathogenicity of certain bacteria. Apigenin is the most widely distributed in the plant kingdom. Apigenin is found in significant amounts as glycosylated in vegetables (*Petroselinum crispum* (Mill.) Fuss, *Apium graveolens* L., and *Allium cepa* L.), fruits (*Citrus sinensis* (Mill.) Pers., 1806), herbs (*Matricaria recutita* L., *Thymus vulgaris* L., *Origanum vulgare* L., and *Ocimum basilicum* L.), and plant-based beverages (tea, beer, and wine). Apigenin has been associated with antiviral effects, together with quercetin, rutin, and other flavonoids. A large number of studies carried out over the years have indicated that apigenin has many interesting pharmacological activities and nutraceutical potential. As an example, its properties as an antioxidant are well known, and it can also be a therapeutic agent to overcome diseases like inflammation, autoimmune disease, neurodegenerative disease, and even several types of cancer (Hostetler et al. 2017; Salehi et al. 2019).

Meanwhile, quercetin is a flavonoid compound found in many plants that has a variety of biological activities. Quercetin research has shown that it has medical applications. Farhadi et al. (2018) report that quercetin and its derivatives showed antibacterial activity against some strains of bacteria, including *Staphylococcus aureus*, methicillin-resistant *S. aureus* (MRSA), and *S. epidermidis*. Yang et al. (2020) report that these compounds demonstrated antimicrobial activity against pathogenic bacteria such as *Pseudomonas aeruginosa*, *Salmonella enteritidis*, *E. coli*, *Proteus*, and *Asp. flavus*. Metwally et al. (2010) isolated and identified quercetin from the leaves of *P. guajava*. The Minimum Inhibitory Concentration (MIC) values of luteolin against various *Trueperella pyogenes* isolates and ATCC19411 were 78 µg/mL. Luteolin has been demonstrated to exhibit good antibacterial activity against *Bacillus subtilis*, *S. aureus*, *Listeria monocytogenes*, *Escherichia coli*, and *Pseudomonas fluorescens* (Guo et al. 2020). The antibacterial mechanism of luteolin against *S. aureus i*nvolves inhibiting the synthesis of nucleic acid and protein, impairing bacterial cell membrane, inducing cell morphological alteration and inhibiting biofilm formation (Wang et al. 2010).

The major catechin found in green tea, epigallocatechin-3-gallate (EGCG), has been shown to have antimicrobial effects against a variety of bacterial pathogens. The MIC_{50} and MIC_{90} values respectively of this compound against 40 clinical isolates of *Stenotrophomonas maltophilia* (nosocomial pathogen) were 256 mg/L when determined by agar dilution and 512 mg/L when determined by broth microdilution. The $MBC_{50/90}$ values were 512 mg/L (minimal bactericidal concentrations for 50% and 90% of the organisms (Gordon and Wareham 2010). Naringenin has antimicrobial activity against a variety of microbial systems, including *E. coli*, *Saccharomyces cerevisiae*, and *E. coli* (Salehi et al. 2019). Myricetin inhibited the biofilm formation of *S. aureus* with MBC_{50} values of 1 g/mL, according to Farhadi et al. (2018). Flavonols, including quercetin, myricetin, kaempferol, and

fisetin, are among the most important classes of flavonoids with antibacterial activity (Buchmann et al. 2022).

In conclusion, this study has identified volatile and non-volatile compounds in ethanolic *P. guajava* leaves extract. There were 15 major volatile compounds identified by GC-MS, i.e., pyrogallol, α-copaene, caryophyllene, aromadendrene, α-humulene**,** alloaromadendrene, γ-muurolene, β-selinene, α-selinene, α-muurolene**,** β-bisabolene, β-bisabolene, (-) globulol, caryophyllene oxide, α-muurolol, and epiglobulol. Furthermore, the non-volatile compounds identified by LC-MS were quinic acid, catechin, apigenin-7-O-β-D-glucuronopyranoside, quercetin, luteolin-7-O-glucuronide, epigallocatechin (4β,8)-gallocatechin, naringenin4′-O-glucopyranoside, and myrecitin. These compounds could be responsible for the antimicrobial property of *P. guajava* leaves extract and could be further isolated to be determined in the future.

ACKNOWLEDGEMENTS

The authors acknowledge the Southeast Asian Regional Center for Graduate Study and Research in Agriculture (SEARCA) for the research scholarship.

REFERENCES

Abu-Lafi S, Rayan M, Masalha M, Abu-Farich B, Al-Jaas H, Abu-Lafi M, Rayan A. 2019. Phytochemical composition and biological activities of wild *Scolymus maculatus* L. Medicines 6 (2): 53. DOI: 10.3390/medicines6020053.

Ampadu GAA, Mensah JO, Darko G, Borquaye LS. 2022. Essential oils from the fruits and leaves of *Spondias mombin* Linn.: Chemical composition, biological activity, and molecular docking study. Evid Based Complement Alternat Med 2022: 7211015. DOI: 10.1155/2022/7211015.

Aparna V, Dileep KV, Mandal PK, Karthe P, Sadasivan C, Haridas M. 2012. Anti-inflammatory property of n-hexadecanoic acid: Structural evidence and kinetic assessment. Chem Biol Drug Des 80 (3): 434-439. DOI: 10.1111/j.1747-0285.2012.01418.x.

Arunmathi C, Malarvili T. 2017. Analysis of bioactive compounds in methanol extract of *Aplotaxis auriculata* rhizome using GC-MS. J Pharmacogn Phytochem 6 (3): 243-247.

Ashraf A, Sarfraz RA, Rashid MA, Mahmood A, Shahid M, Noor N. 2016. Chemical composition, antioxidant, antitumor, anticancer and cytotoxic effects of *Psidium guajava* leaf extracts. Pharm Biol 54 (10): 1971-1981. DOI: 10.3109/13880209.2015.1137604.

Barbalho SM, Farinazzi-Machado FMV, de Alvares Goulart R, Brunnati ACS, Ottoboni AMMB. 2012. *Psidium guajava* (Guava): A plant of multipurpose medicinal plants. Med Aromat Plants 1 (4): 1000104. DOI: 10.4172/2167-0412.1000104.

Berhanu G, Atalel D, Kandi V. 2020. A review of the medicinal and antimicrobial properties of *Carissa spinarum* L. Am J Biomed Res 8 (2): 54-58. DOI: 10.12691/ajbr-8-2-5.

Borah A, Pandey SK, Haldar S, Lal M. 2019. Chemical composition of leaf essential oil of *Psidium guajava* L. from North East India. J Essent Oil-Bear Plants 22 (1): 248-253. DOI: 10.1080/0972060X.2019.1574213.

Brigelius-Flohe R. 2006. Bioactivity of vitamin E. Nutr Res Rev 19 (2): 174-186. DOI: 10.1017/S0954422407202938.

Brusotti G, Cesari I, Dentamaro A, Caccialanza G, Massolini G. 2014. Isolation and characterization of bioactive compounds from plant resources: The role of analysis in the ethnopharmacological approach. J Pharm Biomed Anal 87: 218-228. DOI: 10.1016/j.jpba.2013.03.007.

Buchmann D, Schultze N, Borchardt J, Böttcher I, Schaufler K, Guenther S. 2022. Synergistic antimicrobial activities of epigallocatechin gallate, myricetin, daidzein, gallic acid, epicatechin, 3-hydroxy-6-methoxyflavoneand genistein combined with antibiotics against ESKAPE pathogens. J Appl Microbiol 132 (2): 949-963. DOI: 10.1111/jam.15253.

Carranza MSS, Linis VC, Ragasa CY, Tan MCS. 2019. Chemical constituents and antioxidant potentials of seven Philippine mosses. Malaysian J Anal Sci 23 (6): 950-962. DOI: 10.17576/mjas-2019-2306-04.

Carvalho AMS, Heimfarth L, Pereira EWM, Oliveira FS, Menezes IRA, Coutinho HDM, Picot L, Antoniolli AR, Quintans JSS, Quintans-Júnior LJ. 2020. Phytol, a chlorophyll component, produces antihyperalgesic, anti-inflammatory, and antiarthritic effects: Possible NFκB pathway involvement and reduced levels of the proinflammatory cytokines TNF-α and IL-6. J Nat Prod 83 (4): 1107-1117. DOI: 10.1021/acs.jnatprod.9b01116.

Chai PPK. 2000. Ethnobotanical survey in the Lanjak Entimau wildlife sanctuary. In: Chai PPK, Yaw KS (eds). Proceedings of ITTO Workshop 2000; Development of Lanjak Entimau Wildlife Sanctuary as a Totally Protected Area Phase II. Sarawak, 28 - 29 February 2000. [Malaysia]

Chang CH, Hsieh CL, Wang HE, Peng CC, Chyau CC Peng RY. 2013. Unique bioactive polyphenolic profile of guava (*Psidium guajava*) budding leaf tea is related to plant biochemistry of budding leaves in early dawn. J Sci Food Agric 4 (11): 944-954. DOI: 10.1002/jsfa.5832.

Chan WK, Tan LTH, Chan KG, Lee LH, Goh BH. 2016. Nerolidol: A sesquiterpene alcohol with multi-faceted pharmacological and biological activities. Molecules 21 (5): 529. DOI: 10.3390/molecules21050529.

Chen C, Zhou J, Ji C. 2010. Quercetin: A potential drug to reverse multidrug resistance. Life Sci 87 (11-12): 333-338. DOI: 10.1016/j.lfs.2010.07.004.

Chen-Xing Z, Mi Z, Jing H, Ya-Fang D, Bao-Cai L. 2014. Chemical composition and antioxidant activity of the essential oil from the flowers of *Artemisia austro-yunnanensis*. J Chem Pharm Res 6 (7): 1583-1587.

Costa MdC, Cruz AIC, Ferreira MA, da Rocha Bispo AS, Ribeiro PR, Costa JA, Araújo FM, Evangelista-Barreto NS. 2023. Brown propolis bioactive compounds as a natural antimicrobial in alginate films applied to *Piper nigrum* L. Ciênc Rural 53 (5): e20210805. DOI: 10.1590/0103-8478cr20210805.

Damasceno CSB, de Oliveira LF, Szabo EM, Souza AM, Dias JFG, Miguel MD, Miguel OG. 2018. Chemical composition, antioxidant and biological activity of *Ocotea bicolor* Vattimo-Gil (Lauraceae) essential oil. Braz J Pharm Sci 53 (4): e17298. DOI: 10.1590/s2175-97902017000417298.

Daoutidou M, Plessas S, Alexopoulos A, Mantzourani I. 2021. Assessment of antimicrobial activity of pomegranate, cranberry, and black chokeberry extracts against foodborne pathogens. Foods 10 (3): 486. DOI: 10.3390/foods10030486.

Devi RB, Barkath TN, Vijayaraghavan P, Rejiniemon TS. 2018. GC-MS analysis of phytochemical from *Psidium guajava* Linn. leaf extract and their *in vitro* antimicrobial activities. Intl J Pharm Biol Sci 8 (1): 583-589.

Farhadi F, Khamenh B, Iranshahi M, Iranshahy M. 2018. Antibacterial activity of flavonoids and their structure-activity relationship: An update review. Phytother Res 33 (1): 13-40. DOI: 10.1002/ptr.6208.

Fu J, Gao Y, Xing X. 2022. Preliminary study on phytochemical constituents and biological activities of essential oil from *Myriactis nepalensis* Less. Molecules 27 (14): 4631. DOI: 10.3390/molecules27144631.

García JI, Salvatella L, Pires E, Fraile JM, Mayoral JA. 2014. Addition of ketocarbenes to alkenes, alkynes, and aromatic systems. In: Knockel P (eds). Comprehensive Organic Synthesis. Elsevier, Amsterdam.

Gidik B. 2021. Antioxidant antimicrobial activities and fatty acid compositions of wild *Berberis* spp. by different techniques combined with chemometrics (PCA and HCA). Molecules 26 (24): 7448. DOI: 10.3390/molecules26247448.

Gooré SG, Ouattara ZA, Yapi AT, Békro YA, Bighelli A, Paoli M, Tomi F. 2017. Chemical composition of the leaf oil of *Artabotrys jollyanus* from Côte d'Ivoire. Rev Bras Farmacogn 27 (4): 414- 418. DOI: 10.1016/j.bjp.2017.04.001.

Gordon NC, Wareham DD. 2010. Antimicrobial activity of the green tea polyphenol (-)-epigallocatechin-3-gallate (EGCG) against clinical isolates of *Stenotrophomonas maltophilia*. Intl J Antimicrob Agents 36 (2): 129-131. DOI: 10.1016/j.ijantimicag.2010.03.025.

Graf M, Stappen I. 2022. Beyond the bark: An overview of the chemistry and biological activities of selected bark essential oils. Molecules 27 (21): 7295. DOI: 10.3390/molecules27217295.

Gupta S, Variyar PS. 2016. Nanoencapsulation of essential oils for sustained release: Application as therapeutics and antimicrobials. In: Grumezescu A (eds). Nanotechnology in the Agri-Food Industry Volume 2. Elsevier, Amsterdam.

Harper. 2020. Guava. Online Etymology Dictionary. www.etymonline.com/word/guava.

Haruna MF, Kenta AM, Herawati. 2022. Medicinal plants used by the community of Lipulalongo Village, Banggai Laut District, Central Sulawesi, Indonesia. Asian J Ethnobiol 5: 62-68. DOI: 10.13057/asianjethnobiol/y050107.

Hidayat S, Hikmat A, Zuhud EAM. 2010. Ethnobotanical study of local people at Dukuh Cultural Village Garut Regency, West Java. Media Konservasi 15(3): 139-151.

Hostetler GL, Ralston RA, Schwartz SJ. 2017. Flavones: Food sources, bioavailability, metabolism, and bioactivity. Adv Nutr 8 (3): 423-435. DOI: 10.3945/an.116.012948.

Jaradat N, Al-Lahham S, Abualhasan MN, Bakri A, Zaide H, Hammad J, Hussein F, Issa L, Mousa A, Speih R. 2018. Chemical constituents, antioxidant, cyclooxygenase inhibitor, and cytotoxic activities of *Teucrium pruinosum* Boiss. essential oil. Biomed Res Intl 2018: 4034689. DOI: 10.1155/2018/4034689.

Jegede A. 2019. Top 10 largest guava producing countries in the world. The daily records. www.thedailyrecords.com/2018-2019-2020-2021/world-famous-top-10-list/world/largest-guava-producing-countries-world-fruits-states/ 6566/.

Kamath JV, Rahul N, Kumar CKA, Lakshmi SM. 2008. *Psidium guajava* L.: A review. Intl J Green Pharm 2: 9-12. DOI: 10.22377/ijgp.v2i1.386.

Katewaraphorn J, Aldred AK. 2016. A study of microcapsules containing *Psidium guajava* leaf extract for antibacterial agent on cotton fabric. Intl J Chem Eng Appl 7 (1): 27-31. DOI: 10.7763/IJCEA.2016.V7.536.

Khan S, Richa, Kaur H, Jhamta R. 2019. Evaluation of antioxidant potential and phytochemical characterization using GCMS analysis of bioactive compounds of *Achillea filipendulina* (L.) leaves. J Pharmacogn Phytochem 8 (3): 258-265.

Kiehne A, Engelhardt UH. 1996. Thermospray-LC-MS analysis of various groups of polyphenols in tea. I. catechins, flavonol O-glycosides, and flavone C-glycosides. Z Lebensm Unters Forsch 202 (1): 48-54. DOI: 10.1007/BF01229684.

Lee C, Kim SY, Eum S, Paik JH, Bach TT, Darshetkar AM, Choudhary RK, Hai DV, Quang BH, Thanh NT, Choi S. 2019. Ethnobotanical study on medicinal plants used by local Van Kieu ethnic people of Bac Huong Hoa nature reserve, Vietnam. J Ethnopharmacol 231: 283-294. DOI: 10.1016/j.jep.2018.11.006.

Legault J, Pichette A. 2007. Potentiating effect of β-caryophyllene on anticancer activity of α-humulene, isocaryophyllene and paclitaxel. J Pharm Pharmacol 59 (12): 1643-1647. DOI: 10.1211/jpp.59.12.0005.

Lima BG, Tietbohl LAC, Fernandes CP, Cruz RAS, Botas GDS, Santos MG, Silva-Filho MV, Rocha L. 2011. Chemical composition of essential oils and anticholinesterasic activity of *Eugenia sulcata* Spring ex Mart. Lat Am J Pharm 31 (1): 152-155.

Liu Y, Muema FW, Zhang YL, Guo MQ. 2020. Acyl quinic acid derivatives screened out from *Carissa spinarum* by SOD-affinity ultrafiltration LC-MS and their antioxidative and hepatoprotective activities. Antioxidants 10 (8): 1302. DOI: 10.3390/antiox10081302.

Liyanage NM, Nagahawatta DP, Jayawardena TU, Jayawardhana HHACK, Lee HG, Kim YS, Jeon YJ. 2022. Clionasterol-rich fraction of *Caulerpa racemosa* against particulate matter-induced skin damage via inhibition of oxidative stress and apoptosis-related signaling pathway. Antioxidants 11 (10): 1941. DOI: 10.3390/antiox11101941.

Mancini E, De Martino L, Malova H, De Feo V. 2013. Chemical composition and biological activities of the essential oil from *Calamintha nepeta* plants from the wild in southern Italy. Nat Prod Commun 8 (1): 139-142. DOI: 10.1177/1934578X1300800134.

Mendez D, Kutty A, Moideen A, Prasad SR. 2016. Preferential inhibition of bacterial elastase over human neutrophil elastase by leaf extracts of *Psidium guajava*: An in vitro study. Natl J Physiol Pharm Pharmacol 6 (2): 123-127. DOI: 10.5455/njppp.2016.6.2710201592.

Metwally AM, Omar AA, Harraz FM, Sohafy SME. 2010. Phytochemical investigation and antimicrobial activity of *Psidium guajava* L. leaves. Pharmacogn Mag 6 (23): 212-218. DOI: 10.4103/0973-1296.66939.

Naseer S, Hussain S, Naeem N, Pervaiz M, Rahman M. 2018. The phytochemistry and medicinal value of *Psidium guajava* (guava). Clin Phytoscience 4: 32. DOI: 10.1186/s40816-018-0093-8.

Nguanchoo V, Wangpakapattanawong P, Balslev H, Inta A. 2019. Exotic plants used by the Hmong in Thailand. Plants 8 (11): 500. DOI: 10.3390/plants8110500.

Noriega P, Guerrini A, Sacchetti G, Grandini A, Ankuash E, Manfredini S. 2019. Chemical composition and biological activity of five essential oils from the ecuadorian amazon rain forest. Molecules 24 (8): 1637. DOI: 10.3390/molecules24081637.

Oirere EK, Anusooriya P, Raj CA, Gopalakrishnan VK. 2015. Phytochemical analysis of N-hexane leaf extract of *Alpinia purpurata* (Vieill.) K. Schum using UV-VIS, FTIR and GC-MS. Intl J Pharm Pharm Sci 7 (8): 387-389.

Palariya D, Singh A, Dhami A, Kumar R, Prakash O, Pant AK, Kumar R. 2019. Phytochemical analysis and biological activities of leaves and bark hexane extracts of *Premna mucronata* Roxb. collected from Kumaun hills of Uttarakhand. Intl J Herb Med 7 (5): 35-44.

Paparella A, Shaltiel-Harpaza L, Ibdah M. 2021. β-Ionone: Its occurrence and biological function and metabolic engineering. Plants 10 (4): 754. DOI: 10.3390/plants10040754.

QGIS.org, %Y. 27 March 2023. QGIS Geographic Information System Installation Guide. QGIS Association. Retrieved from http://www.qgis.org.

Rajan S, Suvetha P, Thirunalasundari T, Jeeva S. 2015. Anti-enteric bacterial activity of the traditional medicinal plants of Kanyakumari coast Tamilnadu, India. J Coast Life Med 3 (8): 640-644. DOI: 10.12980/JCLM.3.201514J57.

Rhetso T, Shubharani R, Roopa MS, Sivaram V. 2020. Chemical constituents, antioxidant, and antimicrobial activity of *Allium chinense* G. Don. Future J Pharm Sci 6: 102. DOI: 10.1186/s43094-020-00100-7.

Rukayadi Y, Shim JS, Hwang JK. 2008. Screening of Thai medicinal plants for anticandidal activity. Mycoses 51 (4): 308-312. DOI: 10.1111/j.1439-0507.2008. 01497.x.

Saed NM, El-Demerdash E, Abdel-Rahman HM, Algandaby MM, Al-Abbasi FA, Abdel-Naim AB. 2021. Anti-inflammatory activity of methyl palmitate and ethyl palmitate in different experimental rat models. Toxicol Appl Pharmacol 264 (1): 84-93. DOI: 10.1016/j.taap.2012.07.020.

Salehi B, Venditti A, Sharifi-Rad M, Kręgiel D, Sharifi-Rad J, Durazzo A, Lucarini M, Santini A, Souto EB, Novellino E, Antolak H, Azzini E, Setzer WN, Martins N. 2019. The therapeutic potential of apigenin. Intl J Mol Sci 20 (6): 1305. DOI: 10.3390/ijms20061305.

Sandoval JR, Rodriquez PA. 2013. *Psidium guajava* (guaya). CABI Invasive Species Compedium. www.cabi.org.

Satyal P. 2015. Development of GC-MS Database of Essential Oil Components by the Analysis of Natural Essential Oils and Synthetic Compounds and Discovery of Biologically Active. [Dissertation]. University of Alabama, US.

Seol GH, Kim KY. 2016. Eucalyptol and its role in chronic diseases. Drug discovery from mother nature. In: Crusio WE, Dong H, Radeke HHN, Steinlein O, Xiao J (eds). Advances in Experimental Medicine and Biology. Springer, New York.

Siswadi S, Saragih GS. 2021. Phytochemical analysis of bioactive compounds in ethanolic extract of *Sterculia quadrifida* R.Br. AIP Conf Proc 2353: 030098. DOI: 10.1063/5.0053057.

Soliman FM, Fathy MM, Salama MM, Saber FR. 2016. Comparative study of the volatile oil content and antimicrobial activity of *Psidium guajava* L. and *Psidium cattleianum* Sabine leaves. Bull Fac Pharm Cairo Univ 54 (2): 219-225. DOI: 10.1016/j.bfopcu.2016.06.003 1110-0931.

Swamy MK, Arumugam G, Kaur R, Ghasemzadeh A, Yusoff MM, Sinniah UR. 2017. GC-MS based metabolite profiling, antioxidant and antimicrobial properties of different solvent extracts of Malaysian *Plectranthus amboinicus* leaves. Evid Based Complement Alternat Med 2017: 1517683. DOI: 10.1155/2017/1517683.

Syarpin, Permatasari S, Pujianto DA. 2023. Analysis of phytochemical constituents and antioxidant activity from the fractions of *Luvunga sarmentosa* root extract using LCMS/MS. Biodiversitas 24 (2): 733-740. DOI: 10.13057/biodiv/d240208.

Thenmozhi S, Rajan S. 2015. GC-MS analysis of bioactive compounds in *Psidium guajava* leaves. J Pharmacogn Phytochem 3 (5): 162-166.

Trevizan LNF, do Nascimento KF, Santos JA, Kassuya CAL, Cardoso CAL, do Carmo Vieira M, Moreira FMF, Croda J, Formagio ASN. 2016. Anti-inflammatory, antioxidant and anti-*Mycobacterium tuberculosis* activity of viridiflorol: The major constituent of *Allophylus edulis* (A. St.-Hil., A. Juss. & Cambess.) Radlk. J Ethnopharmacol 192: 510-515. DOI: 10.1016/j.jep.2016.08.053.

Wang J, Zhao J, Liu H, Zhou L, Liu Z, Wang J, Han J, Yu Z, Yang F. 2010. Chemical analysis and biological activity of the essential oils of two valerianaceous species from China: *Nardostachys chinensis* and

Valeriana officinalis. Molecules 15 (9): 6411-6422. DOI: 10.3390/molecules15096411.

Wang L, Wu Y, Xie J, Wu S, Wu Z. 2018. Characterization, antioxidant and antimicrobial activities of green synthesized silver nanoparticles from *Psidium guajava* L. leaf aqueous extracts. Mater Sci Eng C Mater Biol Appl 86: 1-8. DOI: 10.1016/j.msec.2018.01.003.

Wu C. 1998. Structural and Synthetic Studies of Potential Antitumor Natural Products. [Dissertation]. Virginia Polytechnic Institution and State University, United State of America.

Wu M, Brown AC. 2021. Applications of catechins in the treatment of bacterial Infections. Pathogens 10 (5): 546. DOI: 10.3390/pathogens10050546.

Zhang L, Wang Y, Xu M, Hu X. 2017. Chemical composition and antibacterial activity of the essential oil from Chinese wild *Ledum palustre* L. on *Vibrio parahaemolyticus*. Intl J Food Nutr Sci 4 (1): 8-12. DOI: 10.15436/2377-0619.17.1270.