Research Article

Extraction, Profiling, and Characterization of Phytosterols and Triterpenoids from Pili (*Canarium ovatum* Engl.) Pulp Oil Exhibiting Antioxidant and Antibacterial Properties

Nico G. Dumandan [ID],[1] **Annie Cita T. Kagaoan** [ID],[1] **Ranelle D. P. Acda** [ID],[1] **Caren R. Tumambing** [ID],[1] **and Laura J. Pham** [ID] [2]

[1]*National Institute of Molecular Biology and Biotechnology (BIOTECH), University of the Philippines Los Baños College, Los Baños 4031, Laguna, Philippines*
[2]*Biozym Systems Enterprises, Bay 4033, Laguna, Philippines*

Correspondence should be addressed to Nico G. Dumandan; ngdumandan@up.edu.ph

Pili (*Canarium ovatum* Engl.), an indigenous tree found in the Philippines, is highly regarded for its fruit due to its high economic value. During processing, the pulp is often discarded as waste but contains considerable amounts of oil and bioactive minor lipid components. The present study explored the antioxidant and antibacterial properties of saponified diethyl ether extract of pili pulp oil and related this activity to the nature of compounds present in the extract through GCMS. The extract indicated the elution of 18 major compounds which are mostly cyclic triterpenic (*α*-and *β*-amyrin, lupenone, and *β*-amyrone) and phytosterol (*β*-sitosterol, brassicasterol, and stigmasterol) class of compounds. Characterization of the bioactivity of the extract showed high antioxidant activities measured by DPPH radical scavenging (EC$_{50}$: 74.45 ± 1.29 μg/mL) and lipid peroxidation inhibition (EC$_{50}$: 3.02 ± 0.06 μg/mL) activities that were comparable with that of *α*-tocopherol. Moreover, an observed bactericidal activity was demonstrated by the extract against *E. coli* and *S. typhi* with MIC values of 40 and 35 μg/mL, respectively. The observed bioactivity of the pili pulp oil extract can be attributed to these compounds which may provide desirable health benefits.

1. Introduction

Canarium ovatum Engl., locally known as pili, is an indigenous tree commonly found in the Philippines which is cultivated for its edible fruit [1]. Pili nut kernel is the most valuable part of the fruit due to its high economic value owing to its increasing competitiveness in the global confectioneries market [2]. In pili nut processing, its pulp is often discarded as waste, but it contains an appreciable amount of oil and important minor lipid species such as carotenoids, phytosterols, and tocopherols [3, 4]. These nutritionally beneficial minor lipid compounds have gained considerable interest, particularly in their bioactivity which plays an important role in the development of high-value products.

Antioxidants and antimicrobial agents play a significant role in the food sector primarily because bacterial growth and lipid oxidation are the main factors that determine food quality loss and shelf-life reduction. Oftentimes, synthetic additives such as BHA/BHT are commonly added to food products to inhibit the process of lipid oxidation and microbial growth and to extend their shelf-life. However, a shift to naturally derived compounds is seen and increasingly being sought by many companies due to possible adverse effects associated with long-term intake of synthetic compounds [5, 6]. Phytosterols, including other cyclic triterpenes which constitute the majority of unsaponifiable fractions of seeds oils, are known to have several bioactive properties linked to various implications on human health, including anti-inflammatory, antioxidative, antimicrobial,

cholesterol-lowering, and anticarcinogenic activities [7–12]. On the other hand, triterpenoid extracts that are rich in lupeol, betulinic acid, and amyrin have been shown to inhibit the growth of foodborne pathogenic bacteria particularly the methicillin-resistant *S. aureus*, *E. faecalis,* and *P. aeruginosa* are studied by Amoussa et al. [13] and Nzogong et al. [10]. Phenolic compounds have been shown to fuse with extracellular soluble proteins of the microbial cell wall resulting in the suppression of microbial growth and/or oxidative damage [14].

Hence, the present study explored the potential of minor lipid components in pili pulp oil as a source of phytosterols and cyclic triterpenoids with antioxidant and antibacterial properties. The interest in these naturally derived compounds is not only due to their biological activity but also to maximize the economic potential of pili pulp oil.

2. Materials and Methods

2.1. Materials. Pili (*Canarium ovatum* Engl.) fruits were obtained from a local market at Goa, Camarines Sur, Philippines. Microbial strains (*E. coli, P. aeruginosa, S. tyhpi, S. aureus*, and *B. cereus*) used for the antibacterial activity assay were obtained from the Philippine National Collection of Microorganisms (PNCM), BIOTECH, UPLB, College, Los Baños, Laguna, Philippines. All other chemicals and standards were purchased commercially.

2.2. Sample Preparation and Oil Extraction. Pili fruits were manually depulped by blanching in lukewarm water for about 15–20 min. The pulps were then collected and dried in a convection-type oven at 70°C overnight or until moisture content reached about less than 3%. Extraction of oil was carried out by using *n*-hexane at 1 : 4 ratio of dried pulp weight (g) to solvent volume (mL). After 12 h of extraction at room temperature with constant agitation, the oil was recovered by solvent evaporation by using a rotary evaporator.

2.3. Saponification and Fractionation. The unsaponifiable fraction of oil was obtained by saponification following the method of Almeida et al. [15] with some modifications. A 0.3 g of the oil sample was saponified by using 10 mL 3% w/v ethanolic potassium hydroxide at 50°C for 3 h. Then, the solution was cooled by adding 10 mL of distilled water. Subsequent fractionation of the phytosterol and triterpenoids was carried out by repeated liquid-liquid extraction by using 10 mL diethyl ether three times. The organic layers were then combined, washed twice with 10 mL of distilled water, and dried over anhydrous sodium sulfate. The saponified diethyl ether extract (SDEE) of pili pulp oil was then collected upon filtration and solvent evaporation under a stream of nitrogen gas.

2.4. GCMS Profiling. Profiling of SDEE was performed on a Shimadzu GCMS-QP2020 equipped with a Shimadzu AOC-20i Plus auto-injector (Shimadzu Corp., Kyoto, Japan) under electron impact ionization at 70 eV. Separation of components was carried out in SH-Rxi-5Sil MS capillary column with dimensions of 30 m × 0.25 mm ID × 0.25 μm film thickness (Shimadzu Corp., Kyoto, Japan). The initial oven temperature was held at 190°C for 1 min then raised to 300°C at 15°C/min and kept constant for 10 min. Helium was used as the carrier gas with a constant flow rate of 1 mL/min. The injector, MS ion source, and MS interface temperatures were set at 310, 230, and 280°C, respectively. A sample injection of 1 μL was performed in a split mode of 10 : 1 and peaks were detected in full scan acquisition mode from m/z 50 to 500. Identification of the individual components was performed by NIST mass spectral library on the basis of the mass fragment and e/z values of each component. The relative concentration of each peak was computed based on the total ion count.

2.5. DPPH Radical Scavenging Activity. The scavenging activity of SDEE against DPPH radical was evaluated by using the method of [16] with some modifications. Briefly, 3.8 mL of 0.07 mM DPPH solution in chloroform was mixed with 0.2 mL of the sample with varying concentrations. The mixture was incubated in the dark at room temperature for 30 min. Chloroform and α-tocopherol were used as a blank and positive control, respectively. The absorbance of the resulting mixture was measured at 516 nm and the percent radical scavenging activity was calculated based on the following equation:

$$\text{DPPH Radical Scavenging Activity} = \left[1 - \frac{A_{\text{sample}}}{A_{\text{blank}}}\right] x \, 100. \quad (1)$$

The EC_{50} value is defined as the concentration of sample required to scavenge 50% of DPPH radical under the assayed conditions.

2.6. Lipid Peroxidation Inhibition Activity. A mixture of 0.1 mL of 0.1–10 μg/mL of SDEE (in chloroform) and 0.5 mL of 10% egg yolk homogenate (w/v in distilled water) was mixed in a screwcapped tube and the volume was made up to 1.0 mL by adding distilled water. Then, 0.05 mL 0.07 M FeSO$_4$ (in distilled water) was added, and the mixture was incubated at 37°C for 30 min to induce lipid peroxidation. A 0.05 mL of 20% (w/v) trichloroacetic acid solution (TCA) in distilled water and 1.0 mL 0.1% (w/v) thiobarbituric acid solution (TBA) in distilled water was added to the mixture, vortexed, and heated in a water bath at 80°C for 30 min. After cooling, 3 mL of butanol was added, and the mixture was centrifuged at 3000 rpm for 5 min. The absorbance of the upper layer was measured against 3 mL butanol at 512 nm. Chloroform and α-tocopherol were used as blank and positive controls, respectively. The lipid peroxidation inhibition activity was calculated by using the following equation:

$$\text{Lipid peroxidation inhibition \%} = \left[1 - \left(\frac{A_{512\,\text{sample}}}{A_{512\,\text{blank}}}\right)\right] x \, 100. \quad (2)$$

The EC_{50} value is defined as the concentration of sample required to inhibit 50% of lipid peroxidation under the assayed conditions.

2.7. Antibacterial Activity

2.7.1. Disk Diffusion Assay. Qualitative antimicrobial activity of SDEE against *S. aureus, B. cereus, E. coli, S. typhi,* and *P. aeruginosa* was carried out by disk diffusion method [17]. Briefly, a suspension of the test microorganism standardized to 0.5 McFarland with approximately 1.5×10^{8} CFU/mL was uniformly spread onto individual solid media plates of Muller–Hinton agar by using a sterile cotton swab. Disks of 6 mm in diameter were impregnated, until saturation, with 0.1 mg/mL of the extract. The disks were then allowed to dry placing in the inoculated agar. Chloramphenicol and chloroform served as positive and negative controls, respectively. The inoculated plates were incubated at 37°C for 18–24 h. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms.

2.7.2. Minimum Inhibitory Concentration Assay. The MIC of the extract exhibiting sensitivity to the tested microorganism based on the disk diffusion assay was determined following the methods outlined by Eloff [18] with some modifications. First, a stock solution of the extract (1 mg/mL) was prepared in Meuller Hinton broth supplemented with 0.02% Tween 80. The solution was then sonicated for 30 s and vortex homogenized for 2 min to obtain a stable emulsion. Serial dilutions of the extract in broth were prepared in microtubes of 1 mL with a concentration range from 0.1 to 100 μg/mL. Then, 295 μL of each dilution was transferred into a 96-well microplate. A 5 μL of test bacterial suspension (1.0×10^{6} CFU/mL) was inoculated to obtain a final concentration of 1.67×10^{4} CFU/mL and a final volume of 300 μL per well. The inoculum (positive control) and culture medium (negative control) were put into the first column of the microplate, and the chloramphenicol antibiotic control ranging from 0.5 to 75 μg/mL was in the final column. Each plate was wrapped loosely with cling film to prevent dehydration during incubation for 18–24 h at 37 °C. Subsequently, 10 μL of bacterial growth indicator, resazurin at 6.75 mg/mL, was added to wells, which were then incubated for 30 min at 37 °C. The lowest concentration of the extract that visually showed no growth was determined as MIC.

2.8. Statistical Analysis.
All experiments and measurements were carried out in triplicate. The data were presented as mean ± SEM and were analyzed by using one-way ANOVA. Means were compared using Tukey's post hoc comparison test by using R software (version 4.2.1, R Foundation for Statistical Computing, Vienna, Austria). Significant differences between means were determined at $P < 0.05$.

3. Results and Discussion

3.1. Oil Extraction, Fractionation, and GCMS Profiling of the Diethyl Ether Unsaponifiable Extract of Pili Pulp Oil. Extraction of oil revealed $23.92 \pm 0.21\%$ yield, expressed in dry weight basis of pulp. The unsaponifiable fraction of oil obtained from diethyl ether extraction showed $1.0294 \pm 0.0501\%$. These values were slightly lower than our previous findings [3] which can be attributed to the varietal differences of pili fruit used, for which this was also observed in the report of Tugay et al. [4] with 0.47 to 0.53% UM content depending on the fruit's variety. Analysis of the chromatogram obtained from the SDEE extract indicated the elution of 18 major compounds which are mostly triterpenic and phytosterol class of compounds (Figure 1, Table 1).

In terms of relative abundance with respect to total ion count (TIC), β-Amyrin showed the highest abundance of around 34.75%, followed by α-amyrin and β-sitosterol with 20.92 and 15.78%, respectively. Other phytosterols identified in SDEE include brassicasterol, stigmasterol, campesterol, and fucosterol (Figure 2). These were consistent with our previous study where the major sterols present are stigmasterol and campesterol [3]. In addition, it also contains other pentacyclic triterpenes such as lupenone, amyrone, cycloartenol, and betulinaldehyde (Figure 3).

Amyrin, both α-and β-isomers, are generally found in different plant part extracts such as in *C. tramdenum* bark which contains 0.03 mg/g β-amyrin [19]. Areal parts of *M. barteri* and *S. longifolia* also contain high amounts of α-amyrin as reported by Ce et al. [20] and Saeidnia et al. [21]. Other than plant parts, amyrin along with other sterols were also detected in oil samples such as in olive, flaxseed, and camellia seed oils [22, 23].

3.2. Antioxidant Activity. The antioxidant activity of the SDEE was analyzed in terms of scavenging activity against DPPH radical and the ability to inhibit lipid peroxidation in the egg yolk system. As shown in Table 2, SDEE showed comparable EC_{50} values of 3.02 μg/mL with that of α-tocopherol (2.92 μg/mL), a known antioxidant compound, though a moderate activity was observed in terms of its DPPH radical scavenging activity with a slightly higher EC_{50} value of 74.45 μg/mL in SDEE as compared to 65.91 μg/mL in control.

Terpenoids and sterols, in general, have been shown to exert antioxidant properties owing to their hydroxyl group that participates via hydrogen atom transfer or single electron transfer mechanism in quenching reactive oxygen species [24, 25]. It has been demonstrated that phytosterol components such as campesterol, β-sitosterol, and stigmasterol have been shown to exhibit scavenging activities against DPPH radical and provide hepato- and neuro-protection of hydrogen peroxide-induced oxidative stress levels [25, 26]. Isomers of amyrin isolated from *Myrcianthes pungens* leaves revealed high antioxidant activity with antioxidant protection equivalent to the Trolox of 137 and 129% for α-and β-amyrin, respectively, at 500 μg/mL [27].
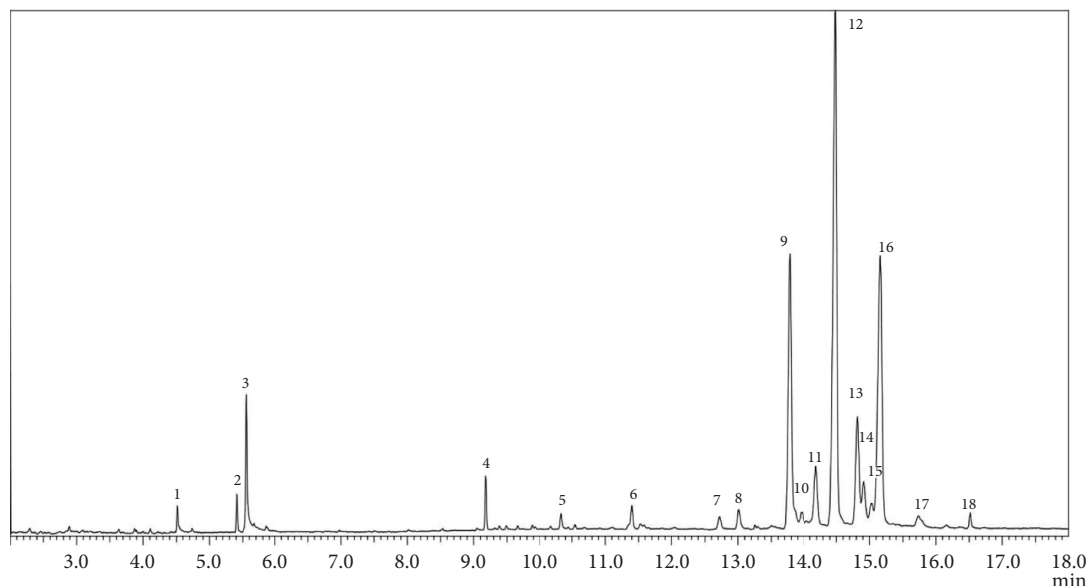
FIGURE 1: GCMS chromatogram of the saponified diethyl ether extract of pili pulp oil. Refer to Table 1 for peak identities.

TABLE 1: GCMS profiling of the saponified diethyl ether extract from pili pulp oil.

Peak no	Name of the compound	R_T (min)	Molecular formula	Molecular weight	Relative abundance (% of TIC)
Phytosterols and triterpenoids					
4	Squalene	9.181	$C_{30}H_{50}$	410.7	1.37
5	Stigmasta-3,5-diene	10.335	$C_{29}H_{48}$	396.7	0.42
6	Brassicasterol	11.400	$C_{28}H_{46}O$	398.7	1.12
7	Campesterol	12.719	$C_{28}H_{48}O$	400.7	0.66
8	Stigmasterol	13.007	$C_{29}H_{48}O$	412.7	0.92
9	β-Sitosterol	13.781	$C_{29}H_{50}O$	414.7	15.78
10	Fucosterol	13.961	$C_{29}H_{48}O$	412.7	0.53
11	β-Amyrone	14.188	$C_{30}H_{48}O$	427.7	4.33
12	β-Amyrin	14.483	$C_{30}H_{50}O$	426.7	34.75
13	Lupenone	14.816	$C_{30}H_{48}O$	424.7	7.19
14	Cycloartenol	14.912	$C_{30}H_{50}O$	426.7	3.15
15	Lanosterol	15.030	$C_{30}H_{50}O$	426.7	1.78
16	α-Amyrin	15.159	$C_{30}H_{50}O$	426.7	20.92
17	β-Amyrin acetate	15.735	$C_{32}H_{52}O_2$	468.8	1.15
18	Betulinaldehyde	16.515	$C_{30}H_{48}O_2$	440.7	0.42
Nonterpenoid compounds					
1	Palmitic acid	4.516	$C_{16}H_{32}O_2$	256.4	0.78
2	Phytol	5.412	$C_{20}H_{40}O$	296.5	0.86
3	Oleic acid	5.554	$C_{18}H_{34}O_2$	282.5	3.85

3.3. Antibacterial Activity. The antibacterial potential of SDEE against 5 common food pathogens is shown in Table 3. The extract was found to exhibit significant inhibitory activity against *E. coli* and *S. typhi* with 10.3 and 11.7 mm zone of inhibition, respectively, at 0.1 mg/mL. The MIC showed that 40 and 35 μg/mL of the extract were able to inhibit the growth of *E. coli* and *S. typhi*, respectively. The extract was not active or did not show antibacterial activity in *S. aureus*, *B. cereus*, and *P. aeruginosa* at 0.1 mg/mL.

The antibacterial activity of SDEE can be attributed to the high amyrin content which was also observed by Abdel-Raouf et al. [28] in several algal extracts containing β-amyrin exerting antibacterial activities against the 5 pathogens tested in this study. In a separate study, hexane extract of *Manilkara subsericea* fruit which is mostly α-andβ-amyrin acetate showed a MIC value of 250 μg/mL against *S. aureus* [29]. Aside from amyrin, Alawode et al. [30] reported that phytosterols, specifically stigmasterol and β-sitosterol that
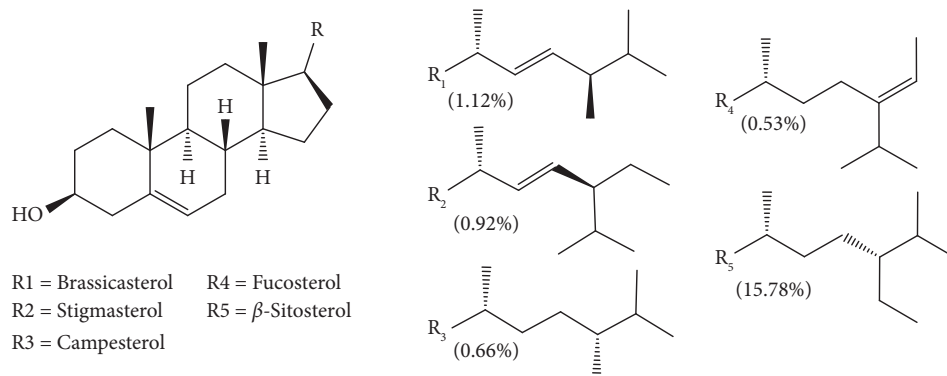
FIGURE 2: Structures of phytosterols identified in the saponified diethyl ether extract of pili pulp oil. Values in parenthesis denote relative abundance in percent of total ion count.
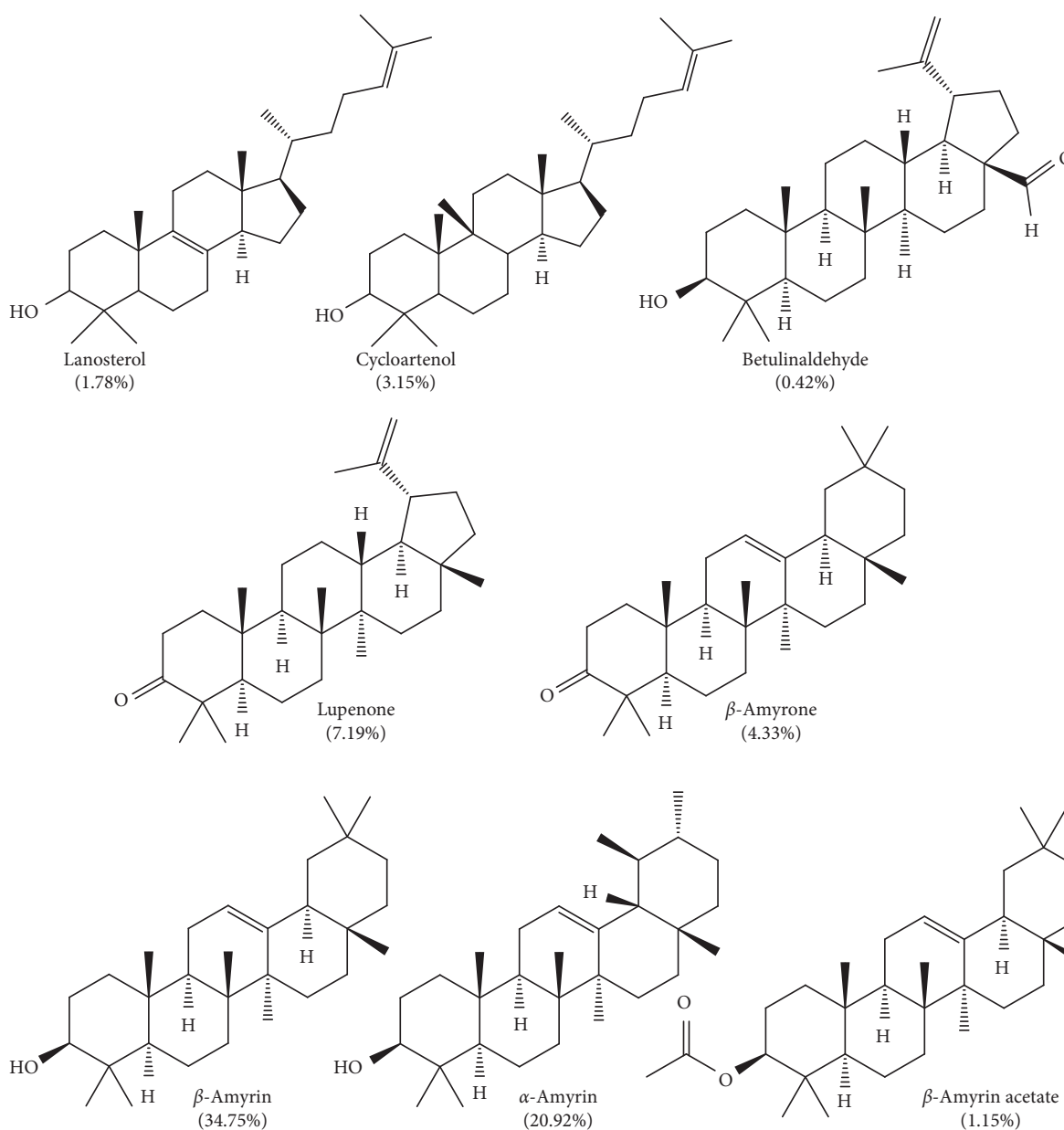

FIGURE 3: Structures of cyclic triterpenoids identified in the saponified diethyl ether extract of pili pulp oil. Values in parenthesis denote relative abundance in percent of total ion count.

TABLE 2: Antioxidant activities of the saponified diethyl ether extract pili pulp oil.

Antioxidant assay	Activity EC_{50} (μg/mL)	
	SDEE	α-tocopherol (control)
DPPH radical scavenging	74.45 ± 1.29^a	65.91 ± 0.05^b
Lipid peroxidation inhibition	3.02 ± 0.06	2.92 ± 0.19

[a-b]Means $\pm$ SEM ($n = 3$) in a row without a common superscript letter differ ($P < 0.05$) as analyzed by one-way ANOVA and the TUKEY HSD test
SDEE–saponified diethyl ether extract of pili pulp oil.

TABLE 3: Antibacterial activity of the saponified diethyl ether extract pili pulp oil.

Pathogen tested	Antibacterial activity			
	SDEE		Chloramphenicol (control)	
	ZOI (mm)	MIC (μg/mL)	ZOI (mm)	MIC (μg/mL)
Escherichia coli	10.3 ± 0.20	40.0 ± 0.50	15.3 ± 0.00	2.25 ± 0.00
Salmonella typhi	11.7 ± 0.10	35.0 ± 0.50	15.7 ± 0.10	2.55 ± 0.00
Staphylococcus aureus		N.A.	15.3 ± 0.10	2.25 ± 0.00
Bacillus cereus		N.A.	20.3 ± 0.10	15.25 ± 0.50
Pseudomonas aeruginosa		N.A.	11.3 ± 0.00	2.00 ± 0.50

ZOI–zone of inhibition MIC–minimum inhibitory concentration N.A. – not active (no zone of inhibition observed) SDEE–saponified diethyl ether extract of pili pulp oil.

were isolated from *Icacina trichantha*, showed high antimicrobial properties against *B. subtilis*, *E. coli*, and *C. albicans*.

The regulatory activity of amyrin including other triterpenoids in disrupting pathways responsible for cell division and protein synthesis, as well as destabilization of bacterial cell membrane and inhibition of cell growth are some of the plausible mechanisms of action of these compounds [31]. Furthermore, these compounds also cause disorganizing effects on cardiolipin-rich domains present in the membrane of *E. coli* as demonstrated by Broniatowski et al. [32].

4. Conclusion

Extraction, saponification, and diethyl ether fractionation of the unsaponifiable matter of pili pulp oil showed strong antioxidant properties as measured by its scavenging activity against DPPH radical and lipid peroxidation inhibition activities and are found to be comparable to α-tocopherol, a known antioxidant compound. The extract also exerts antibacterial activities against *E. coli* and *S. typhi* which are bacterial pathogens of concern, especially in food preparation. GCMS profiling revealed 18 compounds majority of which are cyclic triterpenes and phytosterols such as α- and β-amyrin, lupenone, β-sitosterol, brassicasterol, and stigmasterol. Our results suggest that these bioactive compounds are responsible for the observed bioactivities which may provide desirable health benefits.

Data Availability

Datasets related to this article will be made available upon request.

Disclosure

An earlier version of the manuscript was presented as a preprint in "Extraction Profiling and Characterization of Phytosterols and Triterpenoids from Pili (Canarium ovatum Engl.) Pulp Oil Exhibiting Antioxidant and Antibacterial Properties" in the following link "https://www.authorea.com/doi/full/10.22541/au.166438496.66139421/v1" [33].

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

N.G.D. conceived and designed the experiment and wrote the first draft of the manuscript. A.C.T.K., R.D.P.A., and C.R.T. conducted the experiment. L.J.P. provided technical expertise in the design of the experiment and provided insights into the preparation of the manuscript. All authors contributed to and approved the final draft of the manuscript.

Acknowledgments

References

[1] R. E. Coronel, *Pili Nut Cavarium Ovatum Engl. Promoting the Conservation and Use of Underutilized and Neglected Crops. Rome, Italy: International Plant Genetic Crop Research*, Gatersleben/International Plant Genetic Research Institute (IPGRI), Gatersleben, Germany, 1996.

[2] S. P. Catelo and C. D. Jimenez, "An exploratory study of the economic potential of philippine pili pulp oil from waste pulp," *Journal of Economics, Management & Agricultural Development*, vol. 2, no. 1, pp. 23–37, 2016.

[3] L. J. Pham and N. G. Dumandan, "Philippine Pili: composition of the lipid molecular species," *Journal of Ethnic Foods*, vol. 2, no. 4, pp. 147–153, 2015.

[4] M. M. D. R. Tugay, L. E. Mopera, E. B. Esguerra, and K. A. T. Castillo-Israel, "Physico-chemical and quality characterization of pulp oil from two varieties of pili (*Canarium ovatum* Engl.) from Bicol, Philippines," *Food Research*, vol. 4, no. 6, pp. 1937–1946, 2020.

[5] S. Román, L. M. Sánchez-Siles, M. Siegrist, and M. Siegrist, "The importance of food naturalness for consumers: results of a systematic review," *Trends in Food Science & Technology*, vol. 67, pp. 44–57, 2017.

[6] Y. Zhong, L. Wu, X. Chen, Z. Huang, and W. Hu, "Effects of food-additive-information on consumers' willingness to accept food with additives," *International Journal of Environmental Research and Public Health*, vol. 15, no. 11, p. 2394, 2018.

[7] L. Jiang, X. Zhao, J. Xu et al., "The protective effect of dietary phytosterols on cancer risk: a systematic meta-analysis," *Journal of Oncology*, vol. 2019, Article ID 7479518, 11 pages, 2019.

[8] F. Marangoni and A. Poli, "Phytosterols and cardiovascular health," *Pharmacological Research*, vol. 61, no. 3, pp. 193–199, 2010.

[9] S. A. Mohamed and J. A. Khan, "Antioxidant capacity of chewing stick miswak *Salvadora persica*," *BMC Complementary and Alternative Medicine*, vol. 13, no. 1, p. 40, 2013.

[10] R. T. Nzogong, F. S. T. Ndjateu, S. E. Ekom et al., "Antimicrobial and antioxidant activities of triterpenoid and phenolic derivatives from two Cameroonian *Melastomataceae* plants: *Dissotis senegambiensis* and *Amphiblemma monticola*," *BMC Complementary and Alternative Medicine*, vol. 18, no. 1, p. 159, 2018.

[11] R. E. Ostlund, "Phytosterols and cholesterol metabolism," *Current Opinion in Lipidology*, vol. 15, no. 1, pp. 37–41, 2004.

[12] G. Vilahur, S. Ben-Aicha, E. Diaz-Riera, L. Badimon, and T. Padró, "Phytosterols and inflammation," *Current Medicinal Chemistry*, vol. 26, no. 37, pp. 6724–6734, 2019.

[13] A. M. O. Amoussa, L. Lagnika, M. Bourjot, C. Vonthron-Senecheau, and A. Sanni, "Triterpenoids from *Acacia ataxacantha* DC: antimicrobial and antioxidant activities," *BMC Complementary and Alternative Medicine*, vol. 16, no. 1, p. 284, 2016.

[14] A. Z. Barakat, A. R. Hamed, R. I. Bassuiny, A. M. Abdel-Aty, and S. A. Mohamed, "Date palm and saw palmetto seeds functional properties: antioxidant, anti-inflammatory and antimicrobial activities," *Journal of Food Measurement and Characterization*, vol. 14, no. 2, pp. 1064–1072, 2020.

[15] C. A. S. Almeida, S. R. Baggio, L. R. B. Mariutti, and N. Bragagnolo, "One-step rapid extraction of phytosterols from vegetable oils," *Food Research International*, vol. 130, Article ID 108891, 2020.

[16] K. T. Wang, L. G. Chen, D. S. Chou, W. L. Liang, and C. C. Wang, "Anti-oxidative abilities of essential oils from *Atractylodes ovata* rhizome," *Evidence-based Complementary and Alternative Medicine*, vol. 2011, Article ID eneq006, 2011.

[17] M. A. Wikler, *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically: Approved Standard*, Clinical and Laboratory Standards Institute, Wayne, Pennsylvania, United States, 11th edition, 2006.

[18] J. N. Eloff, "A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria," *Planta Medica*, vol. 64, no. 08, pp. 711–713, 1998.

[19] N. V. Quan, T. D. Xuan, H. D. Tran et al., "Antioxidant, α-amylase and α-glucosidase inhibitory activities and potential constituents of *Canarium tramdenum* bark," *Molecules*, vol. 24, no. 3, p. 605, 2019.

[20] O. Ce, J. O. Amupitan, and R. G. Ayo, "Isolation and biological activity of the triterpene β-amyrin from the aerial plant parts of *Maesobotrya barteri* (Baill)," *Medicinal Chemistry*, vol. 4, no. 11, pp. 729–733, 2014.

[21] S. Saeidnia, L. Ara, H. Hajimehdipoor, R. W. Read, S. Arshadi, and M. Nikan, "Chemical constituents of *Swertia longifolia* Boiss. with α-amylase inhibitory activity," *Res Pharm Sci*, vol. 11, no. 1, pp. 23–32, 2016.

[22] E. F. Varvouni, K. Graikou, O. Gortzi, A. Cheilari, N. Aligiannis, and I. Chinou, "Chemical and biological evaluation of the oil and seedcake from seeds of a Greek cardoon cultivar as potential functional vegetable oil. Comparison with sesame, flaxseed and extra virgin olive oils," *Foods*, vol. 10, no. 11, p. 2665, 2021.

[23] X. Wang, Q. Zeng, V. Verardo, and M. d. M. Contreras, "Fatty acid and sterol composition of tea seed oils: their comparison by the "FancyTiles" approach," *Food Chemistry*, vol. 233, pp. 302–310, 2017.

[24] S. B. Kedare and R. P. Singh, "Genesis and development of DPPH method of antioxidant assay," *Journal of Food Science & Technology*, vol. 48, no. 4, pp. 412–422, 2011.

[25] Y. Yoshida and E. Niki, "Antioxidant effects of phytosterol and its components," *Journal of Nutritional Science and Vitaminology*, vol. 49, no. 4, pp. 277–280, 2003.

[26] A. Prommaban, N. Utama-ang, A. Chaikitwattana, C. Uthaipibull, J. B. Porter, and S. Srichairatanakool, "Phytosterol, lipid and phenolic composition, and biological activities of guava seed oil," *Molecules*, vol. 25, no. 11, p. 2474, 2020.

[27] B. Karen Cardoso, H. Line Marko de Oliveira, U. Zonta Melo et al., "Antioxidant activity of α and β-amyrin isolated from *Myrcianthes pungens* leaves," *Natural Product Research*, vol. 34, no. 12, pp. 1777–1781, 2020.

[28] N. Abdel-Raouf, N. M. Al-Enazi, A. A. Al-Homaidan, I. B. M. Ibraheem, M. R. Al-Othman, and A. A. Hatamleh, "Antibacterial β-amyrin isolated from *Laurencia microcladia*," *Arabian Journal of Chemistry*, vol. 8, no. 1, pp. 32–37, 2015.

[29] C. P. Fernandes, A. L. Corrêa, J. F. R. Lobo et al., "Triterpene esters and biological activities from edible fruits of *Manilkara subsericea* (Mart.) Dubard," *BioMed Research International*, vol. 2013, Article ID 280810, 7 pages, 2013.

[30] T. T. Alawode, L. Lajide, M. Olaleye, and B. Owolabi, "Stigmasterol and β-sitosterol: antimicrobial compounds in the leaves of *Icacina trichantha* identified by GC–MS," *Beni-Suef University Journal of Basic and Applied Sciences*, vol. 10, no. 1, p. 80, 2021.

[31] P. Y. Chung, L. Y. Chung, and P. Navaratnam, "Transcriptional profiles of the response of methicillin-resistant*Staphylococcus aureus* to pentacyclic triterpenoids," *PLoS One*, vol. 8, no. 2, Article ID e56687, 2013.

[32] M. Broniatowski, P. Mastalerz, and M. Flasiński, "Studies of the interactions of ursane-type bioactive terpenes with the

model of *Escherichia coli* inner membrane—Langmuir monolayer approach," *Biochimica et Biophysica Acta (BBA) - Biomembranes*, vol. 1848, no. 2, pp. 469–476, 2015.

[33] N. G. Dumandan, A. C. T. Kagaoan, R. D. P. Acda, C. R. Tumambing, and L. J. Pham, "Extraction, Profiling and Characterization of Phytosterols and Triterpenoids from Pili (Canarium Ovatum Engl.) Pulp Oil Exhibiting Antioxidant and Antibacterial Properties," 2022, [Preprint].