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Research Article

Active Compound Identification in Extracts of *N. lappaceum* Peel and Evaluation of Antioxidant Capacity

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Nephelium lappaceum and its by-products have great potential in the agricultural, pharmaceutical, and food industries. Some studies have shown that *N. lappaceum* by-products exhibit antimicrobial, antioxidant, antidiabetic, and anticancer properties. However, studies focused on identifying these compounds are rare. The availability of polyphenolic compounds can vary according to environmental conditions, soil, plant variety, and agronomic management. Therefore, in this study, the active compounds in extracts of the *N. lappaceum* peel were identified, and their antioxidant properties were evaluated using various extraction solvents and both ultrasonic and boiling extraction techniques. The chemical characterization of the *N. lappaceum* peel exhibited carbohydrate and reducing sugar contents of 12 and 2%, respectively. Phytochemical analysis indicated the presence of flavonoids, tannins, terpenes, and steroids. The total phenolic and flavonoid contents and total antioxidant capacity were the highest in the hydroethanolic extract obtained by ultrasound, with values of 340 mg gallic acid equivalents g^{-1}, 76 mg quercetin equivalents g^{-1}, and 2.9 mmol of Trolox equivalents g^{-1}, respectively. Contrarily, the total anthocyanin content was higher in the acid extract obtained by ultrasound, with a value of 0.7 mg cyanidin-3-O-glucoside equivalents g^{-1}. A total of 18 compounds—including hydroxybenzene, phenolic acid, flavonoids, fatty acids (saturated, unsaturated, and ester), vitamin, arenecarbaldehyde, and phthalate—were identified for the first time in the *N. lappaceum* peel using gas chromatography-mass spectrometry. The identified compounds have been previously isolated from other plants and reportedly exhibit anticancer, anti-inflammatory, antimicrobial, and antioxidant activities. Thus, the *N. lappaceum* peel was shown to be a potential source of bioactive compounds of immense importance in the pharmacological and food industries.

1. Introduction

Nephelium lappaceum, also known as rambutan, is a tropical fruit with excellent nutraceutical properties. In recent years, several studies in Southeast Asia and Australia have revealed that different parts of the fruit, particularly the peel, have powerful antioxidant, antibacterial, and antidiabetic activities, as summarized in Table 1, which may be attributed to the presence of phenolic compounds.

Phenolic compounds are secondary metabolites that exist in all plants as a defense mechanism against stress caused by pathogens or adverse environmental conditions [19]. They act as reducing agents, eliminators of free radicals, and metal chelators [20]. Several studies carried out on phenolic extracts or isolated compounds have shown that they protect against disorders caused by oxidative damage, such as cancer, arteriosclerosis, cardiovascular disorders, and diabetes, by delaying and inhibiting the oxidation of lipids and other molecules [9, 21–25]. These compounds are of interest not only for their antioxidant properties but also for their antiallergic, anti-inflammatory, and antimicrobial properties [26–28].

Presently, about eight thousand phenolic compounds are known to exist in nature [29]. Although phenolic compounds are very diverse, they can be divided into six major classes according to their structure: simple phenols,

Table 1: Biological properties in the extract from the seed, pulp, and peel of *N. lappaceum*.

Material	Extract or solvent	Property	Reference
Peel	Methanolic	Antioxidant including DPPH, lipid peroxidation, ABTS, FRAP, reducing power, b-carotene bleaching, linoleic peroxidation, and free radical scavenging	[1–3]
Peel	Ethanolic	Antidiabetic: significant decrease of 62% in blood glucose levels Antioxidant including ABTS and DPPH assays	[4–6]
Peel	Aqueous	Antioxidant including ABTS and FRAP Antimicrobial activity against *Vibrio cholerae*, *Enterococcus faecalis*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*	[1, 7]
Peel	Ethyl acetate	Antioxidant including ABTS and FRAP	[8]
Peel	Hydroethanolic	Antioxidant including DPPH, ABTS, FRAP, lipid peroxidation, and nitrite and hydroxyl radical scavenging Antiglycation activities	[9, 10]
Peel	Ether	Antimicrobial activity against *V. cholerae*, *E. faecalis*, *S. aureus*, and *S. epidermidis*	[1]
Pericarp	Ethanolic and aqueous	Inhibitory effects on the infection of herpes simplex virus type 1	[11]
Pulp	Ethanolic	Anti-inflammatory: decreasing secretion of tumor necrosis factor alpha (TNF-α) Antioxidant: suppressive effect on reactive oxygen species (ROS) formation	[12]
Seed	Aqueous	Antibacterial: moderate inhibition against *S. aureus*, *S. pyogenes*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa* Antiviral: inhibitory effect on HIV-1 reverse transcriptase (HIV-1 RT) Antitumoral: trypsin/chymotrypsin inhibitory activity	[13, 14]
Seed	Methanolic	Antibacterial: inhibition against *S. aureus*, *S. epidermidis*, *E. coli*, and *P. aeruginosa* Anti-inflammatory, antinociceptive, CNS, and antifungal Antioxidant including DPPH, reducing power, b-carotene bleaching, linoleic peroxidation, and free radical scavenging	[1, 15, 16]
Seed	Ethyl acetate and ethanolic	Antioxidant using DPPH and ABTS assays	[17]
Seed	Hexane, ethyl acetate, butanol, and water	Antidiabetic: α-glucosidase inhibitor activity	[18]

phenolic acids, flavonoids, tannins, stilbenes, and lignans [26, 29–32].

George et al. [33] and Pande and Akoh [34] have reported that phenolic compounds mainly accumulate in the skin or peel, rather than the pulp of fruits and vegetables, making these by-products an attractive source for the isolation of natural active compounds. For example, studies of *N. lappaceum* have revealed the presence of numerous phenolic compounds with important properties in the peel, in even greater quantities than in the seed and pulp [18, 35]. Sun et al. [9] reported the presence of phenolic acids, hydroxycinnamic acids, and flavan-3-ols such as gallic acid, *p*-coumaric acid, catechin, and rutin in the peel. Palanisamy et al. [36] and Thitilertdecha et al. [2] identified a hydrolysable tannin called geraniin in the *N. lappaceum* peel with antihyperglycemic and antioxidant properties. Thitilertdecha et al. [2] further showed that this compound had greater antioxidant capacity than the synthetic antioxidant butylated hydroxytoluene. Such phenolic compounds can also act as antiviral agents that inhibit the replication of human immunodeficiency virus (HIV) [37]. These studies illustrate the promising potential of *N. lappaceum* peels. However, the chemical and biological diversity of these plants depends on factors such as location, variety, management, and climatic conditions [38, 39].

Although the largest commercial production of *N. lappaceum* is concentrated in Thailand, Malaysia, Indonesia, and Philippines [40], there are several countries in the Central American Region, such as Costa Rica, Guatemala, Honduras, Mexico, and Panamá, which are also dedicated to its cultivation. According to the Food and Agriculture Organization of the United Nations (FAO) [41], the global production of *N. lappaceum* reached an estimated 1.4 million tons in 2017, which generated 756 thousand tons of by-products such as peels and seeds [3]. While these by-products have found little or no use so far, they could represent a renewable resource from which many useful biological and chemical products could be obtained, providing an avenue to meet the demand of the food industry for functional foods and reutilization of agricultural by-products.

The economic prospects and market demand of this fruit have seen rapid growth in recent years because studies have shown that it contains polyphenolic bioactive compounds [7]. Although the *N. lappaceum* peel has been documented in previous studies as a material that is rich in bioactive compounds with antioxidant and antihyperglycemic activities, studies identifying the molecules present in its extracts that could be associated with other bioactivities are rare. This is particularly true in Central America. Considering that the availability of polyphenolic compounds can vary according to environmental conditions, soil, plant variety, and agronomic management, studies on these by-products in different locations are necessary. In addition, the ability to obtain polyphenolic compounds may depend on the extraction technique and nature of the extraction solvent used. Polyphenolic compounds range from very polar to very nonpolar compounds, so the extraction solvent plays an important role in their extraction efficiency [42]. Therefore, in this study, we identified the active compounds present in

the extract of the *N. lappaceum* peel and evaluated their antioxidant capacities using different extraction solvents (acid, alkaline, aqueous, ethanolic, and hydroethanolic) and both ultrasonic and boiling extraction techniques. This is one of the first studies utilizing acidic and alkaline media, as well as ultrasound in the extraction of active compounds from *N. lappaceum*. To the best of our knowledge, only Prakash Maran et al. [43] have used ultrasound in the extraction of bioactive compounds (total anthocyanin, phenolic, and flavonoid contents) from *N. lappaceum* L. peels. Our research, focusing on finding such bioactive compounds from renewable resources often considered waste, constitutes an important step towards sustainability within food, pharmacological, and other industries.

2. Materials and Methods

2.1. Raw Materials. A local farmer provided samples of *N. lappaceum* (red variety). The peels were removed from the fruit, washed, and dried for 48 h at 60°C and then milled and stored in dark plastic bags under dry conditions until experimental use for experiments. Sample analysis began immediately after collection.

2.2. Chemical Characterization. The samples were analyzed for the total carbohydrate, reducing sugar, and mineral contents, and phytochemical screening was conducted. All analyses were performed in triplicate. The total carbohydrate content was determined by the phenol-sulfuric acid method. A test tube containing the sample (0.1 g) and HCl (5 mL, 2.5 N) was heated in a water bath (Thomas Scientific, USA) at 95°C for 3 h. The resulting suspension was neutralized with Na_2CO_3, diluted with 5 mL water, and centrifuged (Digicen 21 R centrifuge, Ortoalresa, Madrid, Spain) at 3500 rpm for 5 min. The dilution/centrifugation process was repeated five times. The supernatant volume was adjusted to 100 mL with water, and a 0.1 mL aliquot of this solution was withdrawn. The aliquot was treated with 50 $g{\cdot}L^{-1}$ aqueous phenol (1 mL) and concentrated 96% H_2SO_4 (5 mL), vortexed (MX-S vortex mixer, Scilogex, Thomas Scientific, USA) for a minute, and heated at 30°C for 20 min in a water bath. Finally, the total carbohydrate content was determined by visible-light spectrophotometry (Genesys 10S, Thermo Scientific, USA) at a wavelength of 490 nm, using a calibration curve prepared with glucose as a standard. The reducing sugars were quantified by visible-light spectrophotometry at a wavelength of 540 nm following the dinitrosalicylic acid (DNS) method [44]. Typically, a suspension of the sample (0.1 g) in hot ethanol (8 mL, 800 mL L^{-1}) in a test tube was heated in a water bath at 95°C for 10 min and then centrifuged at 2500 rpm for 5 min. The supernatant was collected, ethanol was evaporated in a water bath at 80°C, and the obtained residue was treated with water (10 mL). A 0.7 mL aliquot of this solution was transferred into a test tube and treated with 1.5 mL of the DNS reagent. The resulting mixture was heated in a boiling water bath for 5 min and then brought to room temperature in a 25°C water bath. Finally, the content of reducing sugars was determined from the absorbance of the cooled mixture at 540 nm, using a calibration curve prepared with glucose as a standard. The DNS reagent was prepared by dissolving 0.748 g of DNS, 0.564 g of crystalline phenol, 21.612 g of Rochelle salt, 1.412 g of NaOH, and 0.564 g of sodium sulfite in 100 mL water. The total nitrogen concentration was determined by the Kjeldahl method (K415-K350-F308 Kjeldahl system, Buchi, Switzerland) according to AOAC Method 988.05 [45]. The phosphorus content was determined by the vanadomolybdophosphoric method described by Kitson and Mellon [46]. For the determination of the mineral content, ashes of a sample were dissolved in hydrochloric acid and the resulting solution was analyzed by atomic absorption spectroscopy (AA-7000 flame atomic absorption spectrophotometer, Shimadzu, Kyoto, Japan) according to AOAC Method 985.35 [45].

Infrared spectra of the samples were measured by attenuated total reflectance (ATR). The spectra were recorded between 4000 and 650 cm^{-1} using a Cary 630 Fourier-transform infrared spectrophotometer (FT-IR) (Agilent Technologies, USA) equipped with a deuterated triglycine sulfate detector. The diamond ATR sensor was cleaned with ethyl alcohol before each sample measurement. The spectral resolution was 4 cm^{-1}, and 64 scans were made for each spectrum.

The sample was subjected to a phytochemical screening by conducting the Salkowski test for sterols, Shinoda and sodium hydroxide tests for flavonoids, Dragendorff, Wagner, and Mayer tests for alkaloids, ferric chloride test for tannins, Rosenthaler test for saponins, and Dimroth test for 5-hydroxy flavones.

2.3. Extraction Method. The extracts were obtained by ultrasound and by boiling from alkaline, acidic, ethanolic, hydroethanolic, and aqueous solutions. For the extractions in acidic, alkaline, aqueous, and hydroethanolic solutions, 1 g of the dry sample was sonicated in 10 mL of the corresponding solvent of 10 $g{\cdot}L^{-1}$ HCl, 10 $g{\cdot}L^{-1}$ NaOH, water, 96% ethanol, or 600 $g{\cdot}L^{-1}$ hydroethanol, respectively, for 20 min using an ultrasonic bath (Elmasonic P60H, Singen, Germany). The extractions were also carried out by boiling for 10 min with the same sample/solvent ratio. Subsequently, the extracts were cooled and filtered [47].

2.4. Phenolic, Flavonoid, and Anthocyanin Content Evaluation. The total phenolic content (TPC) was determined by the Folin–Ciocalteu assay, as described by Miliauskas et al. [39]. A volume of 500 μL of different diluted extracts was mixed with 2500 μL of the Folin–Ciocalteu reagent (0.2 $mol{\cdot}L^{-1}$) and allowed to stand for 5 min. Next, 2 mL of 75 $g{\cdot}kg^{-1}$ sodium carbonate solution was added to the mixtures, which then stood for 2 h at room temperature in the dark. The absorbances of the solutions were then measured at 754 nm using a visible-light spectrophotometer (Genesys 10S, Thermo Scientific, USA). To set up the calibration curve, gallic acid solutions of concentrations ranging from 0 to 12 $\mu g\,mL^{-1}$ were prepared. The results were

expressed as mg of gallic acid equivalents (GAE) per g of dry peel mass.

The total flavonoid content (TFC) was determined by spectrophotometry using the $AlCl_3$ colorimetric method [48], with some modifications. In brief, 50 μL of the extract was mixed with distilled water to prepare a 5 mL solution, followed by the addition of 0.3 mL of 50 g·L^{-1} $NaNO_2$. After 5 min, 0.3 mL of 100 g·L^{-1} $AlCl_3$ was added to the mixture. Shortly thereafter, 2 mL of 1 mol·L^{-1} NaOH was added and the total volume was increased to 10 mL by adding distilled water. After 15 min, the absorbance of the solution was measured at 374 nm using an ultraviolet spectrophotometer (Genesys 10S, Thermo Scientific, USA). The calibration curve was set up using quercetin solutions with concentrations ranging from 0 to 15 μg·mL^{-1}. The flavonoid content in the extract was expressed as mg of quercetin equivalents (QE) per gram of dry peel mass.

The total anthocyanin content (TAC) was estimated by a pH differential method described by Prakash Maran et al. [43] Aliquots of the extracts were adjusted to pH values of 1.0 and 4.5 using 25 mM KCl and 0.4 M CH_3COONa as buffer solution, respectively, and allowed to equilibrate for 20 min. Subsequently, their absorbances were measured at 520 and 700 nm. The TAC of the extracts was calculated using the following equation:

$$\text{Total anthocyanin content, mg} \cdot \text{L}^{-1} = \frac{A \times \text{MW} \times \text{DF} \times 10^3}{\varepsilon}, \quad (1)$$

where A is the difference in the absorbances at a pH of 1.0 ($A_{520\,nm} - A_{700\,nm}$) and a pH of 4.5 ($A_{520\,nm} - A_{700\,nm}$), MW is the molecular weight of cyanidin-3-glucoside (449.2 g·mol^{-1}), DF is the dilution factor, and ε is the molar extinction coefficient of cyanidin-3-glucoside (26,900 L·mol^{-1} × cm^{-1}). Therefore, the TAC was expressed as mg cyanidin-3-O-glucoside equivalents (Cy-3GE) per gram of dry peel mass.

2.5. 2,2′-Azino-bis-(3-ethylbenzothiazoline-6-sulphonic Acid) Radical Cation-Scavenging Activity Determination. The 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assay was performed using the method described by Re et al. [49]. The ABTS radical cation was prepared by reacting equal portions of 7 mM ABTS solution and 2.45 mM potassium persulfate solution. The mixture was incubated at room temperature for 16 h in the dark at 25°C and then diluted with 96% ethanol until its absorbance of 754 nm reached 0.7 ± 0.02. The prepared $ABTS^{\bullet+}$ solution (1900 μL) was mixed with 100 μL of the extract that was diluted and then allowed to stand for 10 min at room temperature in the dark. The absorbance of the sample was measured at 754 nm using a visible-light spectrophotometer (Genesys 10S, Thermo Scientific, USA). The $ABTS^{\bullet+}$ scavenging activity was calculated by comparison with a standard curve of Trolox (40 and 320 mmol·L^{-1} Trolox), and the results were expressed as mmol of Trolox equivalents (TE) per gram of dry peel mass (mmol TE·g^{-1}).

2.6. 1,1-Diphenyl-2-picrylhydrazine Radical-Scavenging Activity Determination. The 1,1-diphenyl-2-picrylhydrazine (DPPH) assay was done as described by Thaipong et al. [50], with some modifications. The DPPH radical solution was prepared by dissolving 13 mg DPPH with 100 mL methanol, and then 10 mL of the solution was diluted with 45 mL of methanol to obtain an absorbance of 0.70 ± 0.02 units at 515 nm using the spectrophotometer (Genesys 10S, Thermo Scientific, USA). The extracts (100 μL) were reacted with 2900 μL of the DPPH solution for 30 min at room temperature in the dark. The absorbance of the sample was measured at 515 nm. The DPPH scavenging activity was calculated by comparison with a standard curve of Trolox (40 and 600 mmol·L^{-1} Trolox). Results are expressed as mmol of Trolox equivalents (TE) per gram of dry peel mass (mmol TE·g^{-1}).

2.7. Compound Isolation. The hydroethanolic extract was reextracted with ethyl acetate and hexane. In this procedure, the extract was evaporated and the residue was resuspended in water. The aqueous extract (3 mL) was then acidified to a pH of 2 with HCl and extracted using liquid-liquid extraction (LLE) with 3 mL ethyl acetate three times. The ethyl acetate phase was treated with activated charcoal to adsorb the pigments. After filtration, the organic phase was dried over anhydrous Na_2SO_4 and evaporated. The residue was resuspended in ethanol for the GC-MS analysis. The LLE process was repeated using hexane.

2.8. GC-MS Analysis. The identification of bioactive compounds from the crude hydroethanol extract and ethanol extract obtained by LLE with ethyl acetate and hexane was performed on a 7890A GC system coupled with a 5975C MS system (Agilent Technologies, USA), fitted with an HP-5MS column (30 m in length × 250 μm in diameter × 0.25 μm in thickness of the film). Pure He was used as the carrier gas at a constant flow rate of 1 mL·min^{-1}. One microliter of the extract was injected in a splitless mode. The ionization of the sample components was carried out at 70 eV. The injector and ion source temperatures were 260°C and 230°C, respectively. The oven temperature ramp was programmed as 60°C at 0 min, 10°C min^{-1}, and 280°C for 30 min. Mass spectra were scanned from *m/z* 45–550 at a rate of 2 scans/s. The total running time of the GC was 52 min. The compounds were identified by comparing their mass spectra with those from the NIST05 Mass Spectral Library.

2.9. Statistical Analysis. Each assay was performed three times per extract to determine their reproducibility. Data were expressed as the mean ± standard deviation values. Two-way analysis of variance (ANOVA) and multiple-comparison test (the Tukey procedure) were used to determine significant differences. The results were deemed significant at $p < 0.05$. Correlations between the total phenolic, flavonoid, and antioxidant capacities were made using Pearson's procedure ($p < 0.05$). Statistical analyses were

conducted using the Statgraphics Centurion XVII software (Statgraphics Technologies, USA).

3. Results and Discussion

3.1. Chemical Composition of the N. lappaceum Peel. The total carbohydrate content in the *N. lappaceum* peel was 120 g·kg^{-1}, which is less than the value of 238 g·kg^{-1} reported by Fila et al. [51] in Nigeria. In addition, the *N. lappaceum* peel is an excellent source of essential elements such as nitrogen, calcium, potassium, phosphorus, magnesium, copper, zinc, manganese, sodium, and iron, as summarized in Table 2. These elements are of considerable interest to the nutraceutical and food industries because they are crucial for the healthy functioning of the human body due to their roles in various vital processes such as cellular functioning, enzymatic activation, genetic expression, and bone and hemoglobin formation [52, 53]. Phosphorus is an important constituent of adenosine triphosphate and nucleic acid and is also essential for acid-base balance and bone and tooth formation. Nitrogen is the basic component of macromolecules such as proteins. Iron is an important component of the cytochromes that function in cellular respiration, and red blood cells cannot function properly without iron in hemoglobin. Magnesium, copper, zinc, iron, manganese, and molybdenum are important cofactors found in the structure of certain enzymes and are indispensable in numerous biochemical pathways [52, 53].

The FT-IR spectrum of the *N. lappaceum* peel is shown in Figure 1. The functional group bands observed in the spectrum are characteristic of the lignocellulosic material. The bands at 3275 and 2925 cm^{-1} correspond to the O–H and C–H stretching vibrations, respectively, of the hydroxyl and carboxylic functional groups in phenols and pyranose. The band at 1707 cm^{-1} is attributed to the –C=O stretching vibrations of the ester carbonyl or carboxylic acid functional group [54], which are characteristic of fatty acids. The –C=O absorptions might correspond to either conjugated or nonconjugated, saturated or unsaturated, amides, acids, or other systems [55]. The bands at 1506 and 1440 cm^{-1} correspond to aromatic C–C stretching, while the peak at 1317 cm^{-1} may be attributed to C–H asymmetric deformation and COO^{-} anion stretching. The bands observed at ~1440–1201 cm^{-1} can be assigned to C–O stretching and O–H deformation vibrations [56]. The absorption band at 1024 cm^{-1} corresponds to the C–O stretching of either an ester or an ether [54], while that at 752 cm^{-1} is attributed to =CH bending.

The phytochemical analysis shown in Table 3 reveals the presence of terpenes/steroids, flavonoids, and tannins. Although alkaloids were not detected in this study, Nethaji et al. [57] have reported the presence of alkaloids, steroids and sterols, glycosides, flavonoids, triterpenoids, and tannins in the methanolic extracts of the *N. lappaceum* epicarp. Lestari et al. [58] have reported that the ethanolic, aqueous, and *n*-butanol extracts of the *N. lappaceum* peel contain compounds such as terpenoids, steroids, flavonoids, sugars, amino acids, and peptides. Thus, the chemical composition of *N. lappaceum* is clearly quite variable, depending on several factors such as the chemical characteristics of the soil, geographic location, environmental conditions, age, and species.

3.2. Total Phenolic, Flavonoid, and Anthocyanin Contents in the Extracts. *N. lappaceum* by-products are rich in phenolic acids, flavonoids, and anthocyanins, as mentioned above. The phenolic acid, flavonoid, and anthocyanins contents in the extracts obtained by ultrasound ranged from 208 to 340 mg·g^{-1}, 44 to 76 mg·g^{-1}, and 0.53 to 0.7 mg·g^{-1}, respectively, while in the extracts obtained by boiling, these same contents ranged from 189 to 315 mg·g^{-1}, 41 to 72 mg·g^{-1}, and 0.15 to 0.68 mg·g^{-1}, respectively (Table 4).

Active compound extraction is a critical process that depends on the solvent, temperature, time, and chemical nature of the sample [48], as well as the extraction technique. In this investigation, we evaluated the solvent and extraction process. To determine the effect of the extraction process and the nature of the extraction solvent, a two-way ANOVA was performed. It was found that there were significant differences at a confidence level of 95.0% ($p < 0.05$) between the TPC, the TFC, and the TAC for the extracts obtained through different extraction processes and solvents (Table 4). To determine the significantly different mean among the solvents at a confidence level of 95.0%, we applied the Tukey test for each extraction method.

For ultrasound, the TPC values for all solvent extractions varied significantly. Meanwhile, the pair of ethanolic and aqueous extracts did not present any significant differences for ebullition. The results obtained by ultrasound were significantly different from those obtained by ebullition. The highest TPC was obtained with the extract produced by ultrasound in hydroethanol, as this solvent allowed for the extraction of both polar and semipolar compounds. These results are similar to the findings in previous studies about extraction of the TPC. Ngo et al. [59] and Dailey and Vuong [42] indicated that extraction solvents play a key role in the extraction of the TPC and that the combined organic solvents and water had a higher recovery yield in comparison with the absolute solvent. The TPC values obtained in this work were similar to, and even higher than, those reported in the literature for the *N. lappaceum* peel extract. While Thitilertdecha et al. [1] reported a TPC of 293.3 mg GAE g^{-1} by extraction with ether, Prakash Maran et al. [43] obtained 5.5 mg GAE g^{-1} of the phenolic material in the aqueous extract by ultrasound. The TPC value reported by Nguyen et al. [3] from the methanolic extract was 128 mg GAE g^{-1}.

For the TFC, the results showed that solvents also significantly affected the extraction yields of flavonoids. The acidic, alkaline, aqueous, ethanolic, and hydroethanolic extracts showed significant differences in all cases, for both extraction methods. The highest TFC for both extraction methods was that of the hydroethanolic extracts, though it was higher for the extracts obtained by ultrasound. The TFC in our study was higher than the values reported previously for the *N. lappaceum* peel extract. Gusman and Tsai [60] showed that, under the two extraction methods of conventional extraction (12 h) and ultrasonic extraction (2 min),

Table 2: Carbohydrate and mineral content of the *N. lappaceum* peel.

Total carbohydrate g·kg^{-1}	Reducing sugar g·kg^{-1}	N	P	K	Ca	Mg	Fe	Cu	Mn	Zn	Na
				g·kg^{-1}					mg·kg^{-1}		
120 ± 10	24 ± 2	9.1 ± 0.2	5.7 ± 0.2	7.6 ± 0.1	0.9 ± 0.1	0.66 ± 0.08	41 ± 2	10 ± 1	160 ± 5	8.7 ± 0.1	45 ± 3

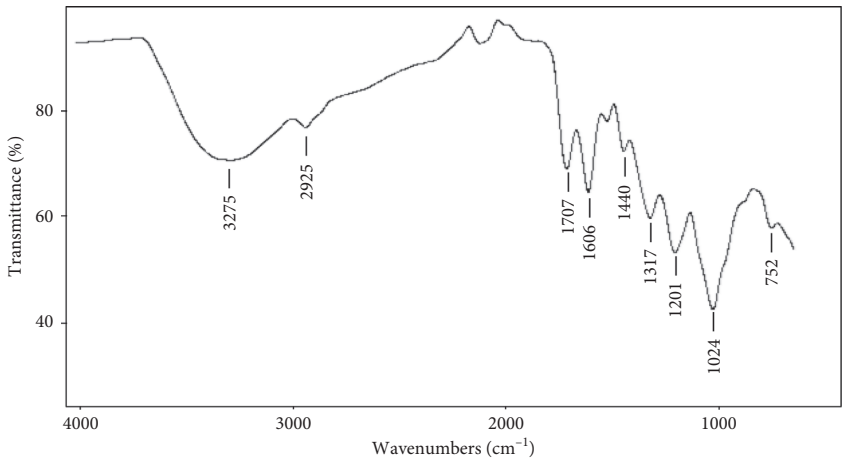

Figure 1: FT-IR spectrum of the *N. lappaceum* peel.

Table 3: Phytochemical analysis of the *N. lappaceum* peel.

Metabolite	Test	Evidence
Alkaloids	Meyer	(−)
	Dragendorff	(−)
	Wagner	(−)
Tannins	$FeCl_3$	(+)
Terpenes/steroids	Salkowski	(+)
Flavonoids	Reaction with NaOH	(+)

Table 4: Total phenolic, flavonoid, and anthocyanin contents based on the extraction method and solvent.

Extraction method	Nature of the solvent	Properties				
		TPC (mg GAE g^{-1})	TFC (mg QE g^{-1})	TAC (mg Cy-3GE g^{-1})	ABTS (mmol TE g^{-1})	DPPH (mmol TE g^{-1})
Ultrasound	Acidic 1%	231 ± 2^{d}	43.7 ± 0.3^{e}	0.70 ± 0.01^{a}	1.91 ± 0.06^{c}	1.65 ± 0.04^{d}
	Alkaline 1%	262 ± 6^{c}	72 ± 2^{b}	ND	2.12 ± 0.03^{b}	1.34 ± 0.02^{e}
	Aqueous	280 ± 2^{b}	54 ± 1^{c}	0.533 ± 0.004^{b}	1.76 ± 0.02^{d}	2.4 ± 0.1^{b}
	Ethanolic	208 ± 1^{e}	48.1 ± 0.3^{d}	0.69 ± 0.01^{a}	1.76 ± 0.02^{d}	1.78 ± 0.02^{c}
	Hydroethanolic 60%	340 ± 4^{a}	76 ± 2^{a}	0.57 ± 0.03^{b}	2.9 ± 0.1^{a}	3.26 ± 0.02^{a}
Boiling	Acidic 1%	189 ± 2^{d}	40.7 ± 0.3^{e}	0.68 ± 0.02^{a}	1.79 ± 0.01^{c}	0.86 ± 0.03^{c}
	Alkaline 1%	258 ± 3^{b}	62.2 ± 0.4^{b}	ND	1.96 ± 0.02^{b}	0.78 ± 0.04^{c}
	Aqueous	235 ± 2^{c}	44 ± 1^{d}	0.18 ± 0.05^{c}	1.54 ± 0.02^{d}	1.38 ± 0.04^{b}
	Ethanolic	233 ± 5^{c}	51 ± 1^{c}	0.15 ± 0.03^{c}	1.73 ± 0.03^{c}	2.3 ± 0.1^{a}
	Hydroethanolic 60%	315 ± 7^{a}	72 ± 1^{a}	0.35 ± 0.04^{b}	2.6 ± 0.1^{a}	1.53 ± 0.02^{b}
p value	Extraction method	0.0026*	0.0001*	0.0001*	0.0001*	0.0001*
	Nature of the solvent	0.0001*	0.0001*	0.0003*	0.0001*	0.0001*

ND: not determined; TPC: total phenolic content; TFC: total flavonoid content; TAC: total anthocyanin content; ABTS: 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid); DPPH: 1,1-diphenyl-2-picrylhydrazine. All values are expressed as mean ± SD ($n = 3$). * p values <0.05 indicate significant differences by two-way ANOVA. Different letters in the same column within each extraction method indicate significantly different values at the 95% confidence level, using the Tukey test.

the TFC values were 7.7 mg QE g^{-1} and 6.4 mg QE g^{-1}, respectively, with hydroethanol as a solvent (40% ethanol). Prakash Maran et al. [43] have reported 1000 μg rutin g^{-1} in the aqueous extract obtained by ultrasound.

The impact of the solvent nature and extraction technique on the extraction of the TAC was also studied. Anthocyanins are the natural plant pigments categorized as flavonoids. Interestingly, the pair of ethanolic and acidic

extracts, and hydroethanolic and aqueous extracts, did not exhibit any significant differences for the TAC when ultrasound was used. In contrast, the acid extract exhibits significant differences from the other extracts obtained by ebullition. Although the TAC obtained by boiling is different from that obtained by ultrasound, the acid extracts presented similar values in both extraction methods. In general, the highest TAC was obtained by the acid extracts. This is reasonable because, typically, anthocyanins are more stable under acidic conditions [61]. For all extracts, these TAC values were intermediate to those reported in the literature. Sun et al. [62] and Maran et al. [43] reported 1.8 mg Cy-3GE g^{-1} and 1 mg Cy-3GE g^{-1} TAC of the ethanolic/aqueous/acidic and aqueous extracts, respectively. These compounds, which are mainly responsible for the red color of the ripe *N. lappaceum* fruit, exhibit antioxidant, anti-inflammatory, antioedema, and antitumor activities [62, 63].

In this investigation, besides traditional extraction solvents such as water and ethanol, acidic and alkaline aqueous media were also used to compare the extraction efficiencies of the active compounds. While the alkaline media make the acidic compounds more soluble in an aqueous medium, the acidic media similarly increase the water solubility of organic bases. Our work is among the few studies that report the extraction of secondary metabolites in *N. lappaceum* using acidic and alkaline media. The results indicated that the compounds in the extracts obtained from *N. lappaceum* could be significantly affected by the extraction solvents, and the variation can be explained by the difference in solubility of different compounds, highlighting the relevance of identifying the optimal extraction solvent for each case. Diverse reports in the literature have shown that extraction of active compounds varies with different solvents used [64–66]. Koffi et al. [66] reported that ethanolic extracts of Ivorian plants yielded a higher amount of phenolic compounds compared to acetone, water, and methanol. Do et al. [65] reported that the *Limnophila aromatica* extract obtained by 100% ethanol showed the highest TPC, TFC, and Trolox equivalent antioxidant capacity (TEAC) extracts compared with pure water, other organic solvents, and aqueous organic mixtures. Contrarily, we found that the extraction with pure solvent (water and ethanol) was less effective than that of aqueous organic mixtures, as reported by Ngo et al. [59] and Dailey and Vuong [42]. In our case, the highest TFC and TPC were those of the hydroethanolic extracts in both extraction methods. In contrast, the TAC was higher in the acid extract.

Although the highest TPC and TFC were obtained in the hydroethanolic ultrasound extracts, and TAC in the acid extract, the TPC, TFC, and TAC from all extracts obtained by both extraction methods were comparable to those reported in the literature as mentioned above [3, 43, 60, 62].

On the contrary, the active compound extraction from *N. lappaceum* also depends on the extraction technique, and generally, the ultrasound technique shows a greater extraction capacity. This technique improves the efficiency of the extraction solvent and has excellent destructive effects on a matrix because it alters the fluid around the solid particles, via cavitation processes, and the internal structure, by disrupting cell walls and/or enlarging its pores, thus allowing the solvent to better penetrate into the cell and release the target molecules [67, 68]. Additionally, this technique is economical and environmentally friendly and presents the ability to produce concentrated extracts in a time-efficient manner [68]. According to Quintero Quiroz et al. [69], the ultrasound technique increased the yield of bioactive compounds and preserved the metabolites with a greater efficiency and thus promoted their functional activities.

3.3. Antioxidant Activity of N. lappaceum. Antioxidant activity plays a crucial role in mediating free radicals and reactive oxygen species (ROS), which are considered harmful to human health [35]. Because multiple oxidation mechanisms exist, no single assay will accurately reflect the entire antioxidant capacity of *N. lappaceum* peel extracts. Thus, to elucidate a complete profile of antioxidant capacity, multiple antioxidant capacity assays are required**.** However, in this study, only assays based on the single electron transfer (SET) mechanism, ABTS and DPPH assays, were evaluated. The ABTS assay has been widely used to evaluate the antioxidant activity of aqueous and lipophilic systems in vitro, while the DPPH assay has been used for evaluating the scavenging activities of antioxidants in lipophilic systems [70, 71].

Although the antioxidant properties of the extract can vary depending on the antioxidant assays used, the nature of solvents and the extraction techniques also have a great impact on the antioxidant properties of extracts. The antioxidant activity, determined using the ABTS and DPPH assays, was expressed as Trolox equivalent antioxidant capacity (TEAC), which indicates the free radical-scavenging activity. The extracts evaluated exhibited good antioxidant capacity with both high inhibitory activity against ABTS•+ radical cations and strong scavenging activity against DPPH radicals. The values obtained by both tests were comparable. The TEAC for the ABTS assay ranged from 1.76 mmol TE g^{-1} to 2.90 mmol TE g^{-1} and 1.54 mmol TE g^{-1} to 2.6 mmol TE g^{-1}, in the extracts obtained by ultrasound and boiling, respectively. Additionally, the TEAC for the DPPH assay ranged from 1.65 mmol TE g^{-1} to 3.26 mmol TE g^{-1} and 0.78 mmol TE g^{-1} to 2.3 mmol TE g^{-1}, in the extracts obtained by ultrasound and boiling, respectively (Table 4). The ANOVA indicated that there was a significant difference, at a confidence level of 95.0%, between the different extraction processes and the solvents used for both assays. The Tukey test showed that there is a significant difference in the TEAC values between the hydroethanolic extract and the rest of the extracts. Meanwhile, the pairs of ethanolic and acidic, and ethanolic and aqueous, extracts did not present any significant differences when ebullition and ultrasound methods, respectively, were used. The differences in antioxidant capacity are attributed to the variation of the types of compounds extracted by the different solvents. Hosseinian et al. [72] indicated that the effect of antioxidant activity varies if antioxidants are water- or lipid-soluble. The highest antioxidant capacity was that of the hydroethanolic extract obtained by ultrasound for both assays. The values obtained

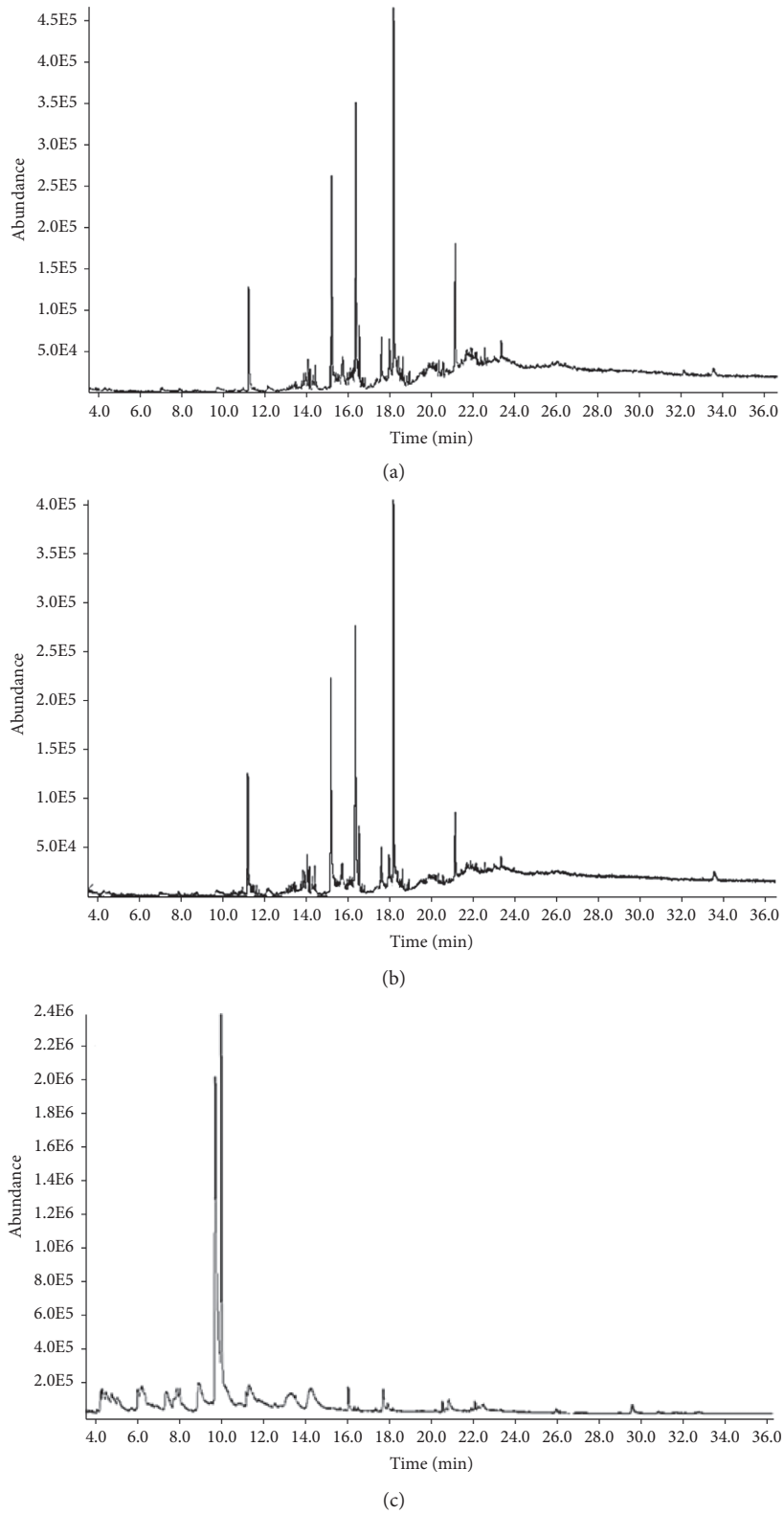

Figure 2: Total ion chromatograms obtained by GC-MS of *N. lappaceum* peel extracts. (a) Ethanolic extract obtained by LLE with ethyl acetate. (b) Ethanolic extract obtained by LLE with hexane. (c) Hydroethanolic crude extract.

are similar to those recently reported by Nguyen et al. [3], 2.16 mmol TE g^{-1} for the ABTS assay and 1.85 mmol TE g^{-1} for the DPPH assay in the methanolic extract of the *N. lappaceum* peel.

Compared with some other fruits and vegetables, the antioxidant capacity of *N. lappaceum* determined through the radical DPPH method is higher than those reported for apples (14 μmol TE g^{-1}), bananas (5.2–11 μmol TE g^{-1}),

Table 5: Active compounds identified in the mass spectra of *N. lappaceum* peel extracts.

Compound (type)	Molecular formula	T_r (min)	Peak area (%)	Molecular weight ($g \cdot mol^{-1}$)	Top peak	Fragment ions (m/z), 2nd and 3rd highest	Extract
5-Methylfuran-2-carbaldehyde (arenecarbaldehyde)	$C_6H_6O_2$	4.1	0.20	110.11	110	109 and 53	Crude
1,2-Benzenediol (hydroxybenzene)	$C_6H_6O_2$	7.4	2.97	110.11	110	64 and 63	Crude
1,2,3-Benzenetriol or pyrogallol (catechin)	$C_6H_6O_3$	9.7	29.17	126.11	126	52 and 80	Crude
3-Hydroxybenzoic acid (phenolic acid)	$C_7H_6O_3$	11.3	7.86	138.12	138	121 and 60	Crude
Hexadecanoic acid or palmitic acid (phenolic acid)	$C_{16}H_{32}O_2$	16.0	1.11	256.43	73	60 and 55	Crude
Hexadecanoic acid, ethyl ester (fatty acid ethyl ester)	$C_{18}H_{36}O_2$	16.4	0.18	284.50	88	101 and 55	Crude
Oleic acid methyl ester (fatty acid methyl ester)	$C_{19}H_{36}O_2$	17.4	0.18	296.5	55	182 and 73	Crude
(9*Z*,12*Z*)-Octadeca-9,12-dienoic acid or linoleic acid (fatty acid)	$C_{18}H_{32}O_2$	17.7	0.29	280.4	67	81 and 55	Crude
9-Octadecenoic acid or elaidic acid (phenolic acid)	$C_{18}H_{34}O_2$	17.8	1.30	282.5	55	73 and 60	Crude
Beta-tocopherol or vitamin E (vitamin)	$C_{28}H_{48}O_2$	26.0	0.40	416.7	416	151 and 207	Crude
2,4-Di-*tert*-butylphenol (alkylbenzene)	$C_{14}H_{22}O$	11.24	7.78 and 9.21	206.32	191	206 and 57	LLE with hexane and ethyl acetate
Isobutyl octyl phthalate	$C_{20}H_{30}O_4$	15.22	15.66 and 16.46	334.45	149	57 and 223	LLE with hexane and ethyl acetate
Hexadecanoic acid, methyl ester (fatty acid methyl ester)	$C_{17}H_{34}O_2$	15.75	4.02 and 2.44	270.5	74	87 and 57	LLE with ethyl acetate
Hexadecanoic acid, ethyl ester (fatty acid ethyl ester)	$C_{18}H_{36}O_2$	16.38	16.54 and 17.79	284.5	88	101 and 55	LLE with hexane and ethyl acetate
Octadecanoic acid, methyl ester or methyl stearate (fatty acid methyl ester)	$C_{19}H_{38}O_2$	17.6	3.55 and 4.17	298.50	74	87 and 57	LLE with hexane and ethyl acetate
Oleic acid, ethyl ester (fatty acid ethyl ester)	$C_{20}H_{38}O_2$	18.0	4.37 and 3.07	310.51	69	55 and 83	LLE with hexane and ethyl acetate
Octadecanoic acid, ethyl ester (fatty acid ethyl ester)	$C_{20}H_{40}O_2$	18.2	24.30 and 25.2	312.5	88	101 and 55	LLE with hexane and ethyl acetate
Diisooctyl phthalate (phthalate ester)	$C_{24}H_{38}O_4$	21.2	8.88 and 5.33	390.6	149	167 and 57	LLE with ethyl acetate

Tr: retention time.

grapes (19–55 μmol TE g^{-1}), kiwis (10 μmol TE g^{-1}), nectarines (12 μmol TE g^{-1}), plums (17–22 μmol TE g^{-1}), broccoli (6–1.8 μmol TE g^{-1}), garlic (0.6–13 μmol TE g^{-1}), onions (0.6 μmol TE g^{-1}), potatoes (3.5–4 μmol TE g^{-1}), peppers (11–12 μmol TE g^{-1}), and spinach (1.8 μmol TE g^{-1}) [73–75]. Similarly, the antioxidant capacity of the *N. lappaceum* peel, determined through the radical ABTS method, is much higher than those reported for apples (10–14 μmol TE g^{-1}), berries (16–31 μmol TE g^{-1}), bananas (9.2 μmol TE g^{-1}), grapes (8.7 μmol TE g^{-1}), kiwis (7.8 μmol TE g^{-1}), nectarines (8 μmol TE g^{-1}), plums (9.8 μmol TE g^{-1}), broccoli (6.8 μmol TE g^{-1}), onions (4–7.2 μmol TE g^{-1}), peppers (3.6–19.8 μmol TE g^{-1}), and spinach (22.4 μmol TE g^{-1} [71, 76].

Antioxidant capacity has an intrinsic relationship with phenolic compounds. Different studies have shown that these compounds can act as powerful antioxidants because of their activity against free radicals and their ability to chelate metals [2, 4, 7]. The Pearson correlation coefficients showed that the TFC and TPC have a strong correlation with antioxidant activity found using an ABTS assay, with $r = 0.84$ and $r = 0.83$, respectively, at a confidence level of 95.0% ($p < 0.05$). However, the correlation with antioxidant activity found using a DPPH assay was lower, with $r = 0.68$ and $r = 0.77$ for the TFC and TPC, respectively. The TAC was found to have a weak correlation with antioxidant capacity, with $r = 0.15$ and $r = 0.10$ found using ABTS and DPPH, respectively. These findings reveal that the antioxidant capacity of the *N. lappaceum* peel was mainly caused by phenolic acids and flavonoids. These results are consistent with the studies on the *Salacia chinensis* root extract, as reported by Ngo et al. [59], which concluded that phenolic compounds and flavonoids are mainly responsible for antioxidant activity, while anthocyanidins have a weak correlation with antioxidant capacity. Although anthocyanins have been reported in some works to have antioxidant properties, it is important to emphasize that these properties depend on their specific chemical structure.

Table 6: Activities and uses reported for the compounds identified in *N. lappaceum* peel extracts.

Compounds	Activity or uses
5-Methylfuran-2-carbaldehyde	Food additive (flavoring agent) [77]
1,2-Benzenediol	Precursor to pesticides, flavors, and fragrance [77]
1,2,3-Benzenetriol (pyrogallol)	Cytotoxic effect on human lung cancer cell lines [78], inhibition or retardation of oxidation reactions, effective antiseptic and antipsoriatic agent [79, 80]
3-Hydroxybenzoic acid	Production of glycol benzoates used as plasticizers [77]
Hexadecanoic acid (palmitic acid)	Novel therapeutic agents that specifically attack multiple myeloma cells and inhibit HIV-1 infection [55, 81]
Hexadecanoic acid, ethyl ester	Food additive (flavoring agent) [77]
Oleic acid methyl ester	Additive for detergents, emulsifiers, wetting agents, stabilizers, textile treatment, plasticizers for duplicating inks, rubbers, waxes, chromatographic reference standard [77]
(9*Z*,12*Z*)-Octadeca-9,12-dienoic acid (linoleic acid)	Improving insulin sensitivity and reducing the risk for type 2 diabetes. Anti-inflammatory effects that may alleviate migraine [82, 83]
9-Octadecenoic acid (elaidic acid)	Food additive [77]
Beta-tocopherol (vitamin E)	Antioxidant [77]
2,4-Di-*tert*-butylphenol isobutyl octyl phthalate	Antifungal, antioxidant, and anticancer [84–86]
Hexadecanoic acid, methyl ester	Anti-inflammatory [87], antifibrotic [88], biodiesel [89], neuroprotector [90], additive for detergents, emulsifiers, wetting agents, stabilizers, resins, lubricants, plasticizers, animal feed, and medical research [91]
Hexadecanoic acid, ethyl ester	Anti-inflammatory [87], antimicrobial [92], acaricidal [93], and flavoring agent or adjuvant [94]
Octadecanoic acid, methyl ester (methyl stearate)	Neuroprotector [90], anti-inflammatory [95], additive for detergents, emulsifiers, wetting agents, stabilizers, resins, lubricants, plasticizers [91]
Oleic acid, ethyl ester	Solvent, plasticizer, lubricant, hydrophobic agent, flavoring [96]
Octadecanoic acid, ethyl ester	Flavoring agent or adjuvant
Diisooctyl phthalate	Plasticizer for vinyl, cellulosic, and acrylate resins and synthetic rubber [97]

In summary, these results show that extraction solvents and extraction technique significantly affected the active compound yield and antioxidant properties of the extracts.

3.4. GC-MS Analysis. GC-MS was used for analyzing volatile organic compounds present in *N. lappaceum*. The bioactive compounds present in the extracts obtained from the *N. lappaceum* peel are summarized in Figure 2 and Table 5. The total ion chromatograms of the three extracts presented in Figure 2 show the retention time and signals that correspond to the active compounds present in the extract. The elution time, molecular formula, molecular weight, and fragment ions are given in Table 5.

A total of 18 compounds were identified, based on their fragmentation patterns, and have been reported for the first time in the *N. lappaceum* peel. These compounds were hydroxybenzenes, phenolic acids, flavonoids, fatty acids (saturated, unsaturated, and ester), vitamins, arene-carbaldehydes, and phthalates, with the specific species including 5-methylfuran-2-carbaldehyde, 1,2-benzenediol, 1,2,3-benzenetriol (pyrogallol), 3-hydroxybenzoic acid, hexadecanoic acid (palmitic acid), hexadecanoic acid, ethyl ester, oleic acid methyl ester, (9Z,12Z)-octadeca-9,12-dienoic acid (linoleic acid), 9-octadecenoic acid (elaidic acid), beta-tocopherol (vitamin E), 2,4-di-*tert*-butylphenol, isobutyl octyl phthalate, hexadecanoic acid, methyl ester, hexadecanoic acid, ethyl ester, octadecanoic acid, methyl ester (methyl stearate), oleic acid, ethyl ester, octadecanoic acid, ethyl ester, and diisooctyl phthalate. These identified compounds have been previously isolated from other plants and are known to exhibit important activities (Table 6). Notably, 1,2,3-benzenetriol exhibits activity against lung cancer cell lines, hexadecanoic acid attacks multiple myeloma cells and inhibits HIV-1 infection, and (9*Z*,12*Z*)-octadeca-9,12-dienoic acid reduces the risk for type 2 diabetes. Among the compounds with antioxidant properties, we identified beta-tocopherol and 2,4-di-*tert*-butylphenol, which likely contribute to the antioxidant capacity observed in the *N. lappaceum* peel.

To the best of our knowledge, there are no studies that identify the compounds reported here. Researchers from China, Malaysia, and Thailand have previously reported the presence of other phenolic compounds such as ellagic acid, gallic acid, corilagin, *p*-coumaric acid, catechin, rutin, and geraniin in *N. lappaceum* peels, found by HPLC-MS, HPLC-UV, and NMR [2, 3, 9, 36]. Recently, researchers in Vietnam have also identified other compounds, such as kaempferol and quercetin [3]. Additionally, researchers in Mexico have been the first to identify ellagitannins, such as brevifolin carboxylic acid, ellagic acid pentoside, castalagin/vescalagin, and galloyl-bis-HHDP-hexoside (casuarinin), in *N. lappaceum* peels [7]. This shows that the chemical diversity of *N. lappaceum* plants varies depending on their geographical location and climate. The chemical pattern of our sample is noticeably different in composition from that of other *N. lappaceum* peels reported in other countries [2, 3, 7, 9, 36].

4. Conclusions

The results presented here indicate that the compounds in the extracts obtained from the *N. lappaceum* peel could be significantly affected by the extraction solvents and extraction technique used. The highest TPC and TFC were

obtained in the hydroethanolic ultrasound extracts, and the highest TAC was obtained in the acid extract. The extracts evaluated exhibited good antioxidant capacity with both high inhibitory activity against ABTS•+ radical cations and strong scavenging activity against DPPH radicals. In this work, we identified 18 compounds that had not previously been isolated from *N. lappaceum* and found that their peels are rich in phenolic acids, flavonoids, and fatty acids. They contain compounds that have excellent antioxidant capacity and are being studied intensively in other plants for their ability to improve human health and counteract chronic diseases. The new compounds can also be used for industrial and food applications, including detergent additives, biodiesel, emulsifiers, wetting agents, lubricants, stabilizers, food additives, and flavoring agents. Additional studies will be carried out to evaluate the biological activities and to identify more specific phytochemicals responsible for these activities.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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