RESEARCH ARTICLE

Enhancing bioactive content in *Malus sylvestris* peel extract for antioxidant source using microwave-assisted extraction (MAE) and predicting the potential of ant nests

[version 1; peer review: 1 approved]

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Abstract

Background

The apple peel is one of the byproducts of apple beverage production that is still underutilized. While considerable information regarding apple peel extract is available, its utilization remains limited. Yet, apple peel contains numerous bioactive components that offer various health benefits. Based on this, information regarding the bioactive components in apple peel and their health potential is required.

Methods

The research consisted of two stages, namely Stage 1, which involved in silico analysis of the bioactive content in apples and predicted the potential of ant nests using the Structural Activity Relationship (SAR) approach Pass Online. In Stage 2, in vitro analysis of apple peel extraction as a natural antioxidant source was conducted using treatment time radiation (3 minutes, 6 minutes, 9 minutes, and 12 minutes). The extract was characterized based on phenolic, flavonoid, antioxidant activity, quercetin, and functional groups using Fourier Transform Infrared Spectroscopy (FTIR).

Results

Stage 1: gave results that several bioactive were identified such as chlorogenic acid, epicatechin, phloridzin, catechin, hyperoside,

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quercitrin, quercetin, and pectin. Based on SAR, the bioactive compounds have functional potential as anti-inflammatory, lipid metabolism regulators, free radical scavengers, lipid peroxidase inhibitors, antihypercholesterolemic, and insulin promoters. Stage 2: Radiation times of the MAE method had a significant effect ($P<0.05$) on the phenolic content, and antioxidant activity and was highly significant ($P<0.01$) on the flavonoid content of *Malus sylvestris.* Radiation time for 12 minutes of MAE gave higher phenolic, flavonoid, and antioxidant activity of *Malus sylvestris* peel extract. The IR spectra of the *Malus sylvestris* extract increased as the radiation time increased.

Conclusions

The optimum radiation time was at 12 minutes with the result of phenolic content at 14.73 mg GAE/g, flavonoid content at 29,62 ppm, antioxidant activity at 95,09%, and the IR spectra at 1031.92 cm^{-1}, 1390.68 cm^{-1}, 2833.43 cm^{-1}, 2945.3 cm^{-1}, 3346.5 cm^{-1}, 3354.21 cm^{-1}.

Keywords
antioxidant, by-product, flavonoid, phenolic, functional group

This article is included in the Agriculture, Food and Nutrition gateway.

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Introduction

In Malang, Indonesia, apples (Malus sylvestris) are a type of fruit that is quite popular and an excellent source of nutrition. Apples contain several vitamins, minerals, fiber, and antioxidant compounds that are important for human health.[1] Apples contain antioxidant compounds that protect the body from damage caused by free radicals.[2,3] Afsharnezhad, M., Shahangian, S. S., Panahi, E., & Sariri, R. (2017). Free radicals are unstable molecules that can damage body cells and cause various chronic diseases, including cancer, heart disease, diabetes, and premature aging.[4] One of the greatest sources of natural antioxidants is found in apple skin. Apple skin (Malus sylvestris) is the outer part of the apple that protects the flesh inside.[5] Apple skin has several layers, including a thin epidermis that protects the fruit from water loss and physical damage. The color can vary from light green, and yellow, to red depending on the type of apple. Apple skin contains a much greater number of polyphenols than the flesh of the fruit.[6,7] So far, the by-product of the processing process in leather has only been used as a substitute for animal feed and plant fertilization, even though it still has the potential to contain micronutrients in the form of natural antioxidants.[8,9]

The content of antioxidant compounds in apple skin varies depending on the type of apple and also growth factors such as the environment and season.[10,11] Flavonoids are a group of polyphenolic compounds that have strong antioxidant properties. These compounds help protect the body from damage caused by free radicals.[12] The phenol content in apple skin also varies based on the type of apple, growing conditions and growth environment.[1] Phenol is a chemical compound that has antioxidant properties. strong. It also provides color, taste, and aroma to fruits. The phenols in apples are mainly concentrated in the skin.[11] The greater the content of phenolic and flavonoid compounds in a plant indicates its strong antioxidant activity. The higher the content of phenolic and flavonoid compounds, the ability to capture free radicals will also increase.[13]

The extraction method for antioxidant compounds can be carried out using conventional and non-conventional methods. Conventional methods have been widely used in the extraction process but require a long time.[14] The advantages of non-conventional methods are that they are environmentally friendly and highly efficient, including membrane-based technology, ultrasound, microwave extraction, and high hydrostatic pressure. The results of the research show that the best extraction method to produce the highest levels of flavonoids in 70% ethanol extract of iler leaves is the microwave-assisted extraction method. Flavonoid levels using the maceration, reflux, microwave-assisted extraction, and Ultrasound-Assisted Extraction methods were 0.41%, 0.45%, 0.75%, and 0.62% respectively.[15] According to the advantages of the extraction method, research about combination maceration and MAE to characterize Malus sylvestris peel extract.

Methods

The research is divided into two stages, namely:

Stage 1: In silico analysis related to the bioactive content of apple peel and the prediction of ant nest potential using the Structural Activity Relationship (SAR) approach Pass Online.

Stage 2: in vitro analysis of apple peel extraction as a natural antioxidant source.

Stage 1a. Apple peel bioactive content

The exploration of bioactive content found in apple peel was conducted through literature searches. Several publications indicate that apple peel contains specific bioactive, as outlined in Table 1. This research is in silico analysis. Subsequently, these bioactive constituents were analyzed to determine their profiles and structures using the PubChem database on the website (https://pubchem.ncbi.nlm.nih.gov/).

Table 1. The bioactive compound of apple peel.

Bioactive	ID	MW (g/mol)	Formula	SMILE
Chlorogenic acid	1794427	354.31	$C_{16}H_{18}O_9$	C1C(C(C (CC1(C(=O)O)O)OC(=O)C=CC2=CC(=C(C=C2)O)O)O)O
Epicatechin	72276	290.27	$C_{15}H_{14}O_6$	C1C(C (OC2=CC(=CC(=C21)O)O)C3=CC(=C(C=C3)O)O)O
Phloridzin	6072	436.4	$C_{21}H_{24}O_{10}$	C1=CC(=CC=C1CCC(=O)C2=C(C=C(C=C2O[C@H]3[C@@H]([C@H]([C@@H]([C@H](O3)CO)O)O)O)O)O)O

Table 1. *Continued*

Bioactive	ID	MW (g/mol)	Formula	SMILE
Catechin	9064	290.27	$C_{15}H_{14}O_6$	C1C(C (OC2=CC(=CC(=C21)O)O)C3=CC(=C(C=C3)O)O)O
Hyperoside	5281643	464.4	$C_{21}H_{20}O_{12}$	C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)OC4C (C(C(C(O4)CO)O)O)O)O)O
Quercitrin	5280459	448.4	$C_{21}H_{20}O_{11}$	CC1C(C(C(C(O1)OC2=C (OC3=CC(=CC(=C3C2=O)O)O) C4=CC(=C(C=C4)O)O)O)O)O
Quercetin	5280343	302.23	$C_{15}H_{10}O_7$	C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O) O
Pectin	1.57E+08	889	$C_{47}H_{68}O_{16}$	CC1CCOC(C1O)(C2CC3C(O2)C=CC(=CC (CC4(CCC(O4) C56CCC(O5)(CC(O6)C7C(=O)CC(O7)(C(C8CCC9(O8)CCCC (O9)C(C(=O)O3)C)O)C)C(=O)O)C)C)C)O

Stage 1b. Predicting the potential of ant nests using the Structural Activity Relationship (SAR) approach with pass online

The compounds present in apple peel were further assessed for their potential using WAY2DRUG PASS Online prediction on the website (http://www.pharmaexpert.ru/passonline/predict.php) as a treatment for obesity. WAY2DRUG Pass Prediction employs SAR analysis to compare the input compounds, specifically those found in ant nests, with compounds known to have specific potential. The prediction value increases as the structural similarity between the compounds rises, indicating a higher likelihood of similar potential. The Pa value (Probability to be Active) is the output prediction value from WAY2DRUG PASS, reflecting the potential of the tested compound. A Pa value exceeding 0.7 suggests a high potential for the compound, such as in the case of anti-inflammatory properties, due to its structural similarity to compounds in the database. We recommend using a cutoff score of 0.5. The Pa value signifies the accuracy of the obtained prediction function; the higher the Pa value, the better the accuracy of the function.[16]

Stage 2. In vitro analysis of apple peel extraction as a natural antioxidant source

Malus sylvestris peels were sourced from Koperasi Bhakti Materia Medica, Batu, East Java, Indonesia. It was subjected to a drying process in an oven set at 50°C for 24 hours, and subsequently ground to yield a powder with a particle size of 1777µm. *Malus sylvestris* peel powder was prepared 10 gr. All substances and materials utilized for extraction and analysis were of analytical grade and procured from Merck, Germany. These included a quercetin standard (Q4951 Sigma-Aldrich) as 1 gr, 50% methanol (019200WT Merck Label Set TR420) as 100 ml, and Whatman No. 4 (WHA2017013) filter paper.

Malus sylvestris extraction and recovery

Malus sylvestris extraction was conducted using the MAE method[17] with a slight modification, employing a Sharp Model R -222Y(S) microwave at a medium (50°C). The *Malus sylvestris* powder was mixed with 50% methanol at a ratio of 5 grams of powder to 100 milliliters of methanol and allowed to soak for 24 hours. Subsequently, the solution underwent extraction in a microwave oven for intervals of 3 (T1), 6 (T2), 9 (T3), or 12 (T4) minutes, with a cycle of one minute on and two minutes off to prevent overheating. The resulting crude extract was filtered through Whatman no. 4 filter paper, and ethanol was removed using a rotary evaporator at a pressure of 55 mm Hg and a temperature of 50°C. The filtrate was further concentrated with a rotary evaporator (IKA RV 10) to eliminate the solvent. Finally, the concentrated filtrate was analyzed according to the method described below.

Measurements of phenolic content

The assessment of Total Phenolic Content (TPC) was conducted using the Folin-Ciocalteu reagent method, following the procedure outlined.[18] A solution of saturated sodium carbonate was prepared and allowed to sit for 24 hours. Standard concentrations of gallic acid, varying from 25 to 200 ppm, were freshly prepared at room temperature for each analysis. A solution consisting of 0.5 mL of 1:10 diluted gallic acid in 4.5 mL of distilled water, 0.2 mL of Folin reagent (Merck), and 0.5 mL of saturated sodium carbonate was created and employed to construct a standard sample analysis curve. The AT-1900 UV spectrophotometer was used to measure the absorbance of each sample at a wavelength of 725 nm. The TPC was calculated using the absorbance values and a linear regression equation, allowing for comparison with the gallic acid standard. The results were expressed as milligrams of gallic acid equivalents (GAE) per gram of dry sample weight (mg/g).

Measurement of flavonoid content

Following the procedure outlined,[19] the method involves preparing and dissolving the sample in a suitable solvent like methanol or ethanol until the desired testing concentration is attained. Next, transfer the sample solution into a test tube or beaker. Add the AlCl3/Potassium acetate reagent solution to the test tube or beaker containing the sample, and gently stir. Allow the mixture to undergo a reaction in darkness for approximately 30 minutes, enabling interaction with the flavonoids. Subsequently, centrifuge the mixture to separate the sediment. Utilize a spectrophotometer to measure the absorbance of the solution at the designated wavelength, typically around 415 nm. Either employ a calibration curve (if utilizing a standard solution) or establish a correlation between the sample's absorbance and the total flavonoid concentration.

Quercetin content

The quercetin testing procedure using HPLC,[20] with modifications. Making a standard quercetin solution begins by weighing 10 mg of standard quercetin, placing it in a 10 mL measuring flask, and adding ethanol to the mark. Create a standard series with concentrations 5; 10; 25; 50; 75 and 100 µg/mL.

Analysis of quercetin in samples begins by taking and filtering the sample with a 0.45 µm filter syringe. Inject 10 µL of sample into HPLC, including the quercetin comparator. Sample analysis for 20 minutes at a wavelength of 370 nm. HPLC conditions (Mobile phase A = 0.5% Phosphoric Acid, Mobile phase B = Methanol, System = Isocratic (Mobile phase A: mobile phase B = 60:40), Detector = VWD, Column = Xbridge C18 5µm (4.6 × 250 mm) and Flow rate = 1 mL/min. Create a standard quercetin regression curve and analyze Quercetin levels using the formula:

$$Sample\ quercetin\ levels\left(\frac{\mu g}{mL}\right)=\frac{Quercetin\ levels\ from\ the\ tool\left(\frac{\mu g}{mL}\right)\times final\ add\ (mL)\times fp}{Sample\ volume\ (mL)}$$

Antioxidant activity

The evaluation of antioxidant activity through the DPPH method follows the protocol established by Molyneux,[21] with slight adjustments. Begin by taking the *Malus sylvestris* extract and dissolving it in a suitable solvent to achieve the desired testing concentration. Dissolve DPPH in methanol solvent to reach the appropriate concentration. Combine the extract solution with the DPPH solution in a test tube or beaker and thoroughly stir the mixture. Allow it to undergo a reaction in darkness for about 30 minutes or until the reaction is complete. Employ a spectrophotometer to measure the absorbance of the solution at the specified wavelength, typically around 517 nm. Subsequently, compute the percentage of antioxidant inhibition.

FTIR analysis

The chemical composition was determined following the method,[22] with slight modifications. The UV-Vis spectrum was captured using a GBS UV/VIS 920 instrument. A one-milligram portion of the *Malus sylvestris* extract underwent dehydration in a vacuum desiccator. Subsequently, it was finely ground and thoroughly mixed with 200 mg of KBr powder, oven-dried, and met analytical reagent grade standards (Merck, DAC, USP). This powdered blend was placed into a die and compressed into a transparent disk.

Results

Stage 1. The bioactive compound of apple peel

Based on a literature review, several bioactives were identified in apple peel. These bioactives are as follows (see Table 1).

This research by in silico analysis. We found that several bioactive apple peels were identified such as chlorogenic acid, epicatechin, phloridzin, catechin, hyperoside, quercitrin, quercetin, and pectin. We refer to the.[23–25] The bioactive compound was chosen because having a functional effect higher than others by predicting the potential of Ant Nests using the Structural Activity Relationship (SAR) Approach with Pass Online. Bioactive compounds have different abilities in functional activity.

Table 1 shows the characteristics of the bioactive compound with the molecular weight (MW) and the chemical structure. It was useful for future research to examine more specific interactions between these components and other compounds, for example, used for functional food purposes in the future.

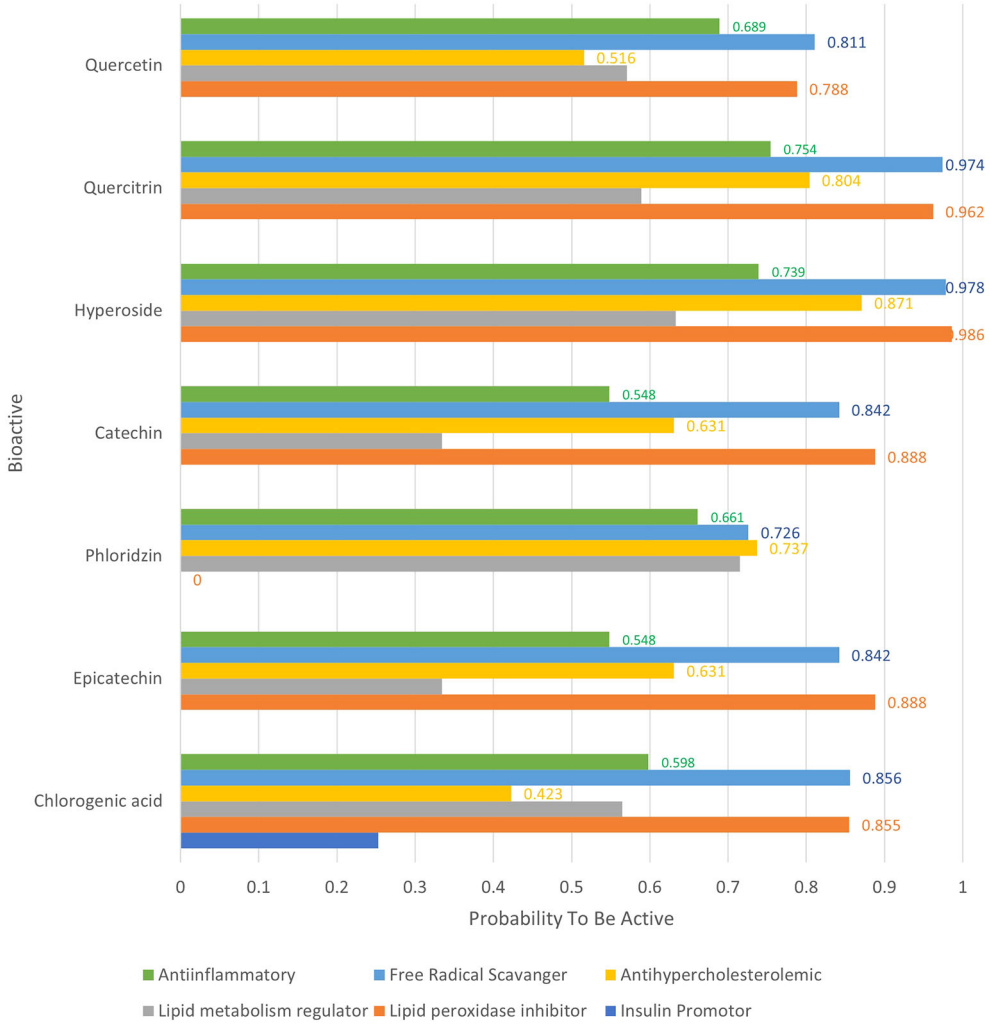

Figure 1. Structural Activity Relationship (SAR) of bioactive apple peel with the functionality.

Predicting the potential of ant nests using the Structural Activity Relationship (SAR) approach with pass online

The research in this section aimed to examine more deeply the use of apple peel in the future if it is used in the functional food sector. Which direction can be taken based on its greatest potential? Figure 1 explains the Pa value (Probability to be Active) of the bioactive components of apple peel on functional properties which include anti-inflammatory, lipid metabolism regulator, free radical scavenger, lipid peroxidase inhibitor, antihypercholesterolemic, and insulin promoter. Apart from that, from Figure 1 you can see the similar functional properties of several components. A Pa value (Probability to be Active) of 0.5 and above is a value high enough to be used as a reference. Furthermore, Figure 2 shows the score relative prediction, the higher the value obtained, means that the bioactive ability is. Figure 3 explains that of all bioactive components, the role of a free radical scanner is higher with a value of 0.754.

Stage 2. In vitro analysis of apple peel extraction as a natural antioxidant source

Antioxidant activity of apple peel extract

Antioxidant activity testing was carried out on apple peel extraction using a combination of maceration and MAE methods with treatment several times (3, 6, 9, and 12 minutes). The results of the antioxidant activity test of apple peel extract using the combination method of maceration and MAE with different treatment times provided a significant difference ($P<0.05$). The results of antioxidant activity using the DPPH method shown in % inhibition can be seen in Table 2.

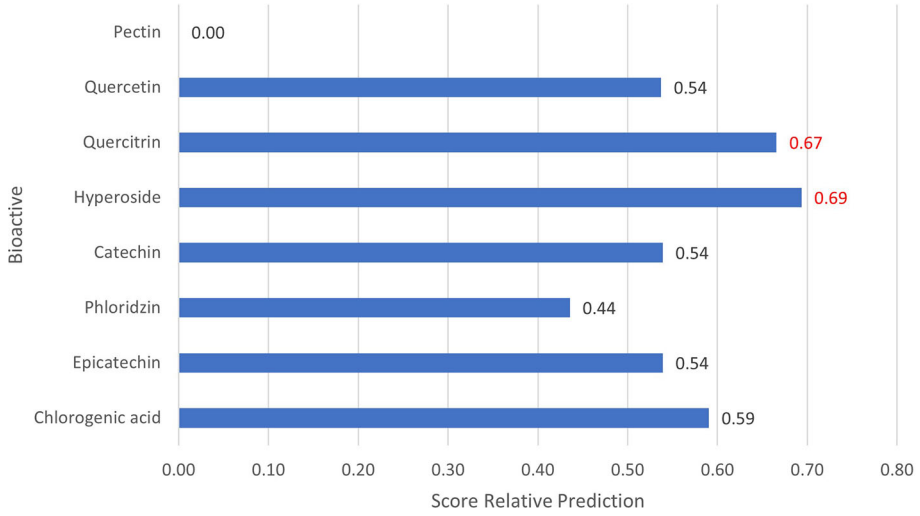

Figure 2. **Exploring the potential of apple peel through SAR approach.**

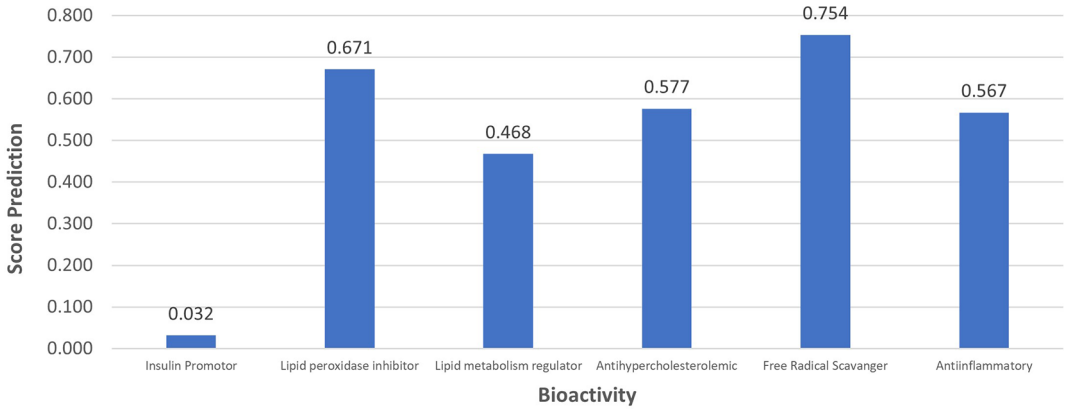

Figure 3. **The potential relative score of apple peel using SAR approach.**

Table 2. **Results of antioxidant activity of apple peel extract.**

Treatment	Antioxidant activity (%) ± SD
T1	93.85 ± 0.38^{a}
T2	94.26 ± 0.52^{a}
T3	95.07 ± 0.24^{b}
T4	95.09 ± 0.20^{b}

Note: Different uppercase letters in the column indicated a significant effect (P<0.05).

Phenolic content of apple peel extract

Phenolic content testing was carried out on apple peel extraction using a combination of maceration and MAE methods with several treatments (3, 6, 9, and 12 minutes). The results of total phenolics can be seen in Table 3. The results of the Phenolic content of apple peel extract using the combination method of maceration and MAE with different treatment times gave a significant difference (P<0.05).

Table 3. Result of phenolic content of apple peel extract.

Treatment	Total phenolic content (GAE mg/g)
T1	13.52 ± 0.64^{a}
T2	14.05 ± 0.48^{ab}
T3	14.72 ± 0.23^{b}
T4	14.73 ± 0.39^{b}

Note: Different uppercase letters in the column indicated a significant effect (P<0.05).

Table 4. Result of flavonoid content of apple peel extract.

Treatment	Total flavonoid content (ppm)
T1	23.76 ± 0.93^{a}
T2	26.04 ± 1.85^{ab}
T3	28.27 ± 2.05^{b}
T4	29.62 ± 2.61^{b}

Note: Different uppercase letters in the column indicated a significant effect (P<0.01).

Flavonoid content of apple peel extract

Flavonoid content testing was carried out on apple peel extraction using a combination of maceration and MAE methods with treatment several times (3, 6, 9, and 12 minutes). The results of flavonoid content can be seen in Table 4. The results of the apple peel extract using the combination method of maceration and MAE with different treatment times gave a highly significant difference (P<0.01) in Flavonoid content.

Chemical structure-Fourier Transform Infrared Spectroscopy (FTIR)

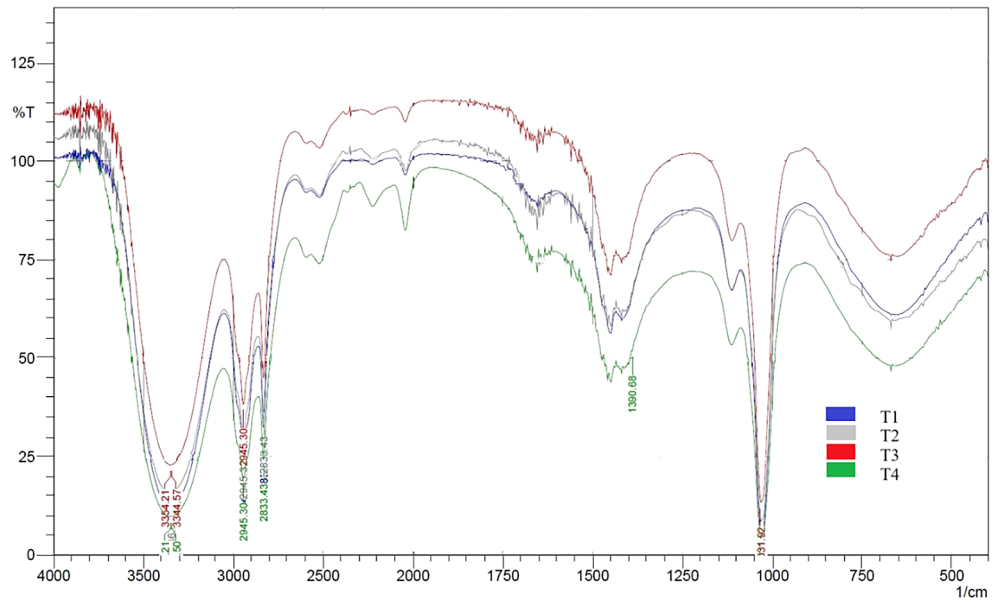

Figure 4. Functional groups of *Malus sylvestris* peel extract. For an explanation of the various symbols, see Table 5.

Quercetin analyzed by High-performance liquid chromatography (HPLC)

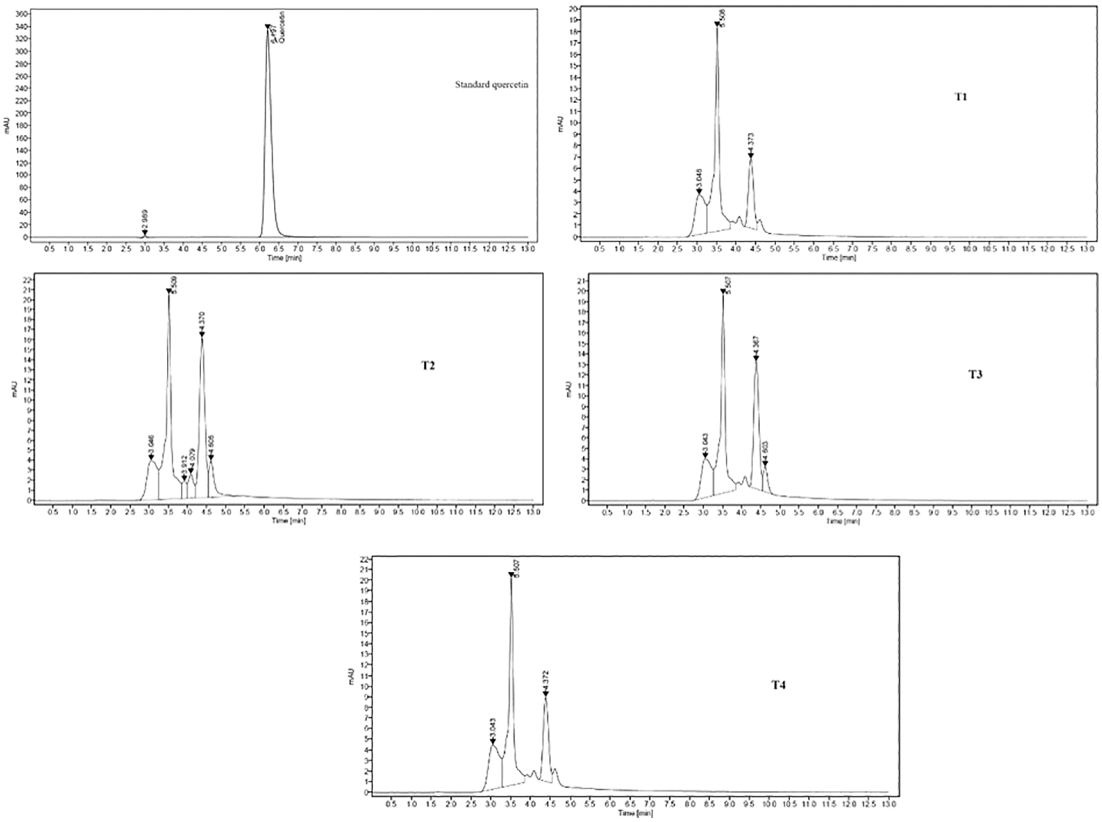

Figure 5. HPLC chromatograms of quercetin standard (standard) and Malus sylvestris peel extract (T1, T2, T3, and T4).

Discussion

Stage 1. The bioactive compound of apple peel

Table 1 serves as the primary reference for the dominant bioactive compounds present in apple peel, intended for further exploration of their potential health benefits. Focusing on eight key bioactive compounds, including Chlorogenic acid, Epicatechin, Phloridzin, Catechin, Hyperoside, Quercitrin, Quercetin, and Pectin, a detailed analysis is conducted to understand their potential positive impact on health.

Prediction of ant nest potential using the Structural Activity Relationship (SAR) approach with pass online[26]

Based on SAR analysis, it has been identified that bioactives present in apple peel exhibit the highest potential as Free Radical Scavengers (0.754). Additionally, these bioactives show potential as Lipid Peroxidase Inhibitors (0.671), Antihypercholesterolemics (0.577), and Lipid Metabolism Regulators (0.468) (see Figure 2). Hyperoside and quercitrin emerge as two bioactives with the highest potential (see Figure 1). Free Radical Scavengers play a crucial role in preventing the formation of reactive oxygen species, thereby eliminating reactive oxygen before it can damage cells. If free radicals surpass the body's capacity, it can lead to oxidative stress, altering the body's physiological conditions and potentially causing various diseases. Maintaining a proper balance between free radicals and antioxidants (radical scavengers) is essential for optimal physiological function.[5] Lipid peroxidation involves a chain reaction of oxidative degradation of lipids. This process occurs when free radicals "steal" electrons from lipids in cell membranes, leading to cellular damage. The mechanism of this process unfolds through a chain reaction of free radical reactions.[2]

Antioxidant activity of apple peel extract

The percentage of antioxidants from the research ranged from 93.85% to 95.09% (Table 2). In this study, antioxidant activity testing was conducted against the free radical 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) from the apple peel extract. The antioxidant activity of the fruit extract was expressed as % inhibition against the DPPH radical. % inhibition was obtained from the difference between the absorbance of the DPPH control and the measured sample using a UV-Vis Spectrophotometer. The research results indicated that the antioxidant activity of the apple peel extract increased with the extraction time. This could be explained by the increase in the amount of antioxidant compounds extracted from the apple peel over time.

The study by Ref. 14 showed antioxidant activity (DPPH inhibition) in avocado seeds using the MAE method with 40% ethanol for 5 minutes, resulting in the highest value of 80.32%, and the lowest was 22.93% for 40% ethanol with 1 minute. Reported on the antioxidant activity.[27] The analysis of variance results showed that the type of solvent significantly influenced ($P<0.01$) the antioxidant activity of lemon peel extract. The highest antioxidant activity was obtained in 70% ethanol solvent, which was 52.72%, while the lowest antioxidant activity was obtained in aquadest solvent, which was 25.35%. Lemon peel extract using ethanol solvent had the highest antioxidant activity, which was 52.64%. Antioxidant activity could be influenced by the amount of flavonoid compounds present in lemon peel extract; the more flavonoid compounds, the higher the antioxidant activity.

In the reference, the research showed that the highest average antioxidant activity was found in the treatment of 40°C temperature for 20 minutes, which was 89.66%, and the lowest average antioxidant activity was obtained in the treatment of 30°C temperature for 10 minutes, which was 79.75% for wuluh starfruit leaf extract with the Ultrasonic-Assisted Extraction (UAE) method.[28]

Based on the obtained references, there was still limited information regarding apple peel extract as an antioxidant source. It could be inferred from several consulted references that the treatment duration of 12 minutes of radiation in this study resulted in the highest antioxidant value, with a recorded percentage of 95.09%.

Total phenolic content of apple peel extract

Based on the analysis results, it shows that apple peel by-product extract with extraction time treatment of 3 minutes, 6 minutes, 9 minutes, and 12 minutes provides total phenols of 13.52-14.73 mg GAE/g sample. Research has been carried out to determine the comparison of extraction time using MAE with different radiation times on antioxidant activity, total phenol, and flavonoid levels of qpple peel. The DPPH free radical scavenging activity test was carried out using visible spectrophotometry ($\lambda = 520$ nm). Determination of total phenol content was carried out using the Folin Ciocalteu method using visible spectrophotometry ($\lambda = 706$ nm) with gallic acid as a comparison. Determination of total flavonoid levels was carried out with the help of AlCl3 using visible spectrophotometry ($\lambda = 503$ nm) with catechin as a comparison. From the research results, the EC50 value, total phenol, and flavonoid content of 5 minutes MAE were respectively 508.32 bpj, 1.14% w/w GAE (Gallic Acid Equivalent) and 1.53% w/w CE (Cathecin Equivalent). Meanwhile, the 20-minute MAE was obtained at 474.11 bpj, 1.72% w/w GAE, and 1.94% w/w CE. The results of the t-test statistical calculation ($\alpha = 0.05$) on the EC50 value, total phenol, and flavonoid levels showed a significant difference between 3 minutes of radiation and 12 minutes. From the results of this study, it was concluded that 12 minutes of radiation was better than 3 minutes.

Total flavonoid content of apple peel extract

Based on the analysis results, it shows that apple peel by-product extract with extraction time treatment of 3 minutes, 6 minutes, 9 minutes, and 12 minutes provides total flavonoids of 23.76-29.62 ppm using a combination of maceration and MAE extraction methods. Supported by the previous literature that 50% methanol with the sonication extraction technique method produces a TFC of 22.05 which is higher than 75% methanol with a TFC of 12.15.[9]

The type of solvent treatment had a very significant effect ($P<0.01$) on the total flavonoids of lemon peel extract.[27] The highest total flavonoids were obtained using 70% ethanol solvent, namely 7.14 mg QE/g extract, and the lowest total flavonoids were obtained using distilled water, namely 4.34 mg QE/g extract. The total flavonoids in lemon peel extract using ethanol solvent show that the ethanol solvent has a similar polarity level and is more effective in dissolving flavonoid compounds in lemon peel, so lemon peel extract using ethanol solvent produces the highest flavonoid compounds. Then extracted for 60 minutes using an ultrasonic bath at a frequency of 47 kHz.

Research has been carried out to determine the comparison of extraction time using microwave assistance (microwave-assisted extraction = MAE) for 5 and 20 minutes on antioxidant activity, total phenol, and flavonoid levels of spoon leaves (Plantago major L.). The DPPH free radical scavenging activity test was carried out using visible spectrophotometry ($\lambda = 520$ nm). Determination of total phenol content was carried out using the Folin Ciocalteu method using visible

Table 5. Functional group of *Malus sylvestris* extract.

Treatment				Group	Chemical structure
T1	T2	T3	T4		
1031.92	1031.92	1031.92	1031.92	C-O stretch C-N stretch	Alcohol Aliphatic amines
			1390.68	C=O	stretching vibration of alkyl ester in pectin,
2831.5	2833.43	-	2833.43	O-H	Carboxylic acids with hydrogen bonds
2945.3	2945.3	2945.3	2945.3	C-H stretch	Alkanes
3346.5	3346.5	3344.57	3346.5	O-H, H-bonded	Phenols, hydrogen bound
-	-	3354.21	3354.21	N-H	Amine/Amide

spectrophotometry ($\lambda = 706$ nm) with gallic acid as a comparison. Determination of total flavonoid levels was carried out with the help of AlCl3 using visible spectrophotometry ($\lambda = 503$ nm) with catechin as a comparison. From the research results, the EC50 value, total phenol, and flavonoid content of 5 minutes MAE were respectively 508.32 bpj, 1.14% w/w GAE (Gallic Acid Equivalent) and 1.53% w/w CE (Cathecin Equivalent). Meanwhile, the 20-minute MAE was obtained at 474.11 bpj, 1.72% w/w GAE and 1.94% w/w CE. The results of the t-test statistical calculation ($\alpha = 0.05$) on the EC50 value, total phenol, and flavonoid levels showed a significant difference between MAE 5 and 20 minutes. From the results of this study, it was concluded that a 20-minute MAE was better than a 5-minute MAE.

Chemical structure-Fourier Transform Infrared Spectroscopy (FTIR)

The purpose of analyzing FTIR was to examine the functional groups in Malus sylvestris peel extract to ascertain the chemical composition and concentration of active compounds within it. Quercetin, the active compound responsible for the plant's pharmacological effects, could be identified through FTIR analysis, revealing functional groups such as Phenolic groups (OH), Carbonyl Groups (C=O), Aromatic C-H Groups, C-O Groups, and Aromatic C=C Groups, even in the presence of quercetin. Consequently, this assessment of functional groups in Malus sylvestris extract via FTIR served to furnish insights into the extract's quality and purity.

Figure 4 and Table 5 exhibited peaks within the IR spectra ranging from 1050-1300 cm^{-1}, indicating the presence of C-O groups in alcohols, esters, and carboxylic acids, as well as C-N stretching in aliphatic amines. Peaks at 2500-2700 cm^{-1} signified O-H groups in carboxylic acids with hydrogen bonds. The peaks at 2850-2970 and 1340-1470 cm^{-1} indicated the presence of C-H bonds in alkanes. Additionally, peaks at 3200-3600 cm^{-1} pointed towards O-H groups associated with phenols and hydrogen bonds. The peak at 3749.62 cm^{-1} demonstrated the presence of a phenolic compound.[29] Notably, one of the pivotal groups signifying the presence of quercetin was situated at the 3200-3600 cm^{-1} range, indicating a hydroxyl group engaged in hydrogen bonding. At T4, the spectral shift observed at 1390.68 cm^{-1}, representing C-O stretching vibrations in quercetin,[30] who asserted that the 1360 cm^{-1} peak refers to C-O groups. This shift towards lower wavenumbers (1296 cm^{-1}) after incorporation into membranes supported the idea of the involvement of polar groups, such as hydroxyl groups, of the flavonoid in binding water in the membrane environment.

The IR spectra of Malus sylvestris peel extract obtained through MAE exhibited distinct profiles with varying radiation times. Specifically, the spectra at 3200-3600 cm^{-1}, from T1 to T4, played a crucial role in indicating the presence of bioactive compounds, notably quercetin. Among these, T4 revealed a greater abundance of spectra associated with bioactive compounds in the Malus sylvestris peel extract compared to the other treatments. This discrepancy was attributed to the influence of different radiation times during the extraction process on the extraction of bioactive compounds. This aligned with the findings,[31] who proposed that microwave energy prompts molecular motion via ion migration and dipole rotation. This rapid movement generates friction, leading to the production of heat energy within the material. Consequently, the cell walls and tissue of the material experienced damage, facilitating the extraction of active compounds.

Quercetin analyzed by High-performance liquid chromatography (HPLC)

Quercetin is a type of flavonoid found in various types of food, including fruits and vegetables. Flavonoids are natural compounds that have antioxidant and anti-inflammatory properties and are known to have various health benefits. Quercetin testing was carried out in the reference, mentioned apples contain quite high levels of quercetin at 340.99 mg/L.[32] Based on this, it is expected that apple skin also contains quite high levels of quercetin.

However, based on the analysis of the quercetin results in Figure 5 using HPLC, no quercetin value was obtained with a detection limit of 0.44 µg/L. The Retention Time (RT) value for quercetin was 6.5 (min) while in treatments T1, T2, T3, and T4 no spectra were found at RT. This is thought to be because the extract contains quercetin below the detection limit. Coupled with the spectrum results, other bioactive components are suspected to appear in apple peel extract with radiation treatment during extraction. The RT that appears cannot be detected in the HPLC database so other analyses are needed, one of which is Gas Chromatography-Mass Spectrometry (GCMS) to determine other compounds in the extract.

Conclusion

Radiation times of the MAE method had a significant effect on phenolic content, flavonoid content, andrographolide content, and antioxidant activity of apple peel extract. Radiation time for 12 minutes of MAE gave higher phenolic, flavonoid, and antioxidant activity of AP. The IR spectra of the apple peel extract increased as the radiation time increased and had the characteristic on Phenols, hydrogen bound 33465.5 cm^{-1}.

Disclaimer

None.

Data availability statement

PubChem: The quercetin data obtained from this study are available in PubChem CID 5280343, https://pubchem.ncbi.nlm.nih.gov/compound/5280343.

Extended data: none.

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There is no section for statistical analysis method, better to explain it.
The research flow is relatively structured. Through the in silico approach, in vitro testing is more targeted. But, the potential of apple peel through SAR approach indicate that quercitrin and hyperoside the most potential component. Why not quantify it using HPLC?

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: food chemistry, food technology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.