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Research Article

Quantification of flavonoids in papaya leaves under combined microwave and solvent extraction techniques: LC-TOF-MS analysis

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Abstract
Carica papaya leaves are a rich source of flavonoids, which have been reported to have anticancer, anti-inflammatory and antioxidant properties. Previous extraction methods, such as supercritical carbon dioxide and solvent extraction, have limitations, including low flavonoid yield and high solvent or time requirements. Therefore, this study integrated microwave pretreatment with solvent extraction (MSE) to enhance recovery efficiency while reducing processing time and solvent consumption. The aim was to optimize the extraction parameters for maximum total flavonoid content (TFC) yield and to characterize its flavonoid compounds. TFC quantification was performed spectrophotometrically using quercetin as the standard. A single-factor experiment was conducted to investigate the effects of individual parameters of particle size (0.425 - 1.180 mm), microwave power (100 - 500 W), solvent type (methanol, ethanol, acetone, chloroform and water), solid-liquid ratio (1:10 - 1:30), and extraction time (1- 8 h) on TFC. The highest TFC of 37.94 mg QE/g DS was obtained under optimum conditions: 0.425 mm particle size, 300 W microwave power, ethanol as the solvent, 1:15 solid-liquid ratio, and 3 hours of extraction time. LC-TOF-MS analysis revealed 5 major flavonoids: isobavachalcone, sakuranin, kaempferol 3-O-beta-D-glucosylgalactoside, kaempferol-3-O-rutinoside and 7-hydroxy-2,4,5-trimethoxyisoflavone. This study demonstrates that optimized MSE is a simple, scalable and energy-efficient approach for isolating valuable flavonoids from *C. papaya* leaves with potential industrial and pharmaceutical applications.

Keywords: flavonoid, combined microwave and solvent extraction, papaya leaves, TFC, LC-TOF-MS

Introduction

Flavonoids are a diverse class of secondary metabolites widely recognized for their potent anticancer [1], anti-inflammatory [2], antioxidant and antibacterial [3] activities. These pharmacological properties offer substantial value to the food, nutraceutical and pharmaceutical industries, where flavonoids serve as a key ingredient in the development of plant-based products with enhanced nutritional and therapeutic potential. Flavonoids are mostly found in various plants, including *Carica papaya* leaves, which are often regarded as agricultural waste. Instead of being discarded, *C. papaya* leaves can be utilized as a sustainable source of bioactive flavonoid compounds.

Various extraction methods have been applied to papaya leaves, such as maceration, microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE) [4], solvent extraction [5] and supercritical carbon dioxide (SC-CO_2) [6]. These methods were reported to have their own advantages and disadvantages. For instance, conventional extraction methods of maceration and solvent extraction are often limited by their long extraction times and high solvent and energy requirements, which have a negative impact on the environment [7]. On the other hand, alternative extraction methods using SC-CO_2, while environmentally attractive, are constrained by the nonpolar nature of carbon dioxide, which leads to a low extraction yield [8]. Similarly, for the UAE method, despite producing a pure extract, the yield obtained is often low. A comparative study by Thilakarathna et al. [9] on the use of UAE and solvent

extraction on plant seeds revealed that the extract yield from UAE is lower than that from the solvent extraction method. Regarding MAE, although the method offers several advantages, such as short extraction time and low solvent and energy consumption, a comparative study between MAE and solvent extraction demonstrated that MAE achieved a lower yield [10] suggesting the effectiveness of the solvent extraction method.

In line with current extraction methods for papaya leaves, a new approach was introduced: exposing the sample to microwave radiation prior to solvent extraction. Several studies have found that this combined method enhances extraction yield and shortens the extraction time. As reported by Zin et al. [11], direct contact of microwave radiation with the plant matrix initiates the release of trapped solutes during the extraction process. Plant material exposed to microwave radiation will experience plant cell wall disruption, improving solvent penetration and thus accelerating the movement of solute into the solvent [12]. Previous research on combining microwave as a pre-treatment method before the extraction process has been widely reported, including microwave and maceration [11], microwave and solid-liquid extraction [13] and microwave and ultrasonic-assisted extraction [14]. According to the authors, exposing plant samples to microwave radiation reduces extraction time and greatly improves the yield obtained.

A previous optimization study on TFC was performed on onion peels using MAE [14]. While this study demonstrated the efficiency of the MAE method, the influence of microwave pre-treatment prior to solvent extraction has not yet been fully explored, especially for papaya leaves. Therefore, detailed extraction experiments using the single-factor method are necessary to systematically evaluate the effect of individual extraction conditions on the total flavonoid content (TFC) yield. Moreover, precise flavonoid characterization was performed in this study using advanced analytical tools, specifically liquid chromatography-time of flight-mass spectrometry (LC-TOF-MS) analysis. This study aims to optimize the extraction conditions for papaya leaves to achieve the highest TFC yield and to characterize the flavonoid profile using the LC-TOF-MS method.

Materials and Methods

Materials

Methanol, ethanol, acetone and chloroform with a purity of 99.7%, used as the extraction solvents, were purchased from Evergreen Engineering and Resources. Quercetin with a purity greater than 95% (Next Gene Scientific Sdn. Bhd.), aluminium chloride with a purity of 99.99% (BT Science Sdn. Bhd.), sodium nitrite and sodium hydroxide (Stabilab Sdn. Bhd.) were used in the total flavonoid content analysis.

Sample preparation

Fresh papaya leaves used in this study were obtained from Johor. The leaves were cleaned of dirt and dust using distilled water. During the extraction process, the water content in the plant sample needed to be reduced to an ideal moisture percentage of 8 to 11% [15]. The moisture in the sample should not be too high, as excessive water will prevent the solvent from reaching the solute during the extraction process. However, the water content should also not be too low to retain the freshness and natural properties of the active compounds in the sample. To achieve the ideal moisture content, the leaves were oven-dried at a mild temperature of 40 °C, and the percentage moisture content was measured using the wet basis method as reported by Raja et al. [15]. The dried sample was then ground to several particle sizes using a mechanical sieve shaker, model NL Scientific.

Total flavonoid content analysis

Total flavonoid content (TFC) was estimated in triplicate using the aluminium chloride colorimetric assay described by Phuyal et al. [16]. Quercetin was used as the flavonoid standard for this study. Briefly, 1 mL of extracted oil was diluted with 70% ethanol and placed in a test tube with 4 mL of distilled water. After 5 minutes, 0.3 mL of 5% sodium nitrite ($NaNO_2$) and 0.3 mL of 10% aluminium chloride ($AlCl_3$) were added to the test tube. After a further 6 minutes, 2 mL of 1 M sodium hydroxide (NaOH) was added. Distilled water was then added to the test tube until the total mixture volume reached 10 mL. The absorbance was measured at 510 nm using a spectrophotometer. The established calibration curve was used to quantify the TFC in the extracts, which was reported as mg quercetin equivalent (QE)/g of dried sample (DS) of plant material.

Experimental design

This study applied microwave radiation prior to the extraction process was based on the methods reported by Tan et al. [17] and Yu et al. [18]. As this is the first report on the use of combined microwave and solvent extraction for flavonoid compounds from papaya leaves, detailed extraction experiments are necessary. Therefore, a single-factor experiment method as described by Konagano et al. [19] was employed to evaluate the effect of individual factors on the response. Five main extraction parameters (i.e., particle size, microwave power, solvent type, solid to liquid ratio and extraction time) were studied with respect to the total flavonoid content (TFC) as the response. **Table 1** presents the sequence of the single-factor experiment method used to evaluate the five parameters step by step. The range of values used for each parameter was selected based on previous literature on the extraction process of flavonoid compounds from various plant materials. As shown in **Table 1**, to investigate the effect of different particle sizes (0.425 mm, 0.600 mm and 1.180 mm), 10 g of

powdered sample was used for each run. The extraction was conducted under constant parameters: 500 W microwave power, methanol as the extraction solvent, a 1:20 solid-liquid ratio and 4 hours of extraction time. The selection of these constant parameters is based on studies by previous researchers, where a high TFC yield was successfully obtained.

LC-TOF-MS analysis

Detailed flavonoid compound analysis was carried out using liquid chromatography time-of-flight mass spectrometry (LC-TOF-MS), conducted at the Institute of Biology System (INBIOSIS), Universiti Kebangsaan Malaysia (UKM), Bangi, Malaysia. Separation was performed using a Thermo Scientific C18 column (AcclaimTM Polar Advantage II, 3 x 150 mm, 3 µm particle size) on an UltiMate 3000 UHPLC system (Dionex). Gradient elution was conducted at a flow rate of 0.4 mL/min and a column temperature of 40 °C using water with 0.1% formic acid (A) and 100% acetonitrile (B) with a total run time of 22 minutes. The injection volume was 3 µL. The gradient started at 5% B (0 – 3 min), increased to 80% B (3 – 10 min), remained at 80% B (10 – 15 min), and returned to 5% B (15 – 22 min). High resolution mass spectrometry was performed using a MicroTOF QIII Bruker Daltonic with ESI negative ionization under the following conditions: capillary voltage, 4000 V; nebulizer pressure, 2.0 bar; drying gas, 8 L/min at 300 °C. The mass range was set from 50 to 1000 m/z. Accurate mass data for the molecular ions, provided by the TOF analyser, were processed using Compass Data Analysis software (Bruker Daltonik GmbH). A maximum error of 10 ppm was applied.

Results and Discussion

Quantification of total flavonoid content at various extraction parameters

Total flavonoid content (TFC) of the extract is expressed as mg of quercetin equivalent per gram of dried sample (mg QE/g DS). TFC quantification was performed using the established standard curve of quercetin, as shown in **Figure 1**. The standard curve exhibited a strong linear relationship described by the regression equation $y = 300.52 + 1.1755$ with an R^2 value of 0.9984, indicating excellent linearity and reliability of the method. This equation was used to calculate the TFC yield for all extracts.

Numerous studies on extraction processes, regardless of the extraction methods applied, have shown that a smaller particle size of the plant sample leads to a higher extraction yield. Total flavonoid content (TFC) extracted using the combined microwave and solvent extraction method shows significant dependence on particle size when sizes of 0.425, 0.600 and 1.180 mm were used with constant extraction parameters of 500 W microwave power, methanol as the extraction solvent, a 1:20 solid-liquid ratio, and 4 hours of extraction time. **Figure 2(a)** illustrates the effect of different particle sizes on the TFC expressed as mg QE/g DS of papaya leaves. As shown, the TFC is the highest at 21.1 mg QE/g DS when the smallest particle size of 0.425 mm was used, followed by 0.600 mm and 1.180 mm, respectively.

Table 1. Sequence of single-factor experiment steps

Particle Size (mm)	Microwave Power (W)	Solvent	Solid-liquid Ratio (g/mL)	Extraction Time (h)	Reference
0.425, 0.600, 1.180	500	MeOH	1:20	4	[20]
Optimal	**100, 200, 300, 400, 500**	MeOH	1:20	4	[21]
Optimal	Optimal	**MeOH, EtOH, acetone, chloroform, water**	1:20	4	[22]
Optimal	Optimal	Optimal	**1:10, 1:15, 1:20, 1:25, 1:30**	4	[23]
Optimal	Optimal	Optimal	Optimal	**1, 2, 3, 4, 5, 6, 7, 8**	[24]

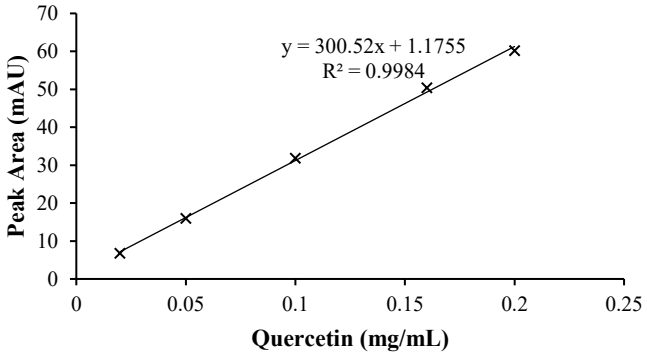


Figure 1. Standard curve of quercetin for the determination of total flavonoid content

The increasing trend of TFC obtained as particle size decreased is consistent with previous findings. Taweekayujan et al. [25] investigated the influence of various particle sizes on the extraction of coffee beans and found that the smallest size achieved the highest yield. According to the authors, the highest yield was attained when the particle size was between 0.250 and 0.425 mm, similar to the result obtained in this study. This range of particle size indicates that most solutes, including flavonoids, are located at the solid surface of the powdered sample, making them easily accessible to the extraction solvent. The higher surface area for solute-solvent contact created by the grinding process provides a shorter travel path for flavonoid solutes to transfer from the solid matrix to the solvent.

Furthermore, the higher TFC observed with the smallest particle size may be attributed to the high microwave power used, i.e., 500 W, which causes rapid internal heating and pressure buildup in the sample. In addition, the high microwave power promotes cell wall rupture, enhances solvent penetration, and accelerates the mass transfer of intracellular compounds into the solvent by increasing internal dielectric heating [26]. Thus, the plant cell wall is ruptured, exposing more oil containing flavonoids. A similar result was reported where a better absorption of microwave energy was achieved for the smaller particles due to the uniform heat distribution. This enhanced the extraction yield of flavonoid compounds for the smaller particles compared to the larger particle size. As shown in **Figure 2(a)**, the TFC of larger particle sizes (i.e., 0.60 mm and 1.18 mm) is relatively lower than that of the 0.425 mm sample due to the lower surface area. The high microwave power of 500 W was not able to increase the flavonoid yield because of incomplete cell disruption and limited interaction between the solid and the solvent. Larger solid particles consisting of a coarser structure restricted the movement of solutes during the extraction process, thereby reducing the TFC yield. The results obtained in this study indicate that sample particle size significantly affects the efficiency of flavonoid extraction, with the 0.425 mm particle size emerging as the optimum parameter for maximizing the TFC yield.

The effect of different microwave power levels, ranging from 100 to 500 W, on the leaf samples was then evaluated using the optimum particle size obtained, while the other extraction parameters remained the same. The purpose of applying microwave radiation to the leaf samples before the extraction process is to damage the plant cell wall, thereby exposing the solute to be extracted in a short time. The influence of increasing microwave power on TFC yield is shown in **Figure 2(b)**. As the microwave power increases from 100 W to 300 W, the TFC yield increases significantly, with the highest TFC obtained being 34.53 mg QE/g DS. The increase in TFC yield is attributed to effective plant cell disruption at the high microwave power used. The intracellular flavonoids in the plant leaves were exposed to the solvent, facilitating TFC enhancement. The high microwave power increased the internal pressure within the plant matrix, leading to a superheating phenomenon in which the water content (i.e., moisture) within the plant sample exhibited micro-explosions that ruptured the plant cell wall, enhancing solvent penetration into the sample [27]. On the contrary, the microwave power used should not be very high. This is evident when the microwave power was increased to 400 W, where the TFC began to decrease from 34.5 to 34.4 mg QE/g DS. In addition, a drastic decrease was observed when the power reached its highest level, i.e., 500 W. This pattern indicates that the optimum microwave power is 300 W, as further increases to 400 W and 500 W are unnecessary and would result in excessive energy usage, potentially causing environmental impact. Lasunon and Sengkhamparn [28], in their study on optimizing microwave power using the microwave-assisted extraction method on tomato waste found that a power level of 300 W was sufficient to extract bioactive compounds from the plant sample.

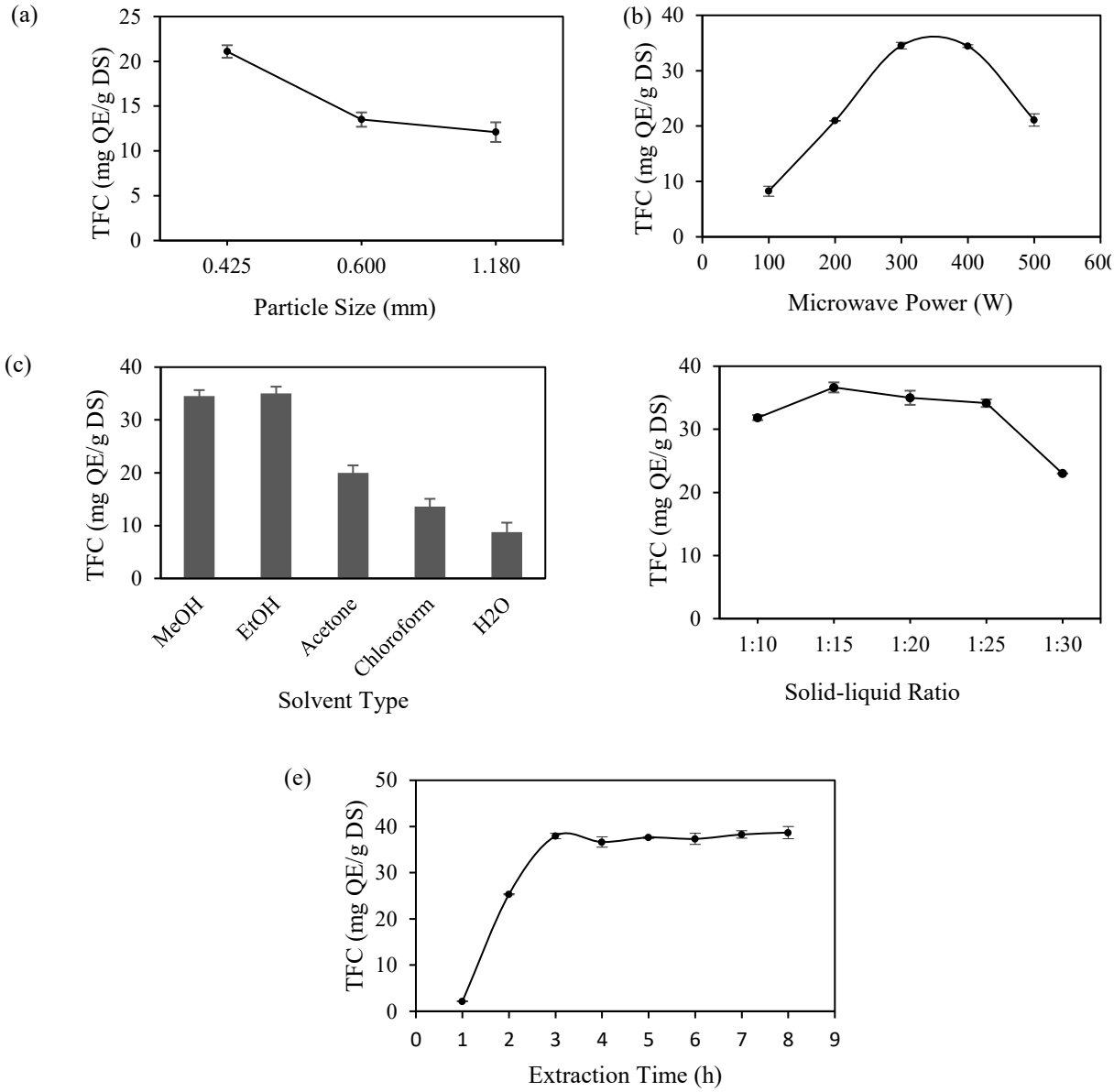


Figure 2. The effect of (a) particle size, (b) microwave power, (c) solvent type, (d) solid-liquid ratio and (e) extraction time on total flavonoid content (TFC) from papaya leaves under combined microwave and solvent extraction (MSE)

The extraction experiment was further continued by evaluating the effect of solvent types on TFC yield, as illustrated in **Figure 2(c)**. Five different solvents with varying polarity indices, namely, methanol, ethanol, acetone, chloroform and water, were investigated. Flavonoids are known as complex compounds which can be classified as moderately polar or polar, depending on their derivatives [29]. Among the solvents used, ethanol extracted the highest TFC (35 mg QE/g DS), followed by methanol, acetone, chloroform and water. There were no significant differences in TFC obtained when using methanol and ethanol. Despite methanol having a higher polarity than ethanol, it achieved a slightly lower TFC yield of 34.53 mg QE/g DS. Therefore, ethanol was chosen as the best solvent for this process. In addition, ethanol is a food-grade solvent and is safer for human products.

Another extraction condition that influences the yield is the solid-liquid ratio. This refers to the mass of the solid sample per volume of solvent used, expressed in g/mL. The study employed different solid-liquid ratios, ranging from 1:10 to 1:30 g/mL. As shown in **Figure 2(d)**, a ratio of 1:15 yielded the highest TFC (36.64 mg QE/g DS) while a ratio of 1:30 resulted in the lowest TFC (23 mg QE/g DS). The TFC increased when the ratio was raised from 1:10 to 1:15, but began to decrease when the ratio was further increased to 1:30. The increase in TFC at a ratio of 1:15 can be explained by the greater penetration of solvent into the ruptured plant cell wall, which dissolves the solute. However, when the ratio increased from 1:15 to 1:30, the TFC yield began to decrease and reached its lowest at a ratio of 1:30. This result indicates that the increasing amount of solvent from 150 mL to 300 mL

decreased the yield. This may be because the equilibrium volume was already achieved when the solid-liquid ratio was 1:15. Most oil was extracted when 150 mL of solvent was used, indicating that the optimum solid-liquid ratio was achieved. This finding suggests that excessive solvent consumption may not be necessary for the extraction process and should be avoided to reduce waste.

Among all extraction parameters that influence extraction yield, the extraction time plays a crucial role in determining the efficiency of the entire process. Theoretically, increasing the extraction time will increase the yield as the solvent has more time to diffuse into the plant matrix and extract the compound. However, if the duration is too long and no significant changes in yield, this will impact the time and energy consumption of the entire process, affecting environmental sustainability by causing unnecessary energy waste. **Figure 2(e)** shows the effect of different extraction times ranging from 1 to 8 hours on TFC yield. As shown, the TFC increased significantly as the extraction time increased from 1 hour to 3 hours, with the maximum TFC obtained being 37.94 mg QE/g DS. As the time was further increased from 3 to 8 hours, the TFC yield reached its plateau phase, indicating that equilibrium had been achieved. At this stage, the diffusion rate balanced the solvent saturation, so prolonging the extraction time was no longer necessary. The sharp increase in TFC from 1 to 3 hours suggests that exposure to microwave energy enhanced the mass transfer of solute into the solvent. More oil was extracted as a result of the exposed oil caused by microwave radiation and the grinding process before extraction. The result is consistent with previous studies, which have shown that applying microwave radiation before extraction shortens the extraction time [13,18]. As reported by the authors, the ability of microwave energy to destroy plant cell walls is a promising method in extraction technology, as it reduces the time required to extract the compound. A shorter process time benefits many industries by lowering energy consumption and reducing operational costs.

The results indicated that the optimum extraction parameters for papaya leaves using combined microwave and solvent extraction are a 0.425 mm particle size, 300 W microwave power, ethanol as the extraction solvent, 1:15 solid-liquid ratio and 3 hours of extraction time. Although the single-factor approach provides a straightforward assessment of individual parameter effects, it does not account for possible interactions among variables. Therefore, future studies should employ statistical optimization techniques such as response surface methodology (RSM) or Box–Behnken design to refine further and optimize the ring system cleavage at 216.0908 m/z. Sakuranin has a flavanone structure as the flavonoid backbone, comprising the three rings found in flavonoid extraction process.

Characterization of flavonoids using LC-TOF-MS analysis

The extract obtained under the optimum extraction conditions was quantified using Liquid Chromatography–Time-of-Flight Mass Spectrometry (LC-TOF-MS) to isolate flavonoid compounds. Flavonoids consist of two benzene rings, known as rings A and B, connected by three carbon atoms. Flavonoid derivatives can be classified into several groups. The identification of flavonoid compounds in the leaf extract of *C. papaya* was carried out using the MS/MS fragmentation pattern in relation to KEGG compound reference standards from the MetFrag online database. The mass-to-charge ratio (m/z) of the characteristic ions and molecular ions was used to identify the composition of each peak. **Figure 3** shows the chromatogram of the detected compounds, and **Table 2** presents the identified flavonoids in *C. papaya* leaves, along with their structures and pharmaceutical benefits.

As shown in **Figure 3**, many compounds were detected in the extract, with 5 identified as flavonoids (i.e., compounds 21, 23, 32, 36, and 38). As presented in **Table 2**, **Compound 21** detected at m/z 323.136 suggested that it originated from a flavonoid precursor. With an open chain between the two benzene rings A and B, the characteristic ions at m/z 323.1352, 457.1935 and 647.2777 identified the flavonoid compound as isobavachalcone. As reported by Sahu et al. [30], the open chain between 2 benzene rings consists of a three-carbon chain with C=C and C=O bonds. The chalcone part in isobavachalcone is the phenyl-CH=CH-C(=O)-phenyl groups, as shown in **Table 2**. The chemical structure also has a five-carbon chain attached to the aromatic ring. Numerous pharmacological activities of isobavachalcone have been identified, including anticancer, antimicrobial, anti-inflammatory, antioxidant and neuroprotective effects [31]. A clinical investigation by Ouyang et al. [32] demonstrated that this compound exhibits significant antibacterial activity against Enterococcus faecalis. Furthermore, several in vitro and in vivo studies have shown that isobavachalcone is able to induce cell death, primarily through apoptosis, in various types of cancer, including leukemia [33], hepatocellular carcinoma (liver cancer) [34], and breast cancer [35].

Compound 23 produced at m/z 447.1513 is identified as a flavanone glycoside by comparison of its mass spectrum data with the database library. The presence of characteristic ions at 216.0908, 447.1513 and 717.2292 supports its identification as sakuranin due to the aglycone characteristic at 447.1513 m/z and core compounds (rings A, B and C, as shown in **Table 2**). The methoxyl group (-OCH_3) and glucopyranoside attached to ring A, along with a hydroxyl group (-OH)

at ring B, confirm the compound as sakuranin [36]. Sakuranin has been reported to exhibit various pharmacological activities, including. antioxidant, anti-inflammatory, antiproliferative and antihepatitis B effects [36, 37]. Recently, a study investigated its hypotensive and vasorelaxant effects in rats [38]. The compound was found to lower the blood pressure and induce smooth muscle relaxation. Due to its significant biological and medical properties, the compound represents a promising plant-based medicine with potential for future drug development and clinical applications.

Compound 32 observed at m/z 609.1497 was identified as kaempferol 3-O-beta-D-glucosyl galactoside with the chemical formula $C_{27}H_{30}O_{16}$. The characteristic fragment ions at 236.0567, 609.1497 and 846.2148 described the compound. M/z 236.0567 represents the core flavonoid structure of the two benzene rings (A and B rings), while m/z 609.1497 confirms the presence of the intact glycosylated flavonoid (i.e. glycosyl group) supporting the identification as kaempferol 3-O-beta-D-glucosylgalactoside. This compound is classified as a kaempferol derivative due to the aglycone core structure of kaempferol in its chemical structure. Previous studies have shown that the compound exhibits notable antidiabetic and anti-obesity effects, improving insulin sensitivity and regulating lipid metabolism [39]. Consumption of foods containing kaempferol can reduce the risk of developing chronic diseases such as cancer and cardiovascular disorders [40].

Similarly, **Compound 36**, detected at m/z 593.155, corresponds to the molecular ion of kaempferol-3-O-rutinoside, a glycosylated flavonol. The MS/MS analysis showed fragment ions at m/z 327.0847, 537.327, and 693.2827. As with **Compound 32, Compound 36** also has its kaempferol aglycone at m/z 327.0847, indicating the presence of rhamnose and glucose. These fragmentation patterns are consistent with the study reported by Dou et al. [41], confirming its identity as a kaempferol derivative. Kaempferol-3-O-rutinoside has been reported to possess multiple bioactivities that benefit human health, including hepatoprotective, antioxidant, cardioprotective and metabolic effects [42]. A previous study conducted by Zheng et al. [43] demonstrated that kaempferol-3-o-rutinoside is able to reduce fever in mice faster than the commercial fever drug ibuprofen.

As illustrated in **Figure 3**, **Compound 38** is also a flavonoid, specifically 7-hydroxy-2,4,5-trimethoxy isoflavone, based on the m/z value of 327.0858 in the mass spectrum. Its chemical structure, shown in **Table 2**, consists of a basic isoflavone skeleton with a hydroxyl group at position 7 of the A-ring – a functional group commonly responsible for antioxidant activity. Additionally, three methoxy groups at positions 2, 4 and 5 of the B-ring further confirm the compound as 7-hydroxy-2,4,5-trimethoxyisoflavone.

The structural features observed in this compound are consistent with the findings reported by Innocent [44], who previously identified a similar compound with an identical chemical structure. The compound has demonstrated notable pharmacologicalproperties. Plant extracts containing this compound have exhibited antiurolithiatic activity, effectively preventing the formation of kidney stones [45]. Furthermore, Tu et al. [46] reported that the compound possesses anti-inflammatory activity, highlighting its various medicinal benefits.

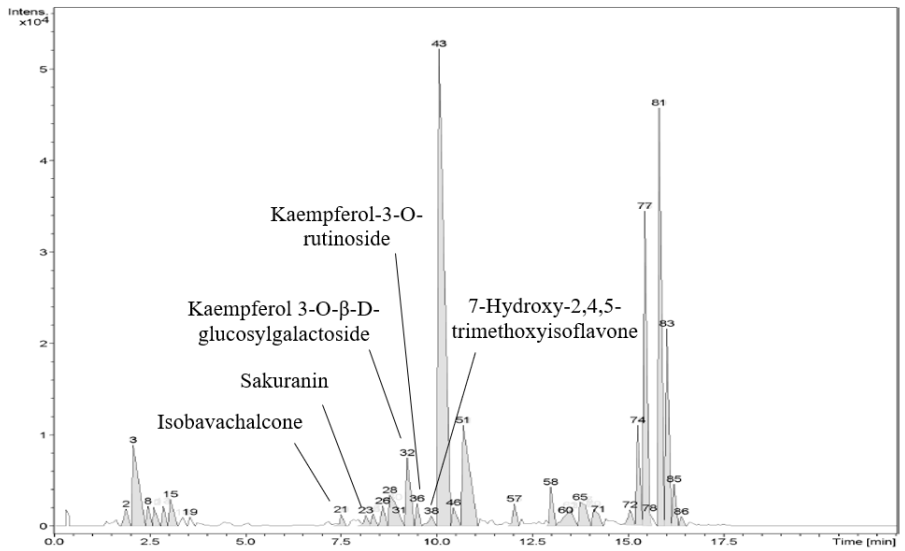
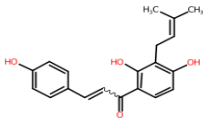
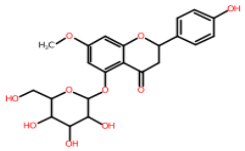
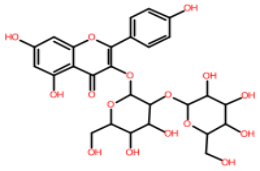
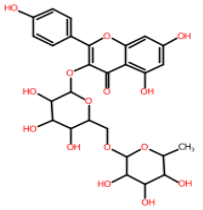
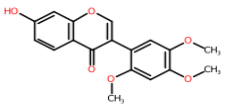


Figure 3. Chromatograph of flavonoid compounds from *C. papaya* leaves

Table 2. Identified flavonoid compounds in *C. papaya* leaves extract using LC-TOF-MS in negative ion mode

Peak No.	RT (min)	S/N	Observed m/z (M^-H^-)	Area	Identified Component	Chemical Formula	Chemical Structure	Pharmacological Effects
21	7.5	34.7	323.136	7584.1	Isobavachalcone (flavanone)	$C_{20}H_{20}O_4$		Anti-cancer, anti-microbial, anti-inflammatory, antioxidative [47]
23	8.1	10.4	447.1513	7268.5	Sakuranin (flavanone)	$C_{22}H_{24}O_{10}$		Antiproliferative, antioxidant, antimicrobial, anti-inflammatory, antiparasitic, antimutagenic, and antiallergic [36]
32	9.2	33.6	609.1497	57156.1	Kaempferol 3-O-beta-D-glucosylgalactoside (flavonol)	$C_{27}H_{30}O_{16}$		Anti-diabetic, anti-obesity [39]
36	9.5	18.2	593.155	14682.2	Kaempferol-3-O-rutinoside (flavonol)	$C_{27}H_{30}O_{15}$		Hepatoprotective [42]
38	9.9	3	327.0858	10411.6	7-Hydroxy-2,4,5-trimethoxyisoflavone (isoflavonoid)	$C_{18}H_{16}O_7$		Antimicrobial activity [48]

Conclusion

This study verified the optimization of combined microwave-solvent extraction for recovering total flavonoid content (TFC) from *C. papaya* leaf and identified the major flavonoid compounds under the optimized conditions using LC-TOF-MS. The highest recovery of TFC was obtained under the following extraction conditions: 0.425 mm particle size, 300 W microwave power, ethanol as the extraction solvent, a solid to liquid ratio of 1:15 g/mL, and an extraction time of 3 hours, resulting in TFC of 37.94 mg QE/g DS. Compared with conventional solvent extraction, the combined method achieved a higher yield, demonstrating a significant improvement in extraction efficiency attributed to microwave-induced cell disruption. Similar improvements were reported in previous studies on the application of microwaves before the extraction process, which enhanced extraction recovery [13, 49].

Five major flavonoid compounds were profiled under these conditions: isobavachalcone, sakuranin, kaempferol 3-O-β-D-glucosylgalactoside, kaempferol-3-O-rutinoside, and 7-hydroxy-2,4,5-trimethoxy isoflavone. The results indicate that *C. papaya* leaf contains a diverse range of flavonoid compounds, suggesting that this plant material can be further studied to understand the pharmacological properties and quantification of the identified flavonoid compounds.

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