Research Article

DOI: http://dx.doi.org/10.53043/2347-3894.acam12008

Analysis of the Phytochemistry, Antioxidant Properties, and Antibacterial Efficacy of Extracted *Rubus rosifolius Sm.* (Sampinit)

Rufo S. Calixtro, Jr.[1]*, Neil Jade G[2], Kozo Watanabe[3] and Maria Nilda M. Munoz[4]*

[1]University of Perpetual Help System Laguna, Binan, Laguna, Philippines
[2]Palude, St. Luke's College of Medicine
[3]Center for Marine and Environmental Studies, Ehime University, Ehime, Japan
[4]University of Perpetual Help System Laguna/Cagayan State University, Binan, Laguna/Tuguegarao City, Cagayan, Philippines

Asian Journal of Complementary and Alternative Medicine. Volume 12 Issue 03

Published on: 13/12/2024

***Author(s) for Correspondence:** Rufo S. Calixtro, Jr., University of Perpetual Help System Laguna, Binan, Laguna, Philippines. Maria Nilda M. Munoz, University of Perpetual Help System Laguna/Cagayan State University, Binan, Laguna/Tuguegarao City, Cagayan, Philippines.

Cite this article as: Calixtro RS, Neil Jade G, Watanabe K, Maria Munoz NM. *Analysis of the Phytochemistry, Antioxidant Properties, and Antibacterial Efficacy of Extracted Rubus rosifolius Sm. (Sampinit).* Asian Journal of Complementary and Alternative Medicine, Vol 12(3), 58-67:2024.

ABSTRACT

The objective of the study is to evaluate the phytochemical content (both qualitative and quantitative), free radical scavenging activity utilizing 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH), and antibacterial characteristics of unripe *Rubus (R.) rosifolius* Sm. in vitro. *R. rosifolius* Sm. is a recently identified wild raspberry in the Philippines, commonly called "Sampinit." The extracted Sampinit fruits with 80% methanol were analyzed qualitatively for the presence of polyphenols (tannins and flavonoids), steroids (terpenoids), and amino acids (n=3). In a separate aliquot, quantitative phytochemical analysis was conducted. The steroid concentration was 607.98 ± 2.87 mg cholesterol equivalent per gram of sample, polyphenol content was 764.31 ± 11.37 mg gallic acid equivalent per 100 grams of sample, and flavonoid concentration was 3.60 ± 0.21 mg quercetin equivalent per gram of sample. The consistency of the extraction method was verified by using high-performance chromatography which exhibited comparable chromatograms and retention time for the injected samples. The average DPPH scavenging activity of the methanolic extract was 92.83% (± 0.16), in contrast to the positive control (beta hydroxycarboxylic acid or BHA) activity of 87.64% (± 9.77). The extract had antibacterial efficacy against *Staphylococcus aureus* but showed no action against *Enterococcus faecalis, Klebsiella pneumoniae*, and *Pseudomonas aeruginosa.* Our findings indicate that unripe Sampinit may serve as abundant sources of phytochemicals, especially phenolic compounds and flavonoids, which possess natural antioxidant and antibacterial properties, with potential usage in various inflammatory diseases such as lung inflammation, cancer, and metabolic disorders.

Keywords: *Wild raspberry; unripe Rubus (R.) rosifolius* Sm.*; phytochemistry; antioxidant; antibacterial*

INTRODUCTION

Raspberries have long been cultivated for centuries for their fruit [1]. The unripe fruit has been used as a traditional remedy for wounds, burns, and inflammation [2-4]. The Sampinit, also known as the wild raspberry (*Rubus rosifolius* J. E. Sm, *Rosaceae),* is a recently found crop of red and delicious fruits that are abundant in nutrients and antioxidants. Although it is mostly offered in its fresh form, it is also processed naturally into jam, juice, and wine, albeit on a smaller scale.

Sampinit is a nutritious food and demonstrates that the fresh and processed goods include a multitude of vital components that are beneficial to the body, including carbon, oxygen, potassium, and calcium. Saponins, a phytochemical that inhibits the proliferation of cells that are associated with colon tumors, are also present in it. Nevertheless, the plant has a number of additional molecules that act as antioxidants, so lowering the chance of developing cancer.

Sampinit is a plant that may reach a height of three meters and produces red berries that are spherical and many in a single container. Its stems are prickly and coated with thorns that range in size from one to four millimeters. Composed of 5-7 leaflets, the leaves are pinnately complex in structure. There are a majority of solitary, terminal or axillary blooms on the inflorescence, which are atop pedicels that are between

0.5 and 3.0 centimeters in length. Photograph on the left illustrates the newly harvested fruits that make up Sampinit.

Research has shown that the genus *Rubus*, which has more than one hundred species, contains considerable quantities of physiologically active components. These components include polyphenolic compounds, which include flavonoids, anthocyanins, tannins, and other similar substances [5]. Several studies have demonstrated that plant extracts that include phenolic compounds possess anti-inflammatory, anticancer, anti-aging, antioxidant, antibacterial, and antiviral properties [6]. The mature Sampinit that was cultivated in the Philippines was subjected to phytochemical research, which revealed that it has anti-proliferative effects and included leucoanthocyanins, saponins, and anthraquinones [7].

Recuenco et al., [8] described the phytochemical contents of ten different indigenous fruits from the Philippines, including "Sampinit". They also reported the total phenolics, flavonoids, and antioxidant and antibacterial activity of these fruits. However, the active components remain to be identified. Polyphenols, flavonoids, tannins, cardiac glycosides, saponins, and terpenoids were discovered in the "Sampinit" fruits that were gathered in Dolores, Quezon. Previous studies have reported that fruits contain some of these compounds [8]. In addition to having modest antioxidant qualities, it has a moderate quantity of total phenolic and total flavonoids, and it is particularly effective against *Escherichia coli,* which is a gram-negative bacterium. The unripe fruits of this fruit-bearing plant have been the subject of a limited number of research that has investigated the entire characterization of the phytochemical contents. Nonetheless, the medicinal benefit of Sampinit has not been fully elucidated.

For this reason, the researchers decided to carry out the study to assess the phytochemical elements of the locally cultivated unripe Sampinit by quantifying the total phenolics, total steroid, and total flavonoid contents. In addition, the antibacterial activities against four different bacterial isolates were determined, as well as the HPLC profiling and the free radicals scavenging activity using DPPH.

MATERIALS AND METHODS

Plant material

The unripe wild raspberry (*Rubus rosifolius* Sm.) fruits were collected at Sitio Upper Malamig, Brgy. San Diego, San Pablo City, Laguna, Philippines. The fruit season usually starts in September and ends in March. For consistency of results, unripe wild raspberry was collected once in bulk enough for 3 extractions for every independent experiment. For standardization, unripe wild raspberry was collected on the same field. It was authenticated by the curator of Botanical Herbarium of the Museum of Natural History, University of the Philippines Los Baños, Laguna.

Extraction of *Rubus rosifolius* Sm.

Fresh Sampinit was oven-dried at 60°C for 3 days or until dry. Ten grams of dried plant materials were mixed with 30 mL of 80% (v/v) methanol (Thermofischer) yielding a concentration of 0.33 g/mL. It was stirred at the setting of 6 at 50°C for 1 hr using hot plate stirrer (Fischer Scientific). The extracts were filtered through a Whatman No. 1 filter paper. The filtrate was collected and stored at -20°C until used. The methanol extract was concentrated to dryness under reduced pressure in GeneVac to yield powdered unripe raspberry extract. The settings used are as follows: low BP, odor reduction: 50 mbar, max temperature: 37 °C, and time to final stage: preset heat flow. The GeneVac was run for a total of 5 hr until the methanolic extracts were completely dried.

Qualitative Phytochemical Analysis

The fruits underwent phytochemical analysis utilizing the methods outlined by Muñoz et al., [9]. A standard concentration of 0.33 g/mL was prepared by dissolving one gram of oven-dried material in 3 mL of 80% methanol and was subsequently submitted to qualitative testing as follows:

Test for Tannins - Ferric Chloride Test. A total of 3-5 drops of Ferric Chloride solution were added to 0.5 mL of the extract. The formation of greenish-brown, brown, and black solution indicates the presence of tannins.

Test for Saponins – Froth Test. A total of 0.5 mL of the extract and 1 mL of distilled water was separately boiled in a water bath for 10 min. While hot, the mixture was filtered and cooled at room temperature. 1.5 mL of the filtrate was diluted with 5 mL of distilled water. It was vigorously shaken for 2 min. Frothing indicates the presence of saponins in the filtrate.

Test for Flavonoids. A total of 0.5 mL of the extract was boiled with 2.5 mL of distilled water for 5 min and was filtered while hot. A few drops of 20% sodium hydroxide solution were added to the cooled filtrate. The change from yellow color to colorless solution upon the addition of acid (10% HCL) indicates the presence of flavonoids.

Test for Combined Anthraquinones. A total of 0.5 mL of sample was boiled with 1 mL of 10% HCl for 5 min. While hot the mixture was filtered and the filtrate was allowed to cool. The cooled filtrate was added with an equal volume of

chloroform. The chloroform layer was transferred into a clean dry test tube using a clean pipette. An equal volume of 10% ammonia solution was added to the chloroform-containing test tube. The mixture was shaken and allowed to separate. The separated aqueous layer was observed for any color change; the delicate rose pink color indicates the presence of anthraquinones.

Test for Alkaloids. A total of 0.5 mL of the extract was evaporated to syrupy consistency. At 2.5 mL of 2% HCL, 0.25 g of powder NaCl was added. The mixture was stirred and filtered. The residue was washed with 2% HCl to achieve the volume of filtrate to 2.5 mL. Dragendorrf's reagent or Mayer's reagent was added to 0.5 mL of the filtrate. The presence of turbidity indicates the presence of alkaloids.

Test for Terpenoids. A total of 0.5 mL of the extract was added with 0.25 mL chloroform.1 mL of concentrated sulfuric acid was added to form a layer. A reddish-brown precipitate coloration at the interface formed indicates the presence of terpenoids.

*Test for Carotenoids***.** A total of 0.5 mL of the extract was added with 2.5 mL of chloroform in a test tube. The mixture was mixed vigorously. The resulting mixture was filtered and 1.5 mL of 85% sulfuric acid was added. A blue color at the interface indicates the presence of carotenoids.

Test for Quinones. A total of 0.5 mL of the extract was added with 1 mL of ethanol. 1 mL of potassium hydroxide was added to the mixture. The formation of blue color indicates the presence of quinones.

Test for Steroids. A total of 0.5 mL of the extract was dissolved in a 2.5 mL of chloroform. Equal volume of concentrated sulfuric acid was then added through the side of the test tube. The upper layer that turned red and the sulfuric acid layer that shows yellow with green fluorescence indicates the presence of steroids.

Test for Coumarin. A total of 0.5 mL of the extract was mixed with few drops of NaOH. 0.5 mL of ethanol was added to the mixture. Formation of yellow color indicates the presence of coumarins.

Test for Xantho-Proteins. A total of 0.5 mL of the extract was mixed with few drops of nitric acid. Few drops of 10% ammonia were added. Formation of red color indicates the presence of Xantho proteins.

Quantitative Phytochemical Analysis

Assessment of Total Phenolic Content - Folin-Ciocalteu Method [10]. Various concentrations (0.0025, 0.0150, 0.0250, 0.0350, 0.0450 mg/mL) of gallic acid solution (Sigma-Aldrich Chemical) were formulated as standards for calibration purposes. For sample preparation, 0.150 mL of distilled water was combined with 0.05 mL of a 0.33 g/mL extract. 1 mL of 10% Folin-Ciocalteu phenol reagent was added. 0.8 mL of 2% Na_2CO_3 was added into the mixture. To prepare the blank, 0.2 mL of distilled water was combined with 1 mL of 10% Folin-Ciocalteu phenol reagent. 0.8 mL of 2% Na_2CO_3 was added to the mixture. The standard, sample, and blank were incubated for 15 min at ambient temperature. The spectrophotometer (Sigmatech) was calibrated to an absorbance measurement of 765 nm. The spectrophotometer measurement was calibrated to zero or 0.000 using the blank sample. The standard and sample measurements were subsequently documented.

Test for Total Steroid Content [11]. For standard calibration preparation, eight test tubes containing varying concentrations of cholesterol standard (Sigma-Aldrich) - 25, 50, 100, 200, 300, 400, 500, and 700 µL, were produced, with each tube supplemented with 2,500 µL of cholesterol developer. The tubes containing the mixture were vortexed for 10 sec and thereafter put in a 37°C water bath for 10 min. The solutions were left to cool at ambient temperature for an additional 10 minutes. For sample preparation, 300 µL of extract was combined with 2.5 mL of cholesterol color developer. The sample was vortexed for 10 seconds and thereafter maintained at room temperature for 10 minutes. A spectrophotometer (Sigmatech) was calibrated to 620 nm for absorbance measurement. Distilled water served as the blank. The spectrophotometer reading was calibrated to zero or 0.000. The standard and sample measurements were documented.

Test for Total Flavonoid Content [12]. Various concentrations (0.0025, 0.0125, 0.0250, 0.0350, and 0.0500 mg/mL) of quercetin standard solution (Sigma-Aldrich) were formulated for calibration purposes. 1000 µL of the produced standards was used. Subsequently, 1000 µL of 2% $AlCl_3$ was included into the mixture. For sample preparation, 10 µL of the extract was combined with 990 µL of 2% $AlCl_3$. To prepare the blank, 10µL of methanol will be combined with 990µL of 2% aluminum chloride solution. The prepared standard, samples, and blank were incubated for 10 minutes at ambient temperature. The spectrophotometer (Sigmatech) was calibrated to 415 nm for absorbance measurement. Distilled water served as the control sample. The spectrophotometer reading was calibrated to zero or 0.000. The standard and sample measurements were documented.

High-Performance Liquid Chromatography (HPLC) profiling

To confirm the reliability of the extraction procedure, three

independently extracted samples were analyzed by HPLC. The desiccated samples (330 mg) were solubilized in 1 mL of 80% methanol (0.33 g/mL). Samples were injected and analyzed with the Agilent Technologies 1260 Infinity Quaternary LC, which was managed and controlled by Openlab CDS ChemStation workstation software. The column utilized was ZORBAX Eclipse XDB-C18, 4.6 x 100 mm, 5-Micron (Agilent). The mobile phase included 1% aqueous formic acid (A) and 1% formic acid in methanol (B). The linear gradient parameters are: 0-30 minutes, 80% to 35% A; 31-33 minutes, 100% B, with a total duration of 33 minutes, a flow rate of 0.5 mL/min, and a sample injection volume of 5 µL. The column temperature was 25 °C, and the DAD range was 210-600 nm. The employed criteria were quercetin, rutin, and gallic acid. These standards were purchased from Sigma-Aldrich.

Free Radical Scavenging Assay Using 2,2-diphenyl-1-picrylhydrazyl (DPPH) [13]

A total of 150 uL freshly prepared 0.1 mM DPPH (Sigma Aldrich Chemical) in MeOH was added to 50 uL diluted methanolic extract of Sampinit (10-fold). The resulting dilution was incubated in the dark at room temperature for 30 min. Using a microplate reader, the absorbance at 515 nm was measured. The % radical scavenging activity was calculated using the equation: % radical scavenging activity = $[(A_{515Control} - A_{515sample})]$ x 100%, where $A_{515Control}$ = absorbance of DPPH solutions without unripe sampinit extract at 0 min, while $A_{515Sample}$ = absorbance of DPPH solutions with unripe Sampinit extract after 10 min. Beta-hydroxycarboxylic acid or BHA was used as a standard. Results were expressed in % radical scavenging activity.

Antibacterial Activity Using Disk Diffusion Assay (Kirby-Bauer Method)

A total of 0.33 g/mL stock extract solution was prepared by dissolving the extract in sterile distilled water and diluting to yield 1:10 dilution. This stock extract was sterilized and filtered using filter paper (0.2 µm) and stored in Eppendorf tubes at 4°C. Test organisms utilized in this research were *Staphylococcus aureus*, *Enterococcus faecalis, Klebsiella pneumoniae, and Pseudomonas aeruginosa.* These standard bacterial isolates were obtained from the UPLB BIOTECH. Test organisms were cultured overnight at 37°C before being used in the antibacterial assay. The unripe extract of Sampinit was tested for antimicrobial activity using the disc diffusion method (Kirby-Bauer method). Sterile commercial blank discs (Fischer Scientific), 8.0 mm diameter, were impregnated with 1:10 extract dilution. Discs were stored at −5°C before use. Overnight broth cultures were adjusted using a turbidimeter to yield approximately 1.0 x 10^8 cfu per mL. Extract-impregnated discs (20 µL) were placed on agar plates and incubated at 37°C for 24 hrs. Sterile distilled water (20 µL) was used as a negative control, while 10% phenol disc (20 µL) was used as a positive control. These were done in 3 replicates. Antibacterial activities were then determined by measuring the clear zone of inhibition to the nearest millimeter (mm) ± S.E.M using a caliper.

RESULTS AND DISCUSSION

Qualitative Phytochemical Analysis

Qualitative phytochemical analysis was performed to screen and identify medicinally active secondary metabolites found in unripe Sampinit. The panel of tests revealed the presence of tannins, saponins, flavonoids, terpenoids, steroids, coumarins, and xantho proteins (amino acids) (Table 1). However, carotenoids, alkaloids, quinone, and combined anthraquinones were not observed (Table 1). Tests were run in triplicates and were reproducible in three independent experiments/extraction.

The unripe Sampinit extract contained almost the same active secondary metabolites (phenolics, tannins, saponins, flavonoids, and terpenoids) as the ripened extract of Sampinit evaluated by Recuenco et al., [8]. Likewise, alkaloids were reported to be nonexistent. However, our results reported greater phenolics, tannins, saponins, and terpenoids contents. These results could provide valuable information on specific phytochemical constituents of the unripe Sampinit extract before more focused or detailed pharmacologic studies.

Table 1. Qualitative Phytochemical Analysis of Unripe *R. rosifolius* Sm. The extract showed positive signals for tannins, saponins, flavonoids, terpenoids, steroids, coumarins, and Xantho proteins (amino acids. Combined anthraquinones, alkaloids, carotenoids, and quinones were not observed.

Tests for:	Result (n=3)		
	Trial 1	Trial 2	Trial 3
Tannins (Ferric Chloride Test)	+++	+++	+++
Saponins (Froth Test)	++	++	++
Flavonoids	+	+	+
Combined Anthraquinones	-	-	-
Alkaloids	-	-	-
Terpenoids	++	++	++
Carotenoids	-	-	-
Quinones	-	-	-
Steroids	+++	+++	+++
Coumarins	++	++	++
Xantho Proteins (Amino Acids)	++	++	++

Quantitative Phytochemical Analysis

Quantitative phytochemical analysis was used to quantitate select secondary metabolites that showed positive results from the screening process. The number of phytochemicals which are found in 0.33 g/mL of 80% methanol extract was quantitatively determined by standard procedures.

Total Phenolic Content

Total phenolic content was measured quantitatively and was expressed as mg gallic acid equivalent (GAE) per 100g of sample. The standard calibration curve has an R^2 of 0.99 (Figure 1). As seen in Table 2, composite data showed that total phenolic content has a mean + SEM of 764.31 + 11.37 mg GAE/ 100 g sample. Experiments were performed in triplicates and were reproducible in three different extracted samples.

Total Flavonoid Content

Total flavonoid content was measured quantitatively and was expressed as mg quercetin equivalent (QE) per g of sample. The standard calibration curve has an R^2 of 0.98 (Figure 2) As seen in Table 3, composite data showed that total flavonoid content has a mean + SEM of 3.60 + 0.21 mg QE/ g sample. Experiments were performed in triplicates and were reproducible in three different extracted samples.

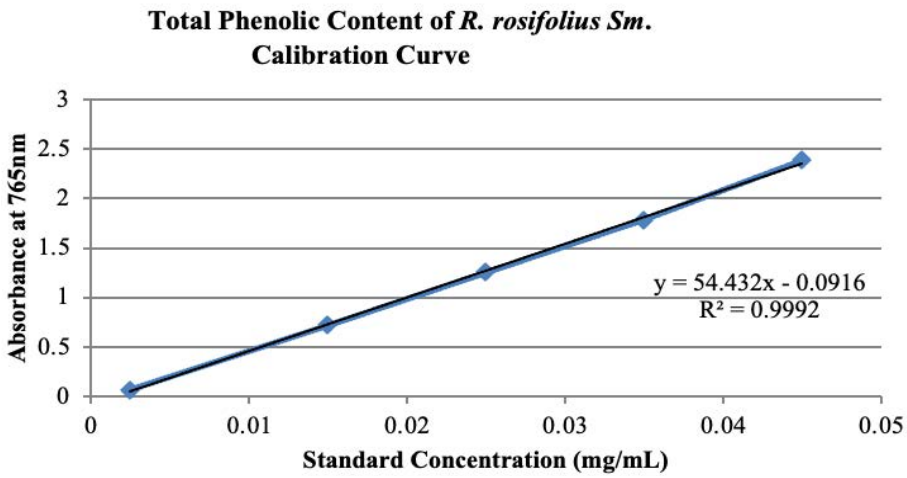

Figure 1. Standard calibration curve of Gallic acid. Total phenolic content was measured quantitatively and gallic acid (GA) was used as reference drug. The correlation coefficient (R^2) indicates the degree of linearity of the calibration curve where R^2 = 0.99. Absorbance was set to 765 nm.

Table 2. Total Phenolic Content of *R. rosifolius* Sm. The composite findings indicated that the overall phenolic content had a mean ± SEM of 764.31 ± 11.37 mg GAE/100 g sample. Experiments were conducted in triplicate (n=3) and were repeatable among three distinct extracted samples.

Extract	Trial	Concentration (mg gallic acid equivalent per 100 g sample)
Total Phenolic	**1**	766.01 ± 12.61
	2	762.31 ± 10.08
	3	764.61 ± 11.42
	Mean	764.31 ± 11.37

Values are expressed as mean ± SEM (n=3)

Table 3. Total Flavonoid Content of *R. rosifolius* Sm. Composite data showed that total flavonoid content has a mean ± SEM of 3.60 ± 0.21 mg QE/ g sample. Experiments were performed in triplicates and were reproducible in three different extracted Sampinit fruits.

Extract	Trial	Concentration (mg Quercetin equivalent per g sample)
Total Flavonoid	**1**	3.14 ± 0.29
	2	4.34 ± 0.82
	3	4.30 ± 0.22
	Mean	**3.60 ± 0.21**

Values are expressed as mean ± SEM (n=3)

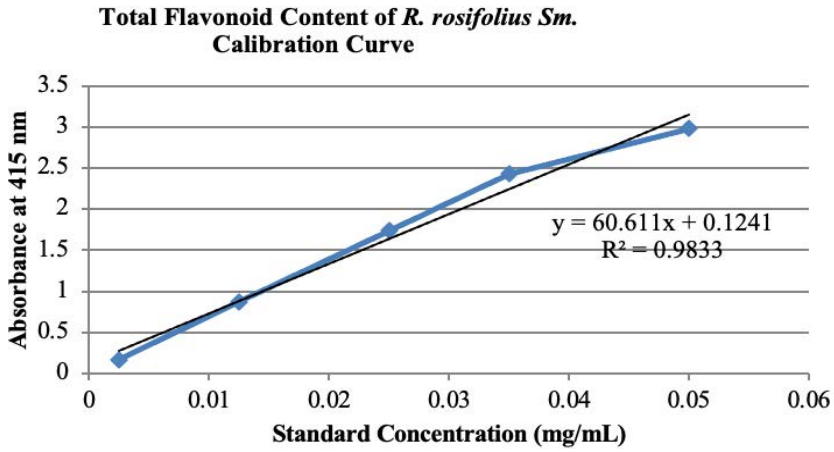

Figure 2. Calibration Curve of Quercetin (QE). Total phenolic content was measured quantitatively and Quercetin was used as reference drug to measure the total flavonoid content in extracted Sampinit. The correlation coefficient (R^2) indicates the degree of linearity of the calibration curve where $R^2 = 0.9833$. Absorbance was set to 415 nm.

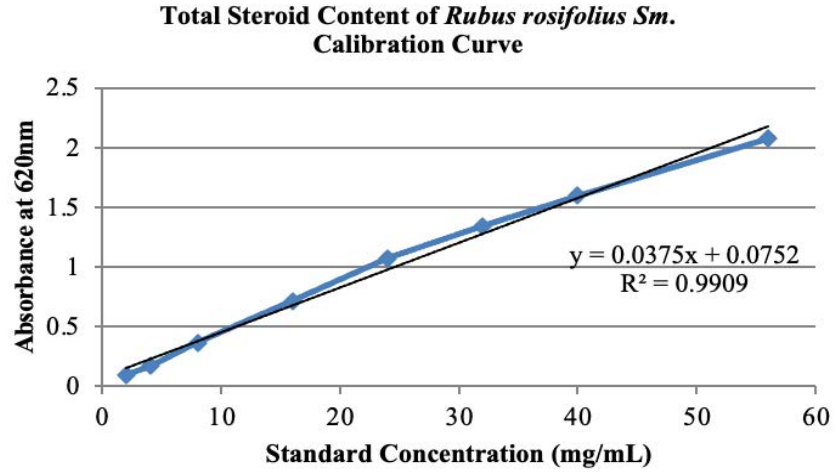

Figure 3. Calibration curve of Steroid. Total steroid content was measured quantitatively and laboratory grade cholesterol was used as reference drug to measure the total steroid content in extracted Sampinit. The correlation coefficient (R^2) indicates the degree of linearity of the calibration curve where $R^2 = 0.9909$. Absorbance was set to 620 nm.

Table 4. Total Steroid Content of *R. rosifolius* Sm. Composite data showed that total cholesterol has a mean ± SEM of 607.98 ± 2.87 in 200 mg% sample. Experiments were performed in triplicates and were reproducible in three different extracted Sampinit fruits.

Extract	Trial	Concentration (Cholesterol standard in 200 mg%)
Total Steroid	**1**	617.82 ± 2.27
	2	601.92 ± 3.87
	3	604.19 ± 1.60
	Mean	**607.98 ± 2.87**

Values are expressed as mean ± SEM (n=3)

Total Steroid Content

Total steroid content was measured quantitatively and was expressed as cholesterol equivalent in 200 mg per g sample. The standard calibration curve has an R^2 of 0.99 (Figure 3). As seen in Table 4, composite data showed that total steroid content has a mean + SEM of 607.98 + 2.87 mg cholesterol equivalent in 200%/ g sample. Experiments were performed in triplicates and were reproducible in three different extracted samples.

The methanolic extract of Sampinit included a total phenolic content of 764.31 ± 11.37 mg GAE/100g extract, a steroidal content of 607.98 ± 2.87 mg cholesterol per 200 mg%, and a flavonoid content of 3.60 ± 0.21 mg quercetin per gram of sample. All data are expressed as the mean ± standard deviation (SD) of three replicates. Phenolic chemicals, including gallic acid, have been documented in substantial quantities within the *Rubus* species [14,15,16]. In conjunction with flavonoids like quercetin, they have been demonstrated in prior research to possess antioxidant properties [17,18]. Compared to other wild raspberry types, the methanolic Sampinit extract cultivated locally exhibited elevated levels of several secondary metabolites. The current findings suggest that unripe Sampinit fruits may serve as alternative abundant sources of these beneficial compounds.

High-Performance Liquid Chromatography

HPLC grade standards, including gallic acid, rutin hydrate, and quercetin, were utilized for the HPLC standard chromatogram (Figure 6). The HPLC profile of the 80% methanolic extract of unripe Sampinit at a concentration of 0.33 g/mL was analyzed (Figure 7). The measurements were both taken at 360 nm. The acquired chromatogram was consistent across three separate trials.

Figure 4 illustrates that the HPLC chromatogram of standards, including gallic acid, rutin hydrate, and quercetin, exhibited peaks at 3.3, 22.6, and 30.1 minutes, respectively. Figure 5 illustrates many peaks, including the peak at par relative to the standard employed.

The findings validated that the methanolic extract of unripe Sampinit had gallic acid, rutin, and quercetin. Previous research has identified and verified that these metabolites are responsible for plant extracts' antioxidant, anti-inflammatory, antibacterial, and antitumor properties [19]. This information can be employed in future research

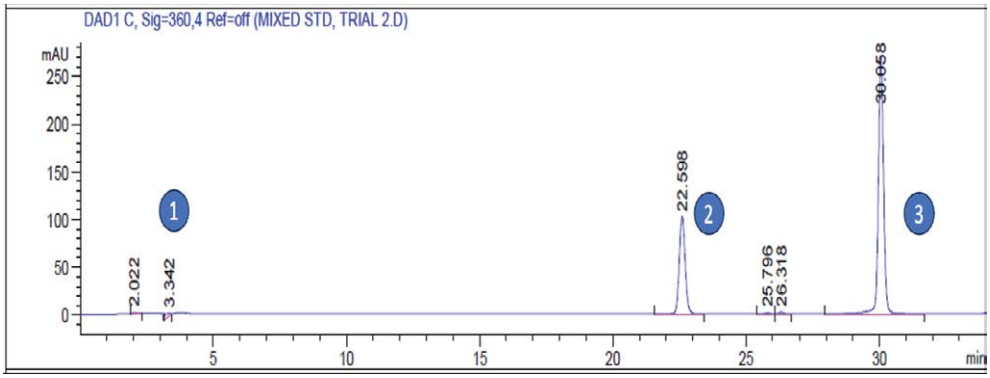

Figure 4. Representative HPLC chromatogram of mixed standard at 360 nm detection. Laboratory grade reference drugs, gallic acid, rutin hydrate and quercetin were injected into the HPLC column and retention times in min were monitored.
(Legend: 1. Gallic acid 2. Rutin Hydrate 3. Quercetin)

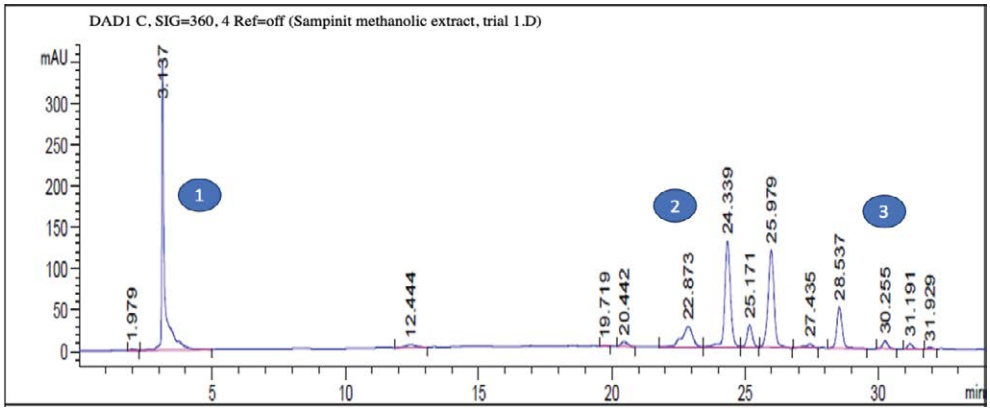

Figure 5. Representative HPLC chromatogram of unripe *R. rosifolius* Sm. extract measured at absorbance = 360nm. Gallic acid demonstrated high amount compared to rutin hydrate and quercetin.
(Legend: 1. Gallic acid 2. Rutin Hydrate 3. Quercetin)

to assess the pharmacological potentials of unripe Sampinit fruits. Moreover, our HPLC profile identified other peaks that suggest the unripe Sampinit fruits harbor other active secondary metabolites, which might serve as reference points for subsequent research. The standards were not available during the studies, leading to their non-identification.

The HPLC profiling of Sampinit fruits conducted in this work comprehensively characterizes their phytochemical composition, elucidating the presence and quantity of beneficial chemicals. This is the first demonstration to analyze the extracted Sampinit by HPLC which could uncover the distinctive nutritional and therapeutic attributes, potentially resulting in novel uses and increased value for these wild raspberry fruits. However, this study has several limitations. Inadequate assessment of other parts, leaves, stem, and ripeness of Sampinit, have been omitted. We conducted quantification of 3 secondary metabolites but we did not reveal the structural formula of the active compounds in Sampinit.

Antioxidant Activity of Unripe Sampinit. using DPPH

DPPH is one of the most widely used methods for screening the antioxidant activity of plant extract [20]. DPPH tests both the lipophilic and hydrophilic compounds and this does not restrict quantifying antioxidants solely based on their nature [21,22]. The reducing capacity of the plant material was indicated by a change from the purplish color of the DPPH solution to a yellow-colored product.

Del Rio et al., [23] asserted that polyphenols have long been acknowledged for their role in preventing chronic illnesses, including cancer and cardiovascular ailments. They explained that oxidative stress contributes to the onset of chronic diseases, caused by increased levels of reactive oxygen species and free radicals that damage critical biological components such as DNA, lipids, and proteins. Plant-derived polyphenols and flavonoids may mitigate harmful effects by functioning as antioxidants, capable of neutralizing free radicals inside cellular structures and diminishing oxidative damage.

The unripe Sampinit extract demonstrated antioxidant action against the DPPH radical (Table 5). The DPPH radical scavenging inhibition of the extract varies from 92.66% to 93.04%, with a mean of 92.83% (± 0.16). The 25 µL concentration of the unripe Sampinit extract had the strongest DPPH radical scavenging activity. This is marginally lower than the scavenging inhibition % of the BHA standard at the identical concentration. The diminished efficacy may result from the obstruction of active antioxidant phytochemicals by other constituents in the heterogeneous extract. Nonetheless, the unripe Sampinit extract demonstrated a superior mean percentage of DPPH radical scavenging inhibition (92.85% compared to 87.64%). The current findings concur with the prior research by Recuenco et al. [8], but with differing numerical activity estimates. Tiwari and Cummins [24] suggested that the inconsistencies could stem from differences in experimental conditions, as well as variations in the methods used for fruit cultivation, collection, and storage. This finding verifies that the many phytochemicals in unripe Sampinit extract may diminish or eliminate destructive free radicals from the cell.

Further, the higher mean percentage of DPPH radical scavenging inhibition of the unripe Sampinit fruits indicates their potent antioxidant capacity, which could make them valuable for health-related applications in preventing oxidative stress-related diseases. This finding highlights the potential of unripe Sampinit as a rich source of natural antioxidants.

Antibacterial Activity of Unripe R. rosifolius Sm. *extract*

The antibacterial activity of the unripe Sampinit extract is shown in Table 6. It only exhibited antibacterial action against *Staphylococcus aureus* BIOTECH 1582 ($\bar{x}$ = 11.20), a gram-positive pathogen. This is lower than the activity demonstrated by the positive control (10% phenol), which had an average inhibition zone diameter of 21.70 mm,

Table 5. Antioxidant Activity of *R. rosifolius* Sm. (Sampinit) using DPPH. The unripe Sampinit extract demonstrated antioxidant action against the DPPH radical at increasing concentrations compare to beta-hydroxycarboxylic acid (BHA), the positive control. The higher mean percentage of DPPH radical scavenging inhibition of the unripe Sampinit fruits indicates their potent antioxidant capacity, which could make them valuable for health-related applications in preventing oxidative stress-related diseases.

Concentration (µg/mL)	**DPPH activity (%)**	
	Unripe *R. rosifolius* Sm. extract (Test sample)	**BHA (positive control)**
10	92.66	70.78
15	92.77	91.90
20	92.85	93.93
25	93.04	93.95
Mean	**92.83 ± 0.16**	**87.64 ± 9.77**

Table 6. Antibacterial Activity of *R. rosifolius* Sm. (Sampinit). *Staphylococcus aureus* BIOTECH 1582 ($\bar{x}$ = 11.20), a gram-positive pathogen. This is inferior to the activity demonstrated by the positive control (10% phenol), which has a mean diameter zone of inhibition of 21.70, signifying greater potency than the unripe Sampinit extract. Standard isolates of *Enterococcus faecalis, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa* exhibited resistance to the unripe Sampinit extract.

Sample	Diameter of Zone of Inhibition (mm)*			
	1	2	3	Mean
Test organism: *Staphylococcus aureus* BIOTECH 1582				
Unripe Sampinit Fruit extract	**11.30**	**11.00**	**11.30**	**11.20**
Positive control: 10% Phenol	**22.40**	**19.40**	**23.20**	**21.70**
Negative Control: Sterile Distilled Water	0.00	0.00	0.00	0.00
Test organism: *Enterococcus faecalis* BIOTECH 10348				
Unripe Sampinit Fruit extract	0.00	0.00	0.00	0.00
Positive control: 10% Phenol	**15.60**	**15.60**	**14.70**	**15.30**
Negative Control: Sterile Distilled Water	0.00	0.00	0.00	0.00
Test organism: K*lebsiella pneumoniae* BIOTECH 1754				
Unripe Sampinit Fruit extract	0.00	0.00	0.00	0.00
Positive control: 10% Phenol	**20.60**	**23.00**	**22.90**	**22.20**
Negative Control: Sterile Distilled Water	0.00	0.00	0.00	0.00
Test organism: *Pseudomonas aeruginosa* BIOTECH 1335				
Unripe Sampinit Fruit extract	0.00	0.00	0.00	0.00
Positive control: 10% Phenol	**28.90**	**29.80**	**28.20**	**29.00**
Negative Control: Sterile Distilled Water	0.00	0.00	0.00	0.00
*Diameter of cylinder cup = 8.0 mm				

*Diameter of cylinder cup = 8.0 mm

indicating greater potency compared to the unripe Sampinit extract. *Enterococcus faecalis*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* standard isolates were resistant to the unripe Sampinit extract.

The present result differs from the results of Recuenco et al., [8], since their Sampinit extract exhibited greater antibacterial action against *Escherichia coli*, a gram-negative bacterium than a gram-positive bacterium – *Staphylococcus aureus*. The difference in antibacterial activity could be due to the different locations where the Sampinit fruit was collected and the different stages of fruit utilized. However, the result agrees with Alvarez et al., [25] regarding activity against *Staphylococcus aureus*. The secondary active metabolites such as phenolic compounds, flavonoids, and steroids confirmed by HPLC profiling may be responsible for the antibacterial action of the extract against the gram-positive *Staphylococcus aureus*. While we determined the effectiveness of Sampinit in antioxidant and antibacterial activities, the full ranges have not been assessed. The exploration of our findings related to antibacterial activity has been narrow. Future studies are needed to recognize the importance of Sampinit in animal models of inflammatory diseases, in mice, which shows similarities between persons with inflammatory disease and induced mice

CONCLUSION

Our research indicated that the locally grown unripe *R. rosifolius* Sm. (Sampinit) fruits might be a substantial source of phytochemicals, such as polyphenols, flavonoids, and steroids. HPLC analysis indicated a significant concentration of rutin in the Sampinit extract, indicating the presence of flavonoids. The minimum quantities of phenols and steroids were detected equivalently. The flavonoid found in Sampinit extract may significantly contribute to its antioxidant and antibacterial activities. Comprehensive scientific study is necessary to identify the bioactive chemicals for the development of the next generation of medicines from natural sources. The application of contemporary purification technology and enhanced methods is essential for the safe and successful use of medicinal plant extracts.

REFERENCES

1. Yang HM, Oh S, Lim SS, Shin, HK, Oh YS, Kim JK (2008) Anti-inflammatory activities of *Rubus coreanus* depend on the degree of fruit ripening. Phytother Res. 22(1):102–107.
2. Kim SK, Kim H, Kim SA, Park HK, Kim W (2013) Anti-inflammatory and anti-bacterial activity of polyphenols isolated from black raspberry. Korean J. Physiol. Pharmacol. Off. J. Korean Physiol. Soc. Korean Soc. Pharmacol. 17:73–79.

3. Lee J, Dossett M, Finn CE (2012) Rubus fruit phenolic research: The good, the bad, and the confusing. Food Chem. 130:785–796.
4. Patel AV, Rojas-Vera J, Dacke CG (2004) Therapeutic constituents and actions of *Rubus* species. Curr. Med. Chem. 11:1501–1512.
5. Bhuyan B, Dutta A (2021) Review of the Phytochemical, Pharmacological, and Traditional Profile on the *Rubus* Genus in North-Eastern and Western Parts of India. Current Trends in Pharmaceutical Research. 8(1):73-87. Retrieved 02 October, 2023, from http://www.dibru.ac.in/ctpr.
6. Rahman MdM, Rahaman MdS, Islam MdR, Mithi FM, Alqahtani T, et al. (2022) Role of Phenolic Compounds in Human Disease: Current Knowledge and Future Prospects. Molecules.27(10):233.
7. https://www.afmis.da.gov.ph/index.php/component/content/article/314.html
8. Recuenco MC, De Luna JRP, Magallano NG, Salamanez KC (2020). Phytochemical Screening, Total Phenolics, and Antioxidant and Antibacterial Activities of Selected Philippine Indigenous Fruits. Phil. J. of Sci. 149 (3-a): 697-706.
9. Munoz MNM, Alvarado UG, Reyes JI, Watanabe K (2021) Acute oral toxicity assessment of ethanolic extracts of *Antidesma bunius* (L.) Spreng fruits in mice. Toxicol. Rep., 2021 Jun 17(8):1289-1299. doi: 10.1016/j.toxrep.2021.06.010.
10. Singleton VL, Orthofer R, Lamuela-Raventós RM (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods in Enzymology, Academic Press, Volume 299, 1999, Pages 152-178, ISSN 0076-6879, ISBN 9780121822002, https://doi.org/10.1016/S0076-6879(99)99017-1.
11. Moreira R, Ferreres F, Gil-Izquierdo Á, Gomes NGM, Araújo L, et al. (2023) Antifungal Activity of *Guiera senegalensis*: From the Chemical Composition to the Mitochondrial Toxic Effects and Tyrosinase Inhibition. Antibiotics (Basel).12(5),869. doi: 10.3390/antibiotics12050869.
12. Da Silva LLL, Pezzini BR, Soares L (2015) Spectrophotometric determination of the total flavonoid content in *Ocimum basilicum* L. (Lamiaceae) leaves. Pharmacog. Mag., 11(41),96-101.
13. Brand-Williams W, Cuvelier ME, Berset C (1995) Use of a free radical method to evaluate antioxidant activity. LWT - Food Sci. and Tech.. 28, (1), 25-30,
14. ISSN 0023-6438, https://doi.org/10.1016/S0023-6438(95)80008-5.
15. Schulz M, Seraglio SKT, Betta FD, Nehring P, Valese AC, et al. (2019) Blackberry (*Rubus ulmifolius* Schott): Chemical composition, phenolic compounds and antioxidant capacity in two edible stages. Food Research International 122:629-634 https://doi.org/10.1016/j.foodres.2019.01.034.
16. Muniyardi K, George E, Sathyanarayanan S, George BP, Abrahamse H, et al. (2019)Phenolics, tannins, flavonoids and anthocyanins contents influenced antioxidant and anticancer activity of Rubus fruits from Western Ghats, India. Food Source and Human Welness 8(1):73-81 https://doi.org/10.1016/jfshw.2019.03.005.
17. Lopez-Corona AV, Valencia-Espinosa I, Gonzales-Sanchez FA, Sanchez-Lopez AL, Garcia-Amizquerta LE, et al. (2022) Antioxidant, Anti-inflammatory, and Cytotoxic Activity of Phenolic Compound Family Extracted from Raspberries (*Rubus idaeus*): A General Review. Antioxidants (Basel) 11(6):1192 doi:10.3390/antiox11061192.
18. Qi W, Qi W, Xiong D, Long M (2022) Quercetin: Its Antioxidant Mechanism, Antibacterial Properties and Potential Application in Prevention and Control of Toxipathy. Molecules 27(19):6545 https://doi.org/10.3390/molecules27196545.
19. Carrillo-Martinez EJ, Flores-Hernandez FY, Salazar-Montes AM, Nario-Chaidez HF, Hernandez-Ortega LD (2024) Quercetin, a Flavonoid with Great Pharmacological Capacity. Mol. 29(5):1000 doi:10.3390/molecules29051000.
20. Mueed A, Shibli S, Al-Quwaie A, Ashkan MF, Alharbi M, et al. (2023) Extraction, characterization of polyphenols from certain medicinal plants and evaluation of their antioxidant, antitumor, antidiabetic, antimicrobial properties, and potential use in human nutrition. Frontiers in Nutrition 10:1125106 https://doi.org/10.3389/fnut.2023.1125106.
21. Nanjo F, Goto K, Seto R, Suzuki M, Hara Y (1996) Scavenging effects of tea catechins and their derivatives on 1,1-diphenyl-2-picrylhydrazyl radical. Free Rad. Biol. Med. 21:895-902.
22. Kulistic T, Radonic A, Katalinic V, Milos M (2004) Use of different methods for testing antioxidative activity of oregano essential oil. Food Chem. 85:633-640.
23. Koleva II, van Boek TA, Linssen JP, de Groot A, Evastatieva LN (2002) Screening of plant extracts for antioxidant activity: A comparative study on three testing methods. Phytochem Anal. 13:8-17.
24. Del Rio D, Rodriguez-Mateos A, Spencer JP, Tognolini M, Borges G, Crozier A (2013) Dietary (poly)phenolics in human health: structures, bioavailability, and evidence of protective effects against chronic diseases. Antioxid. Redox. Signal 18(14): 1818–1892.
25. Tiwari U, Cummins E (2013) Fruits and vegetables. In: Handbook of Plant Food Phytochemicals: source, stability and extraction. Tiwari BK, Brunton NP, Brennan CS eds. West Sussex, UK: John Wiley & Sons. p. 107–129.
26. Alvarez LV, Caunca ES, Nolido JG (2013) Antimicrobial Activity of *Rubus rosifolius* J.E. Sm. (Rosaceae) Fruit Crude Extract. PUP J. Sci. Tech.6(10):1-9.