Open Access Research Journal of
Biology and Pharmacy
Journals home page: https://oarjbp.com/
ISSN: 2782-9979 (Online)

OARJ OPEN ACCESS RESEARCH JOURNALS

(RESEARCH ARTICLE)

Metabolite profiling of betel palm nuts (*Areca catechu* L.)

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Open Access Research Journal of Biology and Pharmacy, 2024, 12(02), 034-049

Publication history: Received on 31 October 2024; revised on 15 December 2024; accepted on 18 December 2024

Article DOI: https://doi.org/10.53022/oarjbp.2024.12.2.0039

Abstract

The nuts of the betel palm (*Areca catechu* L.) are a valuable medicinal component due to their pharmacological effects and the presence of numerous bioactive metabolite compounds. To analyze the composition, abundance, and bioactivity of these metabolites, metabolite profiling was conducted using Gas Chromatography-Mass Spectrometry (GC-MS). The GC-MS analysis resulted in a chromatogram with 88 peaks, identifying 63 compounds, including four dominant ones, as well as other unknown metabolites, yielding a total metabolite compound abundance of 96.66%. The most abundant compound was *arecoline* (31.19%), followed by *phthalic acid di(2-propylpentyl) ester* (4.99%), *9-octadecenoic acid (Z)-methyl ester* (3.51%), and *hexadecanoic acid, methyl ester* (2.40%). The compounds detected in the betel palm nuts included organic acids, alcohols, esters, carbohydrates, fatty acids, steroids, alkaloids, aromatics, phenols, and ascorbic acid. *Arecoline,* which is unique to betel palm nut extract, belongs to the class of pyridine-type alkaloids derived from the shikimic acid biosynthesis pathway. It exhibits a cholinergic effect and possesses various therapeutic properties, including antioxidant, antibacterial, antiallergic, anti-inflammatory, antiparasitic, antihelminthic, and analgesic effects. Therefore, it holds potential for various treatment and therapy applications, particularly for conditions related to the nervous system, cancer, and more.

Keywords: *Areca catechu* L.; betel palm nut; GC-MS; metabolite profile; bioactivity

1. Introduction

The Betel palm (*Areca catechu* L.) is a tropical plant belonging to the Arecaceae family, commonly found in South Asia and Southeast Asia, including countries like the Philippines, Indonesia, Malaysia, Myanmar, China, Bangladesh, and India [1]. It is recognized as a multifunctional medicinal plant because every part of the betel palm can be utilized in medicine, from the roots and leaves to the fruit fibers and various components of the fruit that contain bioactive metabolites [2]. Traditionally, the nuts of the betel palm are used to treat a range of ailments, including boils, diarrhea, dysentery, nosebleeds, malaria, and intestinal worms. Additionally, the fibers from the betel nut can be employed in the treatment of digestive disorders, edema, and beriberi caused by reduced urine output. In Indonesia, betel palm nuts, also known as "pinang," are particularly popular as an ingredient in traditional betel mixtures [3].

Betel palm nuts are utilized for their medicinal properties due to the presence of various bioactive compounds derived from terpenoid, flavonoid, and alkaloid groups. These secondary metabolites exhibit diverse chemical structures that correlate with their bioactivity. The primary alkaloid compounds found in betel seeds include *arecoline, arecaidine, guvacoline,* and *guvasin* [4]. Screening of secondary metabolite compounds in betel palm nuts has revealed the presence of flavonoids, tannins, and terpenoids. Further identification during the study also discovered additional compounds, specifically triterpenoids and glycosides [5].

The methanolic extract of betel palm nuts was analyzed using Liquid Chromatography-Mass Spectrometry (LC-MS), which identified ten compounds [6]. To gain a deeper understanding of these compounds, including their relative

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abundances and bioactivities, a non-targeted metabolite profiling analysis is necessary. This can be achieved through metabolomics profiling using Gas Chromatography-Mass Spectrometry (GC-MS). The aim of this analysis is to thoroughly identify the compounds, assess their abundance and classification, and evaluate the bioactivity of the methanolic extract from betel palm nuts. This study could provide valuable insights into the identified metabolite compounds and their potential future applications.

2. Materials and Methods

2.1. Sampling

Samples of betel palm nuts used in this study were collected from Tanjung Menang Village, Rambang Dangku District, Muara Enim Regency, South Sumatra Province, Indonesia, located at the coordinates -4.004940294766184, 105.46143689250549

2.2. Location research

The research was conducted at the Laboratory of Physiology and Development, the Laboratory of Genetics and Biotechnology, and the Department of Biology, Faculty of Mathematics and Natural Sciences at the University of Sriwijaya, as well as at the Laboratory of Integrated Research and Testing at the University of Gadjah Mada.

2.3. Instruments and Chemicals

The Instruments employed in this study included the GC-MS Column HP-5MS UI and a rotary evaporator. The materials used comprised betel palm nuts and 96% methanol

2.4. Preparation of simplicia

The preparation of the betel palm nut sample began with ripe (old) fruit, from which 2,000 gs of betel palm nuts were obtained. The nuts were then dried in a shaded area for 7 days, avoiding direct sunlight. After drying, 900 gs of the nuts were ground into a powder and sieved to ensure uniform particle size. Next, 450 gs of the powdered simplicia was stored at room temperature until the extraction step.

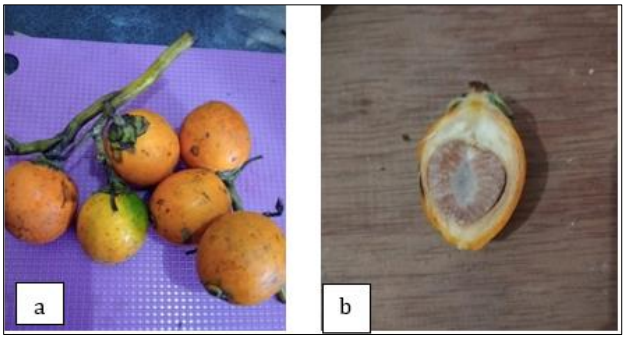

Figure 1 Betel palm fruit: (a) ripe betel palm nuts, (b) betel nut with its fibers

2.5. Extraction and Maceration

For the extraction, the simplicia powder was macerated for two days using a 1:3 ratio of 300 gs of simplicia to 900 mls of 96% methanol. After two days, the filtrate was collected and evaporated using an evaporator at a temperature of 50ºC to obtain a thick extract. This thick extract was then prepared for metabolite profiling analysis.

2.6. Analysis with GC-MS instruments

Metabolite profiling was conducted using GC-MS, where 1 ul of the 96% methanol extract of betel palm nuts was injected according to the protocol for the GC-MS HP-5MS UI Column.

2.7. Data Analysis

The GC-MS analysis provided chromatograms, which were examined for identified chemical components, their chemical structures, retention times, and area abundances. The compounds were identified using *Cromeleon 7* chromatogram

software. Following this, a biosynthesis pathway search was conducted for the dominant compounds using the *PubChem* and *ChEBI* databases, along with a review of existing literature for secondary data on bioactivity.

3. Results and Discussion

3.1. Metabolite Profile of Betel Palm Nuts

The GC-MS chromatogram of the methanol extract from betel palm nuts shows distinctive peaks corresponding to detected metabolite compounds, as illustrated in Figure 2.

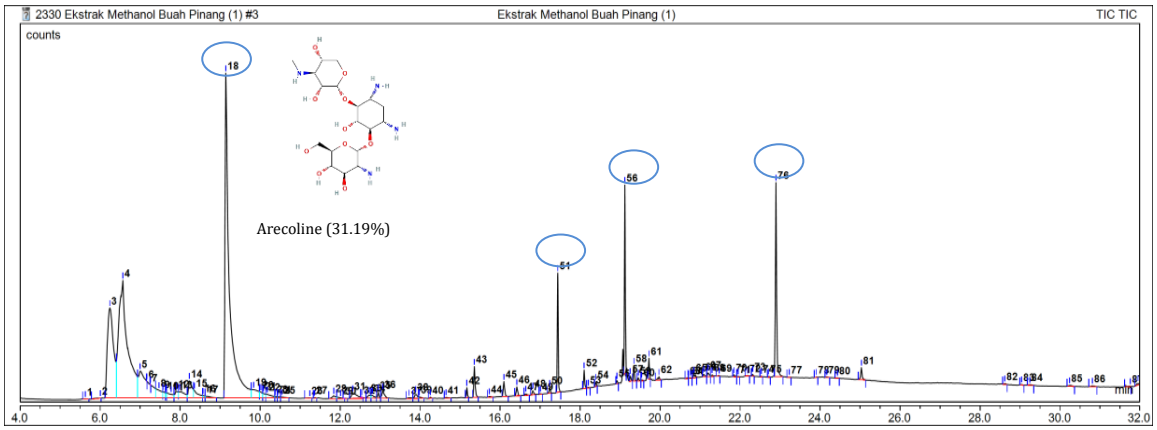

Figure 2 GC-MS chromatogram of methanolic extract from betel palm

The chromatogram presents the abundance of compounds in the methanol extract of betel palm nuts, highlighting different compound peaks that vary based on their abundance and retention times. The analysis identified 88 peaks, with a total abundance of 96.93%. Among these, four main compound peaks are marked with blue circles in the chromatogram shown in Figure 2. Out of the 88 detected peaks, 63 compound component peaks were successfully identified. The four predominant compounds found in the methanol extract of betel palm nuts are as follows: *Arecoline* (31.19%), *Phthalic acid di(2-propylpentyl) ester* (4.99%), 9-*Octadecenoic acid (Z)-methyl ester* (3.51%), and *Hexadecanoic acid, methyl ester* (2.40%).

In addition to *Arecoline*, the extract from betel palm nuts is known to contain other alkaloid compounds, including *Trigonelline; Methyl 1-methyl-1,2,3,6-tetrahydropyridine-3-carboxylate; 3,6-Dimethyl-3,6-dihydro-pyran-2-one oxime;* and *2-Propanol, 1,1'-oxybis-*. According to [7], alkaloids found in betel palm nuts, such as *arecoline, arecolidine, arecain, guvacoline,* and *guvacine*, exert a cholinergic effect. Beyond alkaloids, the extract also contains steroid compounds, amino acids, choline, flavonoids, catechins, and fatty acids (including palmitic, oleic, linoleic, palmitoleic, stearic, and myristic acid), as well as tannins.

Table 1 Metabolite profile, and abundance of metabolite compounds the methanolic extract of betel palm nuts with GC-MS

Retention time (min)	Compound name	Chemical formula	Abundance (%)
5.63	*Cyclopropanetetradecanoic acid. 2-octyl-. methyl ester*	$C_{26}H_{50}O_2$	0.02
6.00	*5-O-Methyl-d-gluconic acid dimethylamide*	$C_9H_{19}NO_6$	0.07
6.24	*2-Propanol. 1.1'-oxybis-*	$C_6H_{14}O_3$	12.35
6.57	*1-Propanol. 2-(2-hydroxypropoxy)-*	$C_6H_{14}O_3$	23.24
7.00	*1-Propanol. 2.2'-oxybis-*	$C_6H_{14}O_3$	6.01
7.17	*1-Propanol. 3.3'-oxybis-*	$C_6H_{14}O_3$	0.00
7.90	*Nipecotic acid. 1-methyl-. methyl ester*	$C_8H_{15}NO_2$	0.46

7.99	*Trigonelline*	$C_7H_7NO_2$	0.81
8.23	*Methyl 1-methyl-1.2.3.6-tetrahydropyridine-3-carboxylate*	$C_8H_{13}NO_2$	1.33
8.35	*Mannosamine*	$C_6H_{13}NO_5$	0.73
8.57	*Acetamide. N-methyl-N-[4-[4-fluoro-1-hexahydropyridyl]-2-butynyl]-*	$C_{12}H_{19}FN_2O$	0.10
8.63	*[1.1'-Bicyclopropyl]-2-octanoic acid. 2'-hexyl-. methyl ester*	$C_{21}H_{38}O_2$	0.17
9.14	*Arecoline*	$C_8H_{13}NO_2$	31.19
9.84	*1.1-Cyclohexane diacetic acid*	$C_{10}H_{16}O_4$	1.18
10.02	*Acetic acid. (3-nitro-2-pyridyl)thio-*	$C_7H_6N_2O_4S$	0.36
10.08	*3.6-Dimethyl-3.6-dihydro-pyran-2-one oxime*	$C_7H_{11}NO_2$	0.24
10.18	*Paromomycin*	$C_{23}H_{45}N_5O_{14}$	0.44
10.55	*Gentamicin a*	$C_{18}H_{36}N_4O_{10}$	0.08
11.36	*Ethanol. 2-(9-octadecenyloxy)-. (Z)-*	$C_{20}H_{40}O_2$	0.02
11.84	*3.7-Diazabicyclo[3.3.1]nonane. 9.9-dimethyl-*	$C_9H_{18}N_2$	0.23
12.01	*Acetamide. N-methyl-N-[4-(3-hydroxypyrrolidinyl)-2-butynyl]-*	$C_{11}H_{18}N_2O_2$	0.13
12.32	*Dimethyl phthalate*	$C_{10}H_{10}O_4$	0.73
12.54	*Tricyclo[4.2.2.0(2.5)]deca-7.9-diene-7.8-dicarboxylic acid. 3-cyano-. dimethyl ester*	$C_{15}H_{15}NO_4$	0.00
12.72	*3-Furanacetic acid. 4-hexyl-2.5-dihydro-2.5-dioxo-*	$C_{12}H_{16}O_5$	0.21
12.81	*10-Heptadecen-8-ynoic acid. methyl ester. (E)-*	$C_{18}H_{30}O_2$	0.21
12.95	*Butylated Hydroxytoluene*	$C_{15}H_{24}O$	0.17
13.06	*Undecanoic acid. 10-methyl-. methyl ester*	$C_{13}H_{26}O_2$	0.41
13.72	*9-Octadecenoic acid. (2-phenyl-1.3-dioxolan-4-yl)methyl ester. cis-*	$C_{28}H_{44}O_4$	0.08
13.89	*Dodecanoic acid. ethyl ester*	$C_{14}H_{28}O_2$	0.26
13.98	*Dodecanoic acid. 2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl ester*	$C_{19}H_{34}O_6$	0.08
14.26	*Estra-1.3.5(10)-trien-17ß-ol*	$C_{18}H_{24}O$	0.04
14.65	*9-Hexadecenoic acid*	$C_{16}H_{30}O_2$	0.06
15.17	*cis-13-Eicosenoic acid*	$C_{20}H_{38}O_2$	0.20
15.36	*Methyl tetradecanoate*	$C_{15}H_{30}O_2$	0.85
15.73	*2-Bromotetradecanoic acid*	$C_{14}H_{27}BrO_2$	0.05
16.09	*Tetradecanoic acid. ethyl ester*	$C_{16}H_{32}O_2$	0.45
16.42	*i-Propyl 12-methyl-tridecanoate*	$C_{17}H_{34}O_2$	0.25
16.64	*Dodecanoic acid. 3-hydroxy-*	$C_{12}H_{24}O_3$	0.08
16.82	*Cyclopenta[g]-2-benzopyran. 1.3.4.6.7.8-hexahydro-4.6.6.7.8.8-hexamethyl-*	$C_{18}H_{26}O$	0.16
17.01	*cis-10-Nonadecenoic acid*	$C_{19}H_{36}O_2$	0.02
17.24	*9-Hexadecenoic acid. methyl ester. (Z)-*	$C_{17}H_{32}O_2$	0.14
17.44	*Hexadecanoic acid. methyl ester*	$C_{17}H_{34}O_2$	2.40

18.10	*Hexadecanoic acid. ethyl ester*	$C_{18}H_{36}O_2$	0.40
18.20	*trans-13-Octadecenoic acid*	$C_{18}H_{34}O_2$	0.03
18.39	*l-(+)-Ascorbic acid 2.6-dihexadecanoate*	$C_{38}H_{68}O_8$	0.11
18.92	*cis-13-Octadecenoic acid*	$C_{18}H_{34}O_2$	0.07
19.12	*9-Octadecenoic acid (Z)-. methyl ester*	$C_{19}H_{36}O_2$	3.51
19.25	*Oleic Acid*	$C_{18}H_{34}O_2$	0.12
19.35	*Heptadecanoic acid. 16-methyl-. methyl ester*	$C_{19}H_{38}O_2$	0.33
19.54	*Octadecanoic acid*	$C_{18}H_{36}O_2$	0.04
19.72	*(E)-9-Octadecenoic acid ethyl ester*	$C_{20}H_{38}O_2$	0.41
20.70	*cis-11-Eicosenoic acid*	$C_{20}H_{38}O_2$	0.04
20.85	*6-Octadecenoic acid. (Z)-*	$C_{18}H_{34}O_2$	0.13
21.09	*cis-Vaccenic acid*	$C_{18}H_{34}O_2$	0.08
21.20	*9-Octadecenoic acid (Z)-. hexyl ester*	$C_{24}H_{46}O_2$	0.15
22.90	*Phthalic acid. di(2-propylpentyl) ester*	$C_{24}H_{38}O_2$	4.99
23.23	*Octadec-9-enoic acid*	$C_{18}H_{34}O_2$	0.03
23.90	*Ethyl iso-allocholate*	$C_{26}H_{44}O_5$	0.02
24.17	*Glycidyl oleate*	$C_{21}H_{38}O_3$	0.04
28.62	*Oleic acid. 3-(octadecyloxy)propyl ester*	$C_{39}H_{76}O_3$	0.04
29.04	*Hexadecanoic acid. 1-(hydroxymethyl)-1.2-ethanediyl ester*	$C_{35}H_{68}O_5$	0.03
30.81	*8.14-Seco-3.19-epoxyandrostane-8.14-dione. 17-acetoxy-3ß-methoxy-4.4-dimethyl-*	$C_{24}H_{36}O_6$	0.07
31.95	*?-Sitosterol*	$C_{29}H_{50}O$	0.10
63 metabolite compounds with total compound abundance (%) 96.93			

The compound with the second highest peak is located at point 76, with an abundance of 4.99%. This compound is *Phthalic acid di(2-propylpentyl) ester*, which belongs to a group of ester compounds known for their allelochemical, antibiotic, antimicrobial, insecticidal, and antidiabetic bioactivities. According to the PubChem database, the *Phthalic acid di(2-propylpentyl) ester* is also referred to by several synonyms, including *di-n-2-propylpentylphthalate, bis(2-propylpentyl) benzene-1,2-dicarboxylate*, and *di(2-propylpentyl) Phthalate*. Note [8] that naturally synthesized *phthalic acid esters* have potential as allelochemicals, antibiotics, and insecticides, which can enhance adaptability.

The methanol extract of betel palm nuts is known to contain a high concentration of fatty acid compounds. Among these, two fatty acids stand out: *9-Octadecenoic acid (Z)-methyl ester* (3.51%) and *Hexadecanoic acid methyl ester* (2.40%). These compounds exhibit various bioactivities, including antioxidant, antitumor, immunostimulant, anticholesteremic, anti-inflammatory, and antibacterial properties. Indicate [9] that derivatives of palmitic acid demonstrate hepatoprotective and anticancer effects. Furthermore, palmitic acid derivatives have been associated with anti-inflammatory, anticancer, antioxidant, hypercholesterolemic, antifungal, antitumor, antibacterial, antiarthritis, and anticoronary properties. These metabolic components hold potential for developing new therapies for various diseases in the future.

3.2. Identification of Metabolite Compound Classes and Bioactivity of Betel Palm Nut Compounds

The compounds detected in the methanol extract of betel palm nuts, as analyzed by GC-MS, belong to several classes including organic acids, alcohols, esters, carbohydrates, fatty acids, steroids, alkaloids, aromatics, phenols, and vitamin C. The classification of these compounds was based on data obtained from *ChEBI* and *PubChem*. Among the alkaloid compounds, *Arecoline* was identified as having the highest abundance at 31.19%. In total, 63 metabolite compounds

were identified in the chromatogram of betel palm nuts, with a notable dominance of alkaloids, alcohols, fatty acids, and esters, as outlined in Table 2.

Table 2 Identification of compound classes and bioactivity of metabolite compounds from betel palm nuts methanolic extract

Relative Area (%)	Compound name	Compound class	Bioactivity	References
0.02	*Cyclopropanetetradecanoic acid, 2-octyl-, methyl ester*	ester (ximenic acid)	Antioxidant, antimicrobial, antiseptic, anticancer, anti-inflammatory, antidiabetic, and antiatherosclerotic	[10]
0.46	*Nipecotic acid, 1-methyl-, methyl ester*	ester (nipecotic acid)	Anticancer, antianemia, antiatherosclerotic, insulin resistance, antidiabetic, anti-inflammatory, and analgesic	[11]
0.17	*[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester*	ester (arachidic acid)	Antidiabetic, anticancer, antihemolytic, Pesticide, skin irritation, and hypocholesterolemia	[12]
0.02	*Ethanol, 2-(9-octadecenyloxy)-, (Z)-*	ester (arachidic acid)	Anticancer, antigonorrhea, and antireverse transcriptase	[13]
0.26	*Dodecanoic acid, ethyl ester*	ester (lauric acid)	Antimicrobial, antioxidant, anticancer, and anticandida	[70]
0.08	*Dodecanoic acid, 2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl ester*	ester (lauric acid)	Antibakterial, antiviral, antiprotozoal, immunity, and antimicrobial	[14]
0.45	*Tetradecanoic acid, ethyl ester*	ester (palmitic)	Anticancer, antidiabetic, anti-inflammatory, hair growth promoter, Alzheimer's treatment and antiatherosclerotic	[15]
0.02	*cis-10-Nonadecenoic acid*	Ester	Antihypertensive, coagulation, antiarrhythmic and antiinfertility	[16]
0.14	*9-Hexadecenoic acid, methyl ester, (Z)-*	Ester	Antimicrobial	[17]
2.40	*Hexadecanoic acid, methyl ester*	ester (palmitic acid)	Antitumor, immunostimulant, antioxidant, anticholesteremic, anti-inflammatory and antibacterial	[18]
0.40	*Hexadecanoic acid, ethyl ester*	ester (asam palmitic acid)	Antioxidant, anti-hypocholesterolemic, Nematicide, pesticide, antiandrogenic, anti-hemolytic	[19])
3.51	*9-Octadecenoic acid (Z)-, methyl ester*	ester (oleic acid)	Antioxidant, anticancer, antimicrobial and antidiabetic	[20]

0.33	*Heptadecanoic acid, 16-methyl-, methyl ester*	ester (stearic acid)	Antioxidant, anticancer, antidiarrheal, anti-inflammatory, Pesticide, nematicide and antimicrobial	[21]
0.41	*(E)-9-Octadecenoic acid ethyl ester*	ester (linoleic acid)	Antibacterial, antibiofilm, antimicrobial, anti-inflammatory, and antidiabetic	[22]
4.99	*Phthalic acid, di(2-propylpentyl) ester*	ester (ester benzic ester)	Allelochemical, antibiotic, antimicrobial, insecticide and antidiabetic	[23]
0.04	*Glycidyl oleate*	ester (oleic acid)	Anticancer	[24]
0.81	*Trigonelline*	Alkaloids	Antimicrobial, anticarcinogenic, antihyperglycemic, and antidegranulation	[25]
1.33	*Methyl 1-methyl-1,2,3,6-tetrahydropyridine-3-carboxylate*	Alkaloids	Anti-inflammatory and antioxidant	[26]
31.19	*Arecoline*	alkaloids pyridine (nicotinic acid)	Antibacterial, antioxidant, antiallergic, anti-inflammatory, antiparasitic, antihelminthic, and analgesic	[27]
0.24	*3,6-Dimethyl-3,6-dihydro-pyran-2-one oxime*	Alkaloids	Antioxsidant	[28]
12.35	*2-Propanol, 1,1'-oxybis-*	Alkaloids	-	-
0.07	*5-O-Methyl-d-gluconic acid dimethylamide*	Aromatic	Antioxidant and antifungal	[29]
0.23	*3,7-Diazabicyclo[3.3.1]nonane, 9,9-dimethyl-*	aromatic (pyrimidine)	Analgesic	[30]
0.16	*Cyclopenta[g]-2-benzopyran, 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-*	aromatic (galactocide)	Pesticide	[31]
0.10	*Acetamide, N-methyl-N-[4-[4-fluoro-1-hexahydropyridyl]-2-butynyl]-*	organic acid (ascorbic acid)	Antioxidant, antimicrobial, anti-inflammatory	[32]
1.18	*1,1-Cyclohexane diacetic acid*	organic acid (ascorbic acid)	Antiepileptic, anti-pain Chronic post-herpetic, anti-pain diabetic neuropathy, anti-neuropathic pain Central and antinociceptive	[33]
0.36	*Acetic acid, (3-nitro-2-pyridyl)thio-*	organic acid (ascorbic acid)	Anti-inflamatory	[34]
0.13	*Acetamide, N-methyl-N-[4-(3-hydroxypyrrolidinyl)-2-butynyl]-*	organic acid (ascorbic acid)	Antioxidant, antimicrobial, anti-inflammatory	[34]

0.21	*3-Furanacetic acid, 4-hexyl-2,5-dihydro-2,5-dioxo-*	organic acid (ascorbic acid)	Antioxidant dan antibacterial	[35]
0.73	*Mannosamine*	carbohydrates (monosaccharides)	Antitumor	[36]
0.44	*Paromomycin*	carbohydrates (monosaccharides)	Antibiotics	[37]
0.08	*Gentamicin a*	carbohydrates (monosaccharides)	Antibiotics	[38]
0.17	*Butylated Hydroxytoluene*	phenol (butyl hydroxy toluene)	Antioxidant, anti-inflammatory, antidiabetic, immunoregulating, anticancer, antimicrobial, and antiseptic	[39]
0.11	*l-(+)-Ascorbic acid 2,6-dihexadecanoate*	vitamin C (ascorbic acid)	Antioxidant, anticoagulant, anti-inflammatory, antitumor, anticonvulsant, antidiabetic, antidiarrheal, antiglaucoma, antiseptic, antistroke, antituberculosis, hypolipidemic, termiticide and antiviral agent	[40]
0.04	*Estra-1,3,5(10)-trien-17ß-ol*	Steroids	Antioxidant and antiatherosclerotic	[41]
0.02	*Ethyl iso-allocholate*	Steroids	Antibacterial, antimicrobial, anti-inflammatory and anti-asthmatic	[42]
0.07	*8,14-Seco-3,19-epoxyandrostane-8,14-dione, 17-acetoxy-3ß-methoxy-4,4-dimethyl-*	Steroids	Antidiabetic	[43]
0.10	*?-Sitosterol*	Steroids	Anti-inflammatory, antioxidant, antiproliferative, antibacterial, antimalarial and anticancer	[44]
23.24	*1-Propanol, 2-(2-hydroxypropoxy)-*	Alcohol	-	-
6.01	*1-Propanol, 2,2'-oxybis-*	Alcohol	-	-
0.21	*10-Heptadecen-8-ynoic acid, methyl ester, (E)-*	fatty acids (α-linolenic acid)	Antiatherosclerotic, anticancer, anti-inflammatory and antiallergic, neuroprotective agent, immunostimulant	[45]
0.41	*Undecanoic acid, 10-methyl-, methyl ester*	fatty acids (undesylenic acid)	Antioxsidant	[46]
0.08	*9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis-*	fatty acids (oleic acid)	Antiandrogenic, anticancer, anti-inflammatory, anemiagenic, dermatitigenic, and hypocholesterolemic	[47]
0.06	*9-Hexadecenoic acid*	fatty acids (palmitoleic acid)	Anticancer, antidiabetic, antiobesity,	[48]

			antiatherosclerotic, and antihepatic steatocy	
0.20	*cis-13-Eicosenoic acid*	fatty acids (paulinic acid)	Anti-inflammatory	[49]
0.85	*Methyl tetradecanoate*	fatty acid (myristic acid)	Antioxidant, hypocholesterolemic, and anticancer	[18]
0.05	*2-Bromotetradecanoic acid*	fatty acid (myristic acid)	Antifungal, antiviral, anticancer, antiparasitic, and immunity	[50]
0.25	*i-Propyl 12-methyl-tridecanoate*	fatty acid (palmitic acid)	Antibacterial, antiviral, antifungal, and anti-inflammatory	[51]
0.08	*Dodecanoic acid, 3-hydroxy-*	fatty acid (lauric acid)	Antibacterial, antifungal, and antiviral	[52]
0.03	*trans-13-Octadecenoic acid*	fatty acid (elaidic acid)	Antibacterial, antifungal, and anticancer	[53]
0.07	*cis-13-Octadecenoic acid*	fatty acid (elaidic acid)	Antibacterial, anti-inflammatory, anticancer, and antifertility	[54]
0.12	*Oleic Acid*	fatty acid (oleic acid)	Antioxidant, anticancer, antitumor, and anti-inflammatory	[55]
0.04	*Octadecanoic acid*	fatty acid (stearic acid)	Anticancer and anti-inflammatory, anesthetic, anthelmintic, and antioxidant	[40]
0.04	*cis-11-Eicosenoic acid*	fatty acid (gondoic acid)	Antibacterial, and antifungal	[56]
0.13	*6-Octadecenoic acid, (Z)-*	fatty acid (petroselinic acid)	Antioxidant, anticancer, antimicrobial, immunoregulatory and antiviral	[10]
0.08	*cis-Vaccenic acid*	fatty acid (vaccenic acid)	Antibacterial, antioxidant, antidiabetic, insulin, anti-inflammatory, arachidonic acid inhibitor and hypocholesterolemia	[57]
0.15	*9-Octadecenoic acid (Z)-, hexyl ester*	fatty acids (selacholeic acid)	Antioxidant and antimicrobial	[58]
0.03	*Octadec-9-enoic acid*	fatty acid (elaidic acid)	Antitumor, antibacterial, Antiviral, antifungal, anticancer and anti-inflammatory	[59]
0.04	*Oleic acid, 3-(octadecyloxy)propyl ester*	fatty acid (oleic acid)	Anti-inflammatory, antioxidant, antiproliferation, antibacterial, and anticancer	[60]
0.03	*Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester*	fatty acid (palmitic acid)	Antioxidant, antibacterial, and antifunga	[61]

According to Table 2, betel palm nuts contain a variety of metabolite compounds, each with potential bioactivities. These compounds serve multiple functions, including antioxidant, anticancer, antibacterial, antiviral, anti-inflammatory, antitumor, antibody production, antidiabetic, antihelminthic, antimalarial, immunostimulant, antihypertensive, anticoagulant, antimicrobial, and analgesic properties. Additionally, several other bioactivities are currently being researched for their potential applications in treatment and health.

Anticancer bioactivity involves identifying natural compounds that can serve as treatments or therapies for combating cancer cells. This term refers to the capability of a compound or natural material to inhibit the growth of cancer cells or even induce their death. Research conducted [62], highlights several active compounds derived from betel palm nut (*Areca catechu* L.), including *protoanthocyanidin, arecaidine, arecoline, guvacoline, isoguvacine*, and *guvacine*, which have shown potential in enhancing sensitivity to anticancer treatments. Among these, proanthocyanidin stands out due to its strong affinity as an NFκB inhibitor, making it particularly effective against breast cancer cells.

3.3. Abundance of Betel Palm Nut Metabolite Compound Classes

The abundance of metabolite compounds in the methanol extract of betel palm nuts, categorized by compound class, is illustrated in Figure 2 below.

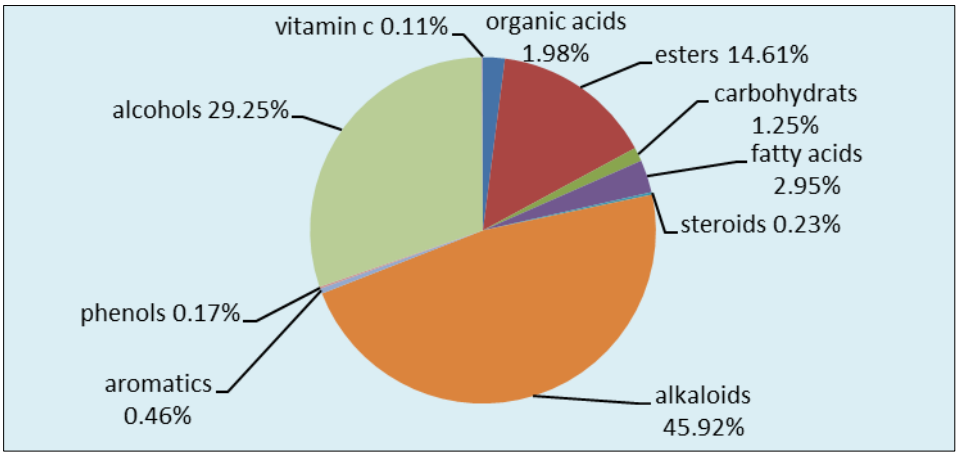

Figure 3 Abundance of Betel Palm Nuts Methanolic Extract Metabolite Compounds by Compound Class

According to Figure 3, there are ten classes of compounds identified in the methanol extract of betel palm nuts. Each class contains a different number of compound types. The results from the GC-MS analysis indicate that the alkaloid class dominates in terms of abundance, comprising 45.92% of the total compounds analyzed. Following the alkaloid class, the other classes present include esters, fatty acids, steroids, alcohols, and several additional groups.

Chemical bioactive compounds are produced naturally by plants through primary or secondary metabolism. These organic compounds serve various biological functions, such as defending against predators (insects and fungi), competing for resources, and protecting plants from environmental stressors like pollution and drought. Additionally, these bioactive compounds can contribute to human health by helping to prevent or combat various diseases.

One notable compound, *arecoline*, which makes up 31.19% of the total, is a pyridine-type alkaloid derived from the shikimic acid pathway. *Arecoline* exhibits multiple beneficial bioactivities, including antioxidant, antibacterial, antiallergic, anti-inflammatory, antiparasitic, antihelminthic, and analgesic properties.

Another significant group of compounds found in the methanol extract of betel palm nuts falls under the fatty acid class. This includes both saturated and unsaturated fatty acids. One prominent unsaturated fatty acid identified is oleic acid, also known as *omega-9*, which is commonly found in vegetable oils, particularly olive oil. According to, [55] oleic acid is a monounsaturated fatty acid (C18 omega-9) present as an ester in triglycerides and glycerophospholipids. Research indicates that *omega-9*, particularly oleic acid, may reduce cancer risk, as several studies have demonstrated its ability to inhibit cell proliferation in various tumor and cancer cell lines and its anti-inflammatory effects [63].

The results of the GC-MS analysis of betel palm nuts are presented in Table 2. A total of 20 metabolite compounds from the fatty acid class were identified, including: *10-Heptadecen-8-ynoic acid, methyl ester, (E)-*; *Undecanoic acid, 10-methyl-*

, methyl ester; *9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester; cis-*, *9-Hexadecenoic acid; cis-13-Eicosenoic acid*; *Methyl tetradecanoate*; *2-Bromotetradecanoic acid*; *Octadecanoic acid*; *i-Propyl 12-methyl-tridecanoate*; *Dodecanoic acid, 3-hydroxy-*; *trans-13-Octadecenoic acid*; *cis-13-Octadecenoic acid*; *Oleic acid*; *cis-11-Eicosenoic acid*; *6-Octadecenoic acid, (Z)-*; *cis-Vaccenic acid*; *9-Octadecenoic acid (Z)-*, *hexyl ester*; *Octadec-9-enoic acid*; *Oleic acid, 3-(octadecyloxy)propyl ester*; dan *Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester*. Among these compounds, *octadecanoic acid*, also known as stearic acid, is a saturated fatty acid recognized for its numerous potential bioactivities. These include anticancer, anti-inflammatory, anesthetic, anthelmintic, and antioxidant properties.

In Table 2, there are 17 ester compounds, including *Cyclopropanetetradecanoic acid 2-octyl methyl ester; Nipecotic acid 1-methyl methyl ester; [1,1'-Bicyclopropyl]-2-octanoic acid 2'-hexyl methyl ester; Ethanol 2-(9-octadecenyloxy)-(Z); Dimethyl phthalate; Dodecanoic acid ethyl ester; Dodecanoic acid 2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl ester; Tetradecanoic acid ethyl ester; 9-Octadecenoic acid (Z)-methyl ester; cis-10-Nonadecenoic acid; Heptadecanoic acid 16-methyl methyl ester; (E)-9-Octadecenoic acid ethyl ester; 9-Hexadecenoic acid methyl ester (Z); Phthalic acid di(2-propylpentyl) ester;* and *Glycidyl oleate*. According [64], esters are compounds formed from the reaction between carboxylic acids and alcohols. These derivatives of alkanoic acids have lower boiling points than carboxylic acids and can dissolve in organic solvents. Esters are commonly found in many fruits and are known for their fragrant aromas.

Another known class of compounds is organic acids. In total, five organic acid compounds were identified, accounting for 1.98% of the overall abundance, as shown in Figure 3. The compounds within this class include *Acetamide, N-methyl-N-[4-[4-fluoro-1-hexahydropyridyl]-2-butynyl]-; 1,1-Cyclohexane diacetic acid; Acetic acid, (3-nitro-2-pyridyl) thio-; 3-Furanacetic acid, 4-hexyl-2,5-dihydro-2,5-dioxo-;* and *Acetamide, N-methyl-N-[4-(3-hydroxypyrrolidinyl)-2-butynyl]-*. These organic acids were extracted from mature betel palm nuts. During the ripening process, various biochemical, physiological, and organoleptic changes occur, including alterations in carbohydrate levels, sugar content, phenolic compounds, organic acids, color, texture, and volatile aroma compounds. The organic acids found in betel palm nuts exhibit various bioactivities, such as antioxidant, antibacterial, anti-inflammatory, anti-epileptic properties, and relief from chronic post-herpetic pain, diabetic neuropathy pain, central neuropathic pain, and nociception. According [65], organic acids can be categorized based on the type of carbon chain (aliphatic, alicyclic, aromatic, or heterocyclic), saturation, substitution, and the number of functional groups. Examples of organic acids commonly found in food include malic acid, lactic acid, fumaric acid, pyroglutamic acid, oxalic acid, ascorbic acid, citric acid, and tartaric acid.

As shown in Figure 3, there is a class of vitamin C compounds known as *l-(+)-Ascorbic acid 2,6-dihexadecanoate*, which exhibits a variety of potential bioactivities. These include antioxidant, anticoagulant, anti-inflammatory, antitumor, anticonvulsant, antidiabetic, antidiarrheal, antiglaucoma, antifertility, anti-gastric, antimalarial, antiprotozoal, antiatherosclerotic, antifatigue, anti-hepatic, antihypertensive, anti-plague, antiproliferative, antiseptic, antistroke, antituberculosis, hypolipidemic, neuroprotective, neurotransmitter, termiticide, and antiviral effects. According[66], vitamin C, or *L-ascorbic acid*, acts as a natural antioxidant that is soluble in water. Its hydrophilic properties can reduce the ability to stabilize fats and oils.

Additionally, the methanol extract of betel palm nuts contains three metabolites classified as carbohydrates: *mannosamine; paromomycin;* and *gentamicin A*. Together, these compounds represent 1.25% of the extract and are illustrated in Figure 2. All three compounds demonstrate bioactivity as antibiotics and antitumor agents. According to research [67], carbohydrates can be categorized into polysaccharides, aldehydes, ketones, and their derivatives. The classification of carbohydrates includes monosaccharides, disaccharides, and polysaccharides. Monosaccharides, regarded as simple carbohydrates, consist of a small number of carbon atoms and cannot be hydrolyzed into other carbohydrates under mild conditions. These monosaccharides are colorless, soluble in water, and insoluble in nonpolar solvents.

Another group of compounds known as steroids plays a vital role in various biological processes. They are essential for maintaining salt balance, regulating metabolism, and improving the function of sexual organs, as well as other functions that differentiate between sexes. Plant-derived steroids exhibit anticarcinogenic effects and can help reduce cholesterol levels. According [68], steroids are a category of secondary metabolites with significant potential for bioactivity, including bioinsecticide, antibacterial, antifungal, and antidiabetic properties.

In Figure 3, additional metabolites found in betel palm nuts include compounds from the phenol class, specifically *Butylated hydroxytoluene* (*BHT*). Phenolic compounds are present in nearly all plants and play a crucial role in various physiological processes related to oxidative stress. These compounds can interact with proteins and bind to free radicals. Research [69], emphasizes the importance of phenolic compounds in food products, particularly their bioactivity—the effects these compounds have on the human body after exposure. Phenolic compounds demonstrate

potential health benefits, including antioxidant, anticarcinogenic, and antimicrobial properties, making them valuable for overall health.

4. Conclusion

The analysis of the metabolite profile from the methanolic extract of betel palm nuts was conducted using gas chromatography-mass spectrometry (GC-MS) and revealed a diverse array of metabolite compounds. A total of 88 peaks were detected, which led to the identification of 63 distinct compounds. Among these, four compounds were particularly predominant: *arecoline* (31.19%), *phthalic acid di(2-propyl pentyl) ester* (4.99%), *9-octadecenoic acid (Z)-methyl ester* (3.51%), and *hexadecanoic acid methyl ester* (2.40%). *Arecoline*, a compound unique to betel palm nut extract, belongs to the class of pyridine-type alkaloids derived from the shikimic acid biosynthesis pathway. This compound exhibits cholinergic activity and possesses various bioactive properties, including antioxidant, antibacterial, antiallergic, anti-inflammatory, antiparasitic, antihelminthic, and analgesic effects.

Further research is recommended to investigate the metabolite profile of betel palm nuts concerning the ripeness of the fruit, utilizing additional metabolomic analyses.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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