AsPac J. Mol. Biol. Biotechnol. 2024
Vol. 32 (4) : 231-244

Acidified water extraction increases carpaine yield from *Carica papaya* L. Leaf

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Received 22nd August 2024 / Accepted 27th October 2024 / Published 31st December 2024

Abstract. *Carica papaya* leaves have been traditionally used in various medicinal applications. The major alkaloid, carpaine, is widely studied for its anti-thrombocytopenic activity. However, extraction of carpaine from *Carica papaya* leaves remains challenging, and the existing methods may not yield high amount of carpaine due to the presence of various interfering compounds. High carpaine yields are essential for studying on its anti-thrombocytopenic and immunomodulatory effects, assessing its efficacy and safety as a potential malaria drug candidate. This study aims to extract and isolate carpaine from *Carica papaya* leaves using alcohol extraction method and acidified water extraction method performed on dried powdered leaves and compare their percentage yield of carpaine. The percentage yield of carpaine isolated from acidified water extraction was 12.01%, while the yield for alcohol extraction was 2.18%. Acidic condition enhances carpaine's water solubility through protonation, converting carpaine in its free base form to salt form which improves extraction efficiency. Heat and stirring further optimize the acidified water extraction process. Characterization of isolated carpaine was performed with various analytical techniques to confirm the structure of carpaine isolated.

Keywords: acid-base extraction, characterization, elucidation, natural product, purification, spectroscopy

INTRODUCTION

Carica papaya, commonly called papaya, belongs to the family of Caricaceae, and it is an herbaceous succulent plant. Generally, papaya tree grows rapidly, is often short-lived, produces latex, and is semi-woody. The latex produced can be used to tenderize meat due to the presence of the enzyme papain (Sekeli *et al*., 2018). It is commonly found in tropical and subtropical countries (Koul *et al*., 2022), such as Tropical America, Southern Mexico, Tropical Africa, Philippines, India, Indonesia, Australia, and Malaysia (Yogiraj *et al*., 2014). Papaya is not only a tropical fruit consumed worldwide but also currently known for its benefits in treating diseases. Humans can use most parts of the papaya plant for medical purposes, such as from the extracts of their leaves, latex, roots, barks, flowers, fruit, and seeds (Rahmani & Aldebasi, 2016).

Plant-derived medications can treat diseases that can help prevent and protect humans against and cure various pathological conditions (Dini *et al*., 2019), which can be fatal such as cancer, malaria, and dengue. Carpaine is a naturally occurring alkaloid found in leaves, seeds (Saran & Choudhary, 2013), roots and barks of *Carica papaya* tree (Julianti *et al*., 2014). The concentration of carpaine can vary among different parts of the papaya plant, but it is particularly abundant in the leaves. According to Shinde and Hase (2020), carpaine has high biological activities such as antitumor, anticancer, and antimicrobial properties. Besides, carpaine can also lower the

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heart rate and blood pressure, promote movement of the intestines, relax the uterus, and dilate the bronchioles (Haldar *et al.*, 2020). Furthermore, the alkaloid carpaine can be used as a medication against dengue infection (Majumder *et al.*, 2020). Research and extraction of carpaine to become an effective drug molecule are becoming interesting nowadays due to its potential to treat the fatal disease of dengue infection, especially in this tropical country, Malaysia, where dengue fever is a nationwide and year-round threat.

Although carpaine possesses various medicinal properties, the efficiency and effectiveness of extracting carpaine from *Carica papaya* leaves remain challenging due to the complex matrix of the plant material and the presence of various interfering compounds. Since carpaine has not yet been successfully synthesized in the laboratory and can only be obtained from *Carica papaya* leaves, optimizing the extraction process is essential to obtain higher yields. Existing extraction methods may not yield a sufficient amount of carpaine, hindering comprehensive pharmacological studies. Furthermore, the optimal extraction method for maximizing carpaine yield is still unknown, making the development of a reliable and reproducible extraction technique crucial for further research on its therapeutic potential.

While many studies focus on the biological activities of carpaine, few have explored which extraction method is the most efficient for achieving a high yield of carpaine. Additionally, to the best of our knowledge, no studies have been done to compare the percentage yield of carpaine between acidified water extraction and alcohol extraction. Hence, this study introduces a novel approach by evaluating and comparing these two extraction methods. Furthermore, limited research has been conducted on acidified water extraction of carpaine despite water being recognized as the greenest solvent which is a more environmentally friendly extraction agent (Ivanović *et al.*, 2020) and significantly less expensive as compared to alcohol extraction. Therefore, more studies should focus on acidified water extraction and ways to optimize the yield of carpaine.

Optimizing a cost-effective, environmentally friendly extraction method like acidified water extraction could pave the way for large-scale production, which is important to the pharmaceutical industry. Carpaine makes up 63% of the total alkaloid content in *Carica papaya* leaf extract and is recognized as a key contributor to its anti-thrombocytopenic effects (Shrivastava *et al.*, 2022). Many studies have been done to show the anti-thrombocytopenic property of carpaine (Tambe *et al.*, 2021; Zunjar *et al.*, 2016). Thrombocytopenia refers to a condition where the platelet count in the blood is lower than normal. It is due to inhibition or improper platelet production induced by dengue or malaria (Zunjar *et al.*, 2016; Munir *et al.*, 2022). Zunjar *et al.* (2016) have verified that a pure sample of carpaine exhibited exceptional anti-thrombocytopenic properties. Furthermore, a patient bitten by dengue-carrier mosquitoes orally administered aqueous leaf extracts of *Carica papaya* for 5 days showed an increase in the platelet count (Majumder *et al.*, 2020). Anti-plasmodial activity of carpaine *in-vitro* has been done by Teng *et al.* (2019), where carpaine exhibited good activity against *Plasmodium falciparum* and showed no toxicity to healthy, uninfected human red blood cells. Hence, discovering an optimal method for carpaine extraction could enable large-scale production, offering significant potential for developing drugs to treat both malaria and dengue. This would not only help to fight against these fatal diseases but also provide a natural alternative for drug development, reducing reliance on synthetic drugs.

Acidified water extraction or acid-base extraction is a widely used technique for isolating alkaloids, as their solubility varies significantly with changes in pH (Lam *et al.*, 2024). Carpaine, an alkaloid with a secondary amine group, is particularly susceptible to protonation in an acidic condition, converting it into a positively charged organic salt. This protonated form enhances its solubility in aqueous solvents, facilitating efficient separation from hydrophobic compounds and other plant materials (Lam *et al.*, 2024). When solutions containing these alkaloid salts are made basic, the alkaloids shift into their less soluble neutral forms. This change causes them to aggregate with other hydrophobic compounds, reducing their contact with water. (Lam *et al.*, 2024). Consequently, these hydrophobic complexes can be easily extracted into a nonpolar

solvent. This property makes acid-base extraction a valuable approach for isolating carpaine.

In this study, acidified water extraction was performed by macerating papaya leaf powder in hot acidified water while stirring with a magnetic stirrer. The increase in temperature can help in photochemical yield by enhancing solvent penetration into cellular walls (Bitwell *et al.*, 2023). Concentrated hydrochloric acid (HCl) was added to distilled water to lower the pH to 2-3, ensuring the solution was sufficiently acidic to convert alkaloids into their salt form, which facilitated their extraction into the aqueous solution. The advantage of acidic extraction is changing the alkaloids into small organic salts, which are more soluble in water. However, the main drawback is the presence of water-soluble impurities and difficulty concentrating (Adejoke *et al.*, 2019). After extraction, the pH of the solution was adjusted to approximately 11, converting the alkaloids back into their free base form. This allowed them to be extracted by non-polar dichloromethane, separating them from the aqueous layer.

In alcohol extraction, ethanol, which is a universal extraction solvent is used in this study to extract carpaine from *Carica papaya* leaves. The dried plant powder was soaked in the mixture of ethanol/water/HCl at the ratio of volume of 89:10:1 for 24 hours with periodically stirring and repeated for 3 more times with changing fresh new solvent. By changing the solvent several times, most of the desired plant compounds will be extracted completely. Extraction efficiency can be increased by increasing the extraction duration for the solute to reach equilibrium inside and outside of the solid material (Zhang *et al.*, 2018). The advantages of alcohol extraction are that both free alkaloids and alkaloid salts can be dissolved in alcohol, and fewer polysaccharides and proteins will be extracted. However, its disadvantage is that more fat-soluble impurities are co-extracted (Adejoke *et al.*, 2019).

MATERIALS AND METHODS

The extraction of alkaloid carpaine was done in two different methods. The first method was done by extraction using a mixed solvent of ethanol/water/HCl at a ratio of volume of 89:10:1 and the second method was extraction using acidified water. The aim is to compare the percentage yield of carpaine from each method.

Material

Carica papaya leaves were obtained from local farmers. The chemicals that were used in this study were distilled water, dichloromethane (CH_2Cl_2), hexane (C_6H_{14}), chloroform ($CHCl_3$), ethyl acetate ($CH_3CO_2CH_2CH_3$), acetone (CH_3COCH_3), methanol (CH_3OH), ethanol (CH_3CH_2OH), hydrochloric acid (HCl), bismuth nitrate, glacial acetic acid (CH_3COOH), potassium iodide (KI), silica gel 60 (0.040 to 0.063 mm), column chromatography (230 to 400 mesh particle size), thin layer chromatography silica gel 60 F_{254}, petroleum ether (C_6H_{14}) and ammonium hydroxide (NH_4OH). All the chemicals and materials were procured from Merck and Sigma-Aldrich.

Preparation of Dragendorff's reagent

Solution 1 was produced by combining 10 mL of glacial acetic acid and 0.85 g of bismuth nitrate in 40 mL of distilled water. Then, solution 2 was prepared by dissolving 8 g of potassium iodide in 20 mL of water. By mixing solutions 1 and 2 with 2 mL of glacial acetic acid and 10 mL of distilled water, Dragendorff's reagent was formed.

Preparation of Carica papaya leaves powder

Mature *Carica papaya* leaves were washed thoroughly with distilled water to remove any dust (Zunjar *et al.*, 2016). The leaves were then dried in an electric blast drying oven at a temperature of 50°C until a constant mass was obtained. Then, the dried leaves were milled and ground to obtain a fine powder with a 0.38 mm (40 mesh) particle size (Wang *et al.*, 2015).

Extraction of carpaine from Carica papaya leaves

Alcohol extraction

One kilogram of papaya leaf powder was macerated with 3 L of mixed solvent of ethanol/water/HCl at the volume ratio of 89:10:1 for 24 hours at room temperature. The extract was then filtered with muslin cloth to separate the ethanol extract with plant residue and a new solvent mixture was added in to macerate the

plant material again. Maceration was done with a total of 4 times using fresh new solvent mixture or until no alkaloid was present (detected by Dragendorff's reagent) to maximize the extraction process. The extract was then removed and stored in a place away from light and at room temperature (Wang *et al.*, 2015). All the extracts were combined and evaporated with a rotary evaporator at 50°C and 60 rpm (Susilawati *et al.*, 2021) to obtain semisolid extract. Then, the semisolid extract was dissolved in 1000 mL of water/HCl solution with a ratio of 98:2 in volume. The solution was filtered and underwent liquid-liquid extraction with 300 mL of petroleum ether to remove fat material (Wang *et al.*, 2015) and other non-polar compounds. The extraction process was repeated 3 times. The aqueous fraction was adjusted to pH 8.0 to 9.0 with NH_4OH solution and the mixture was further extracted with excess chloroform (Wang *et al.*, 2015). Extraction with chloroform was done thrice until no alkaloid was detected in the aqueous fraction. The chloroform fractions were then pooled and evaporated with a rotary evaporator at 50°C and 60 rpm (Susilawati *et al.*, 2021). The dry chloroform extract containing carpaine and some impurities was obtained. Thin-layer chromatography (TLC) was used to confirm the purity of the extract. The extract was spotted onto a TLC plate with mobile phase using hexane: chloroform (1:9 v/v). Then, the TLC plate was exposed to Dragendorff's reagent to visualize the colour change. In the presence of alkaloid, such as carpaine, the TLC plate showed orange to brown spots. The extract was then further purified using column chromatography. The flowchart of alcohol extraction process is shown in Figure 1.

Acidic water extraction

Five hundred grams (500 g) of powdered *Carica papaya* leaves were extracted using acidified distilled water, adjusted to a pH of 2 to 3 with concentrated HCl. The extraction was heated by a hot plate at a temperature of 60°C for 6 hours, with a magnetic stirrer throughout the process. The maceration process was done once, and the extract was filtered with muslin cloth, combined, and adjusted to pH 11 using NH_4OH solution. The extract was then partitioned with dichloromethane 3 times or until no alkaloid remained (Devi *et al.*, 2020). The dichloromethane fractions were combined and concentrated in a rotary evaporator at 50°C and 60 rpm. TLC plate was used to detect the presence of alkaloid with mobile phase using methanol: chloroform (1:9 v/v). The TLC plate was then exposed to Dragendroff's reagent. The extract was then further purified using column chromatography (Haldar *et al.*, 2020). The flowchart of acidified water extraction process is shown in Figure 2.

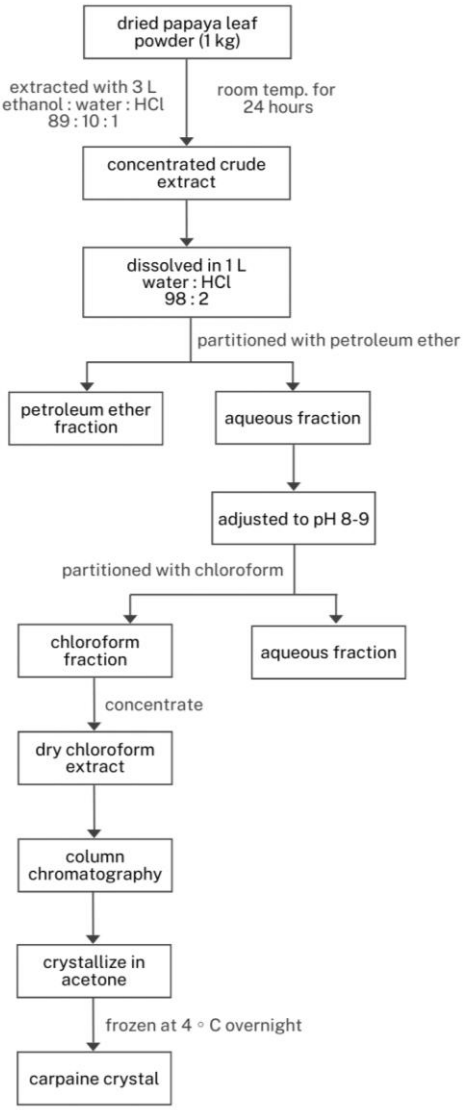

Figure 1. Flowchart of alcohol extraction process

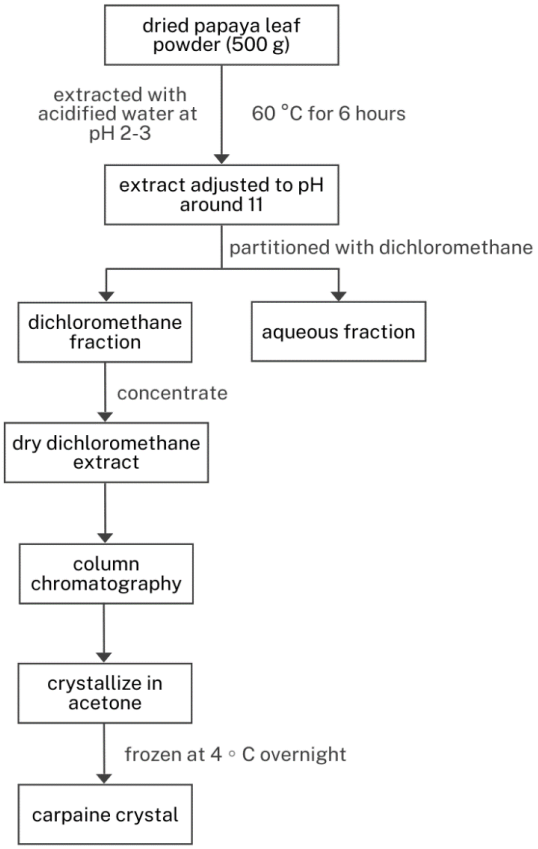

Figure 2. Flowchart of acidified water extraction process

Column chromatography

Column chromatography was used to separate a mixture of compounds using a glass column packed with silica gel. The mixture was separated based on the molecular weight and polarity. The adsorbent used for gravity chromatography was silica gel Merck Kiesel gel 60 Art. No. 9385.1000 of particle size 0.040-0.063 mm and Sigma Lipophilic Sephadex LH-20. Hexane and silica gel were combined and then poured into the column to prepare it. Before being used, the silica gel was given time to settle and was left overnight. Either the wet packing method or the dry packing method, the sample was introduced onto the top of the column. The column was then eluted with a suitable mixture of solvent by increasing the solvent polarity, which was hexane, chloroform, ethyl acetate, acetone and methanol respectively. Fractions that dripped off the column were saved in 10 mL aliquots. Then, the fraction was transferred to sample vials and TLC was used to monitor it after the solvent evaporated using a rotary evaporator. Each TLC fraction was exposed to Dragendorff's reagent to identify the presence of carpaine.

Crystallization of carpaine

After determination of alkaloids in each vial using TLC plate and sprayed with Dragendroff's reagent, the vials potentially containing carpaine and other impurities were combined and dissolved in acetone and frozen at 4°C overnight to crystallize. Solid carpaine was obtained by separating with liquid impurities. Further purification was done by washing with cold acetone to obtain pure carpaine.

Identification of isolated carpaine

The isolated and purified light yellow carpaine crystal was identified by using nuclear magnetic resonance (NMR) spectroscopy, Fourier transform infrared (FTIR) spectroscopy and melting point checking and was compared with literature data to confirm the purity of the carpaine extracted.

Nuclear magnetic resonance (NMR)

The isolated carpaine's identity was confirmed using NMR spectroscopy. Proton (H^1)-NMR and carbon (C^{13})-NMR were recorded in deuterated chloroform on a Joel 500 instrument, operating at 500 (Wang *et al.*, 2015).

Fourier transform infrared (FTIR)

Infrared spectra were determined by using UATAR on 100 Series Perkin Elmer FTIR SPECTRUM BX Spectrophotometer.

Melting point

A melting point tube containing carpaine was inserted into Digital Melting Point Apparatus and the temperature was recorded when the crystal started to melt.

RESULTS AND DISCUSSION

Extraction and isolation of carpaine

In this study, dried powdered *Carica papaya* leaves were used. Two different maceration methods with different solvent types were performed to determine which method gives a higher percentage yield of carpaine. In method one (alcohol extraction), extraction was performed with 1 kg of leaf powder and macerated in 3 L of solvent mixture of ethanol/water/HCl at a volume ratio of 89:10:1 for 24 hours at room temperature, and the maceration was done 4 times with fresh new solvent mixture. In method two (acidic water extraction), 500 g of leaf powder were macerated in acidified distilled water in the presence of heat for 6 hours with a magnetic stirrer. The value of extract yield was calculated from the percentage of mass of crude extract divided by the weight of dried leaf powder (Devi *et al.*, 2020). The percentage of extract yield from each extraction process is presented in Table 1 and Figure 3.

The highest percentage of extract yield was obtained from the first method with alcohol extraction. During method one, extraction was performed 4 times with changing the solvent after macerating for 24 hours each time until no alkaloid was detected using Dragendorff's reagent; meanwhile, in method two, only one maceration was done in the presence of heat. Multiple sequential extractions of a sample were performed in alcohol extraction, assuming that each extraction stage removes a significant portion of the remaining compound. (Golet *et al.*, 2002). Therefore, more plant compounds were extracted in method one as compared to method two, causing a higher percentage yield of extract. Using aqueous ethanol as the maceration solvent as in method one greatly affects the extract yield. The presence of some water can accelerate the mass transfer process by making the solvents more polar, which improves their solubility, causing the plant material to swell and increasing the surface area available for solute-solvent interaction (Fu *et al.*, 2014).

The TLC plate was used to confirm the presence of alkaloids in the crude extract, using a solvent system of hexane and chloroform in a 1:9 ratio. The presence of alkaloids is indicated by the formation of an orange spot on the TLC plate after being sprayed with Dragendorff's reagent. Figure 4(A) displays the TLC profile of the crude extract from method one, where a green spot suggests the presence of impurities. In method two, the TLC was performed using a solvent system of chloroform and methanol in a 9:1 ratio, comparing the pure carpaine obtained from method one (as shown in Figure 4(B)). With the chloroform: methanol system at a 9:1 ratio, the Rf value of pure carpaine was determined to be 0.35.

Table 1. Percentage of extract yield in different extraction method

Extraction method	Alcohol extraction	Acidified water extraction
Weight of crude extract	5.419 g	1.632 g
Percentage yield	0.54%	0.33%

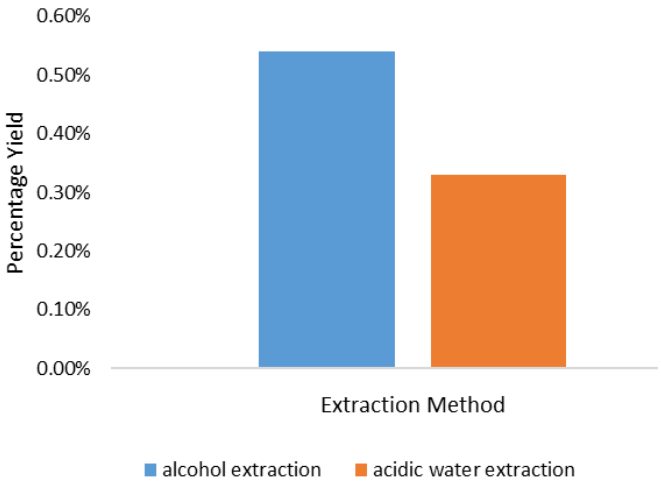

Figure 3. Percentage of extract yield in different extraction method

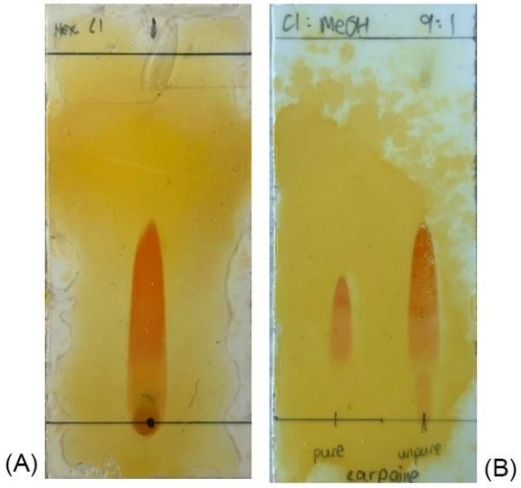

Figure 4. TLC profile. (A) method one, (B) method two.

After purifying carpaine using column chromatography, the compound was dissolved in acetone and left overnight at 4°C in the freezer to crystallize. This crystallization step is highly effective for separating crystalline carpaine from impurities, as the carpaine forms crystals while the impurities remain dissolved in the acetone. Further purification was achieved by washing the carpaine crystals with cold acetone to remove any residual impurities. The purified carpaine crystals were then weighed to determine the yield. Table 2 below presents the percentage yield of carpaine from both extraction methods. The percentage yield of carpaine was calculated by dividing the mass of carpaine obtained by the mass of crude extract.

The highest yield of the alkaloid carpaine was obtained from method 2, which involved acidified water extraction. In method 1, alcohol extraction was used, and the advantage is extracting alkaloids in both their free base and salt forms. Furthermore, alcohol extraction is less likely to extract impurities like polysaccharides and proteins, but the disadvantage of this method is the co-extraction of fat-soluble impurities (Yubin *et al.*, 2014). Therefore, the macerate had to be treated with petroleum ether to remove fat-soluble and other non-polar impurities. Removal of impurities by extractions can result in the loss of the target compound, contributing to a lower yield of carpaine. In contrast, acidified water extraction in method 2 can change alkaloids into their salt forms, which increase their solubility in water. However, the drawbacks include difficulties in concentrating the extract and the presence of more water-soluble impurities (Yubin *et al.*, 2014). In method one, the yield of carpaine from 1 kg of dried powdered *Carica papaya* leaves was 0.118 g, giving a yield of 0.118 g/kg, and a carpaine yield percentage of 2.18%. In comparison, method two yielded 0.196 g of carpaine from 500 g of dried powder, equating to a yield of 0.392 g/kg and a percentage yield of 12.01%. Therefore, the yield of carpaine from method two was significantly higher than that of method one.

Table 2. Percentage yield of carpaine

Method	Mass of carpaine yield	Percentage yield
Alcohol extraction	0.118 g	2.18%
Acidic water extraction	0.196 g	12.01%

Several factors contributed to the lower carpaine yield in method 1 (alcohol extraction). Although maceration was repeated several times, resulting in a larger amount of crude extract compared to method 2, the yield of carpaine was lower. This may be due to the loss of the compound during the liquid-liquid extraction steps. In method 1, the crude extract underwent multiple liquid-liquid extractions, first with petroleum ether to remove non-polar impurities, and then with chloroform to isolate carpaine. These multiple purification steps can lead to substantial losses of carpaine. Furthermore, while ethanol effectively extracts alkaloids in both their free base and salt forms, the subsequent extraction with non-polar petroleum ether may have removed the free base form of carpaine, contributing to the lower yield. Although petroleum ether helps eliminate fat-soluble impurities, it inadvertently resulted in the loss of the desired carpaine compound. In addition, the use of 2% HCl aqueous solution to dilute the concentrated extract may not have been sufficient to convert all the carpaine to its salt form. As a result, some carpaine likely remained in its free base form. Since the free base form of carpaine is non-polar, it may have been removed during the petroleum ether extraction, further lowering the yield. To minimize the loss of carpaine, it is crucial to ensure that all of it is converted into its water-soluble salt form. Therefore, checking the pH of the solution with a pH meter is essential. This would confirm that the solution is sufficiently acidic to convert all the carpaine to its salt form, thereby improving the overall yield of the compound. This study of extracting carpaine using aqueous ethanol was done by Wang *et al.* (2015) and their yield was 0.93 g/kg, which is significantly higher than the yield in this study. The result might be due to the differences in the geographic origin and the environmental conditions of the plant used. Another alcohol extraction was done by Susilawati *et al.* (2021) using 70% ethanol as the extraction solvent. They obtained 7.5 mg of carpaine from 2 kg of dried leaves, which is lower than the yield obtained in our alcohol extraction method. In their procedure, the concentrated extract was dissolved in 500 mL of 2% HCl and extracted with *n*-hexane to remove non-polar compounds. However, this step likely resulted in the loss of carpaine, as the acid concentration may not have been sufficient to convert all the free base carpaine into its salt form. Consequently, some of the free base carpaine might have been removed by *n*-hexane—a similar issue observed in our study.

Hence, the second method, which involved extraction with acidified water, yielded a higher amount of carpaine from *Carica papaya* leaves as compared to the alcohol extraction method, primarily due to fewer extraction and purification steps. Acidic conditions are beneficial for alkaloid extraction, as they promote the formation of water-soluble protonated alkaloids (Rachmaniah *et al.*, 2021), while leaving behind non-polar impurities. The extraction process was further enhanced by the presence of heat and stirring. High temperatures can increase solubility and improve the extraction process (Zhang *et al.*, 2018). It leads to cell disruption and thus increases the solute's solubility in the solvent (Tuan *et al.*, 2023). Furthermore, agitation boosts the mass transfer coefficient and improves the convective mass transfer, resulting in a higher extraction yield (Zainol *et al.*, 2023). Following the extraction, the pH was adjusted to around 11, converting the alkaloid salts back into their free base form, which is more soluble in organic solvents like dichloromethane. A liquid-liquid extraction with dichloromethane was then performed, effectively isolating alkaloids like carpaine into the organic layer while water-soluble impurities remained in the aqueous layer. The advantages of acidified water extraction include reduced use of organic solvents, shorter maceration time, and fewer extraction and purification steps. Thus, the reduced number of purification steps in method two resulted in a higher carpaine yield.

Although acidified water extraction yielded a higher amount of carpaine, this study did not explore how different extraction parameters influence the overall yield. Factors such as the age of the leaf used, surface area of the plant material, the extraction temperature, pH and extraction time could be important considerations for future research. The age and surface area of *Carica papaya* leaves used have a significant effect on the amount of carpaine yield. Based on the study done by Yap *et al.* (2021), blended young leaves contained the highest amount of carpaine (333 µg/g) as compared to blended old leaves (279 µg/g). Therefore, it is recommended to extract

carpaine from young *Carica papaya* leaves. Furthermore, blended young leaves yielded more carpaine than unblended young leaves, which are 333 μg/g and 176 μg/g respectively. This proved that reducing particle size (by blending) can facilitate carpaine extraction process. Smaller surface areas enhance solvent penetration and solute diffusion (Zhang *et al.*, 2018). In terms of extraction temperature, a study done by Julianti *et al.* (2014) found that the highest carpaine yield was achieved at 90°C and the yield slightly decreased at higher temperatures, which was likely due to thermal degradation. Solubility test was done by Tambe *et al.* (2021), where they found that carpaine's solubility in aqueous systems is pH dependent. The solubility of carpaine was found to be 35.12 μg/mL in acidic pH and 18.55 μg/mL in alkaline pH (Tambe *et al.*, 2021). Therefore, carpaine exhibits better solubility in an acidic aqueous system.

Characterization of carpaine

For the characterization of carpaine, various characterization techniques were used to elucidate the structure of carpaine isolated. The isolated carpaine was characterized by determining its melting point, 1D NMR and FTIR. In this study, the average melting point of carpaine examined was found to be 117.5°C, while the actual melting point of carpaine typically ranges from 119 to 121°C. A study done by Yap *et al.* (2021) obtained carpaine with melting point at 119°C. Melting point is a useful indicator of purity, as the presence of impurities can affect the melting point of a crystal. The presence of impurities in a compound can lower the melting point of it due to the disruption of the regular packing of molecules in the crystal lattice, which is a process called melting point depression. Carpaine obtained in this study was pale-yellow needle-like crystalline solid.

The ^{1}H-NMR data obtained are δ 4.718, 2.821, 2.543, 2.369, 2.271, 1.984, 1.177-1.631, 1.002, while the ^{13}C-NMR obtained are δ 173.5, 70.4, 56.1, 53.6, 37.4, 34.6, 29.8, 29.2, 28.8, 28.7, 26.4, 25.5, 25.4 and 18.7. The NMR data obtained are comparable with those found in the literature, as shown in Figure 5 and Table 3 below, indicating that the carbon NMR results match with the published data. In the ^{1}H-NMR spectrum, the peak at 4.718 ppm is the most downfield, as the proton attached to the carbon is directly bonded to an oxygen atom, resulting in most deshielding effects, as shown in Figures 6 and 7 below. The peak at 4.718 ppm is then followed by peaks at 2.821 ppm and 2.543 ppm, which are also highly deshielded due to the protons attached to carbons that are directly bonded to a nitrogen atom. In the ^{13}C-NMR spectrum, the most significant peak is at 173.5 ppm, which is the most downfield. This peak corresponds to a carbonyl carbon, which is consistent with the structure of carpaine, as shown in Figures 8 and 9 below.

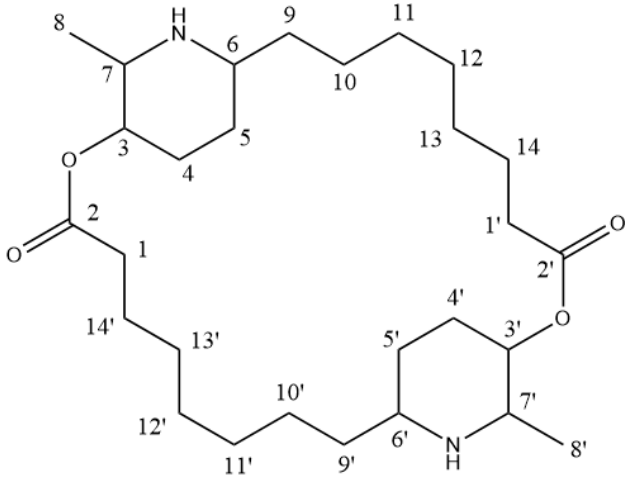

Figure 5. Proton and carbon positions with corresponding NMR shifts in Table 3.

Table 3. ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$)

Position	^{13}C(δ)	^{13}C(δ)*	^{1}H(δ)
1, 1’	34.6	34.7	2.369
2, 2’	173.5	173.6	-
3, 3’	70.4	70.4	4.718
4, 4’	25.4	25.5	1.984
5, 5’	28.7	28.77	1.177-1.631
6, 6’	56.1	56.1	2.543
7, 7’	53.6	53.7	2.821
8, 8’	18.7	18.8	1.002
9, 9’	37.4	37.5	1.177-1.631
10, 10’	26.4	26.5	1.177-1.631
11, 11’	29.8	29.8	1.177-1.631
12, 12’	29.2	29.2	1.177-1.631
13, 13’	28.8	28.82	1.177-1.631
14, 14’	25.5	25.6	2.271

***** Obtained from Yap *et al.*, 2021

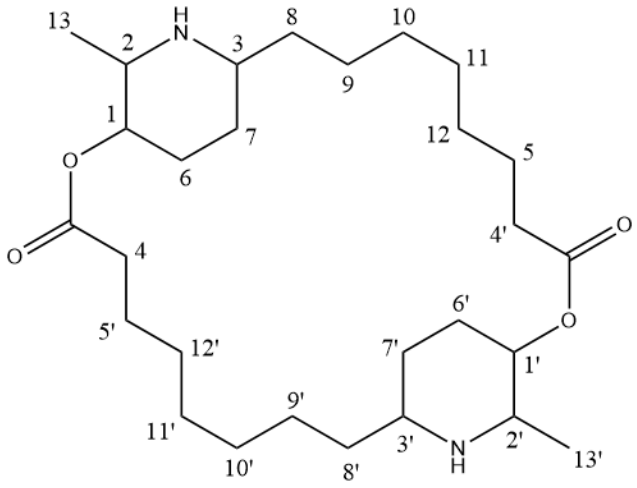

Figure 6. Proton positions of carpaine for ^{1}H

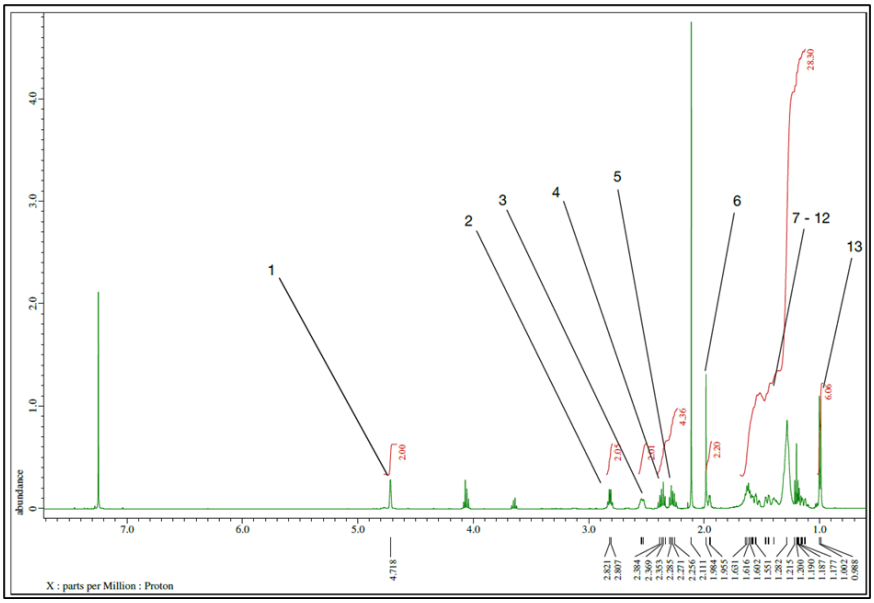

Figure 7. ^{1}H NMR of carpine (500 MHz, $CDCl_3$).

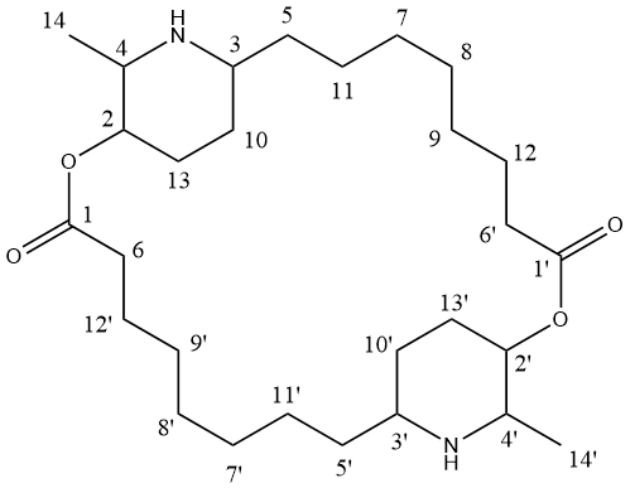

Figure 8. Carbon positions of carpaine for ^{13}C NMR

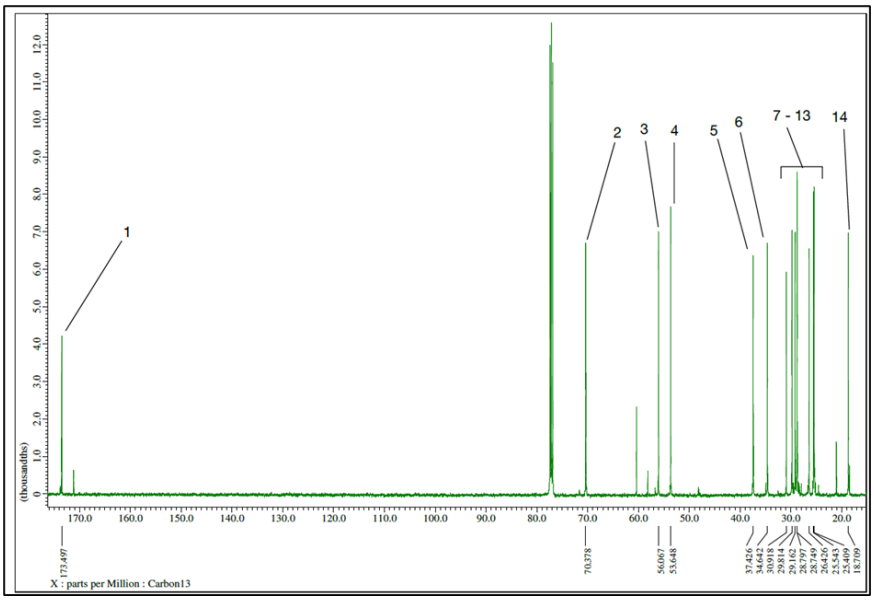

Figure 9. ^{13}C NMR of carpaine (100 MHz, $CDCl_3$).

According to the FTIR spectrum, the peak at 3322.64 cm^{-1} indicates the presence of N-H stretching, as shown in Figures 10 and 11 below. The presence of only one peak at this signal shows that the nitrogen atom is in secondary amine, while primary amine has two N-H bonds and therefore will show two peaks in the range of about 3200 – 3600 cm^{-1}. The result of FTIR showing one peak at 3322.64 cm^{-1} correlates with the carpaine structure with the nitrogen atom bonded to two other carbons in the ring showing a secondary amine. The peak at 1711.62 cm^{-1} indicates the presence of a C=O stretch, which corresponds to the ester functional group in the carpaine structure. The signals at 2921.17 cm^{-1} and 2852.42 cm^{-1} are due to alkane C-H stretching, which is also present in the carpaine structure.

Therefore, based on the data and results obtained from the melting point, ^{1}H-NMR, ^{13}C-NMR and FTIR spectrum, those results correlate with the literature data and correspond to the structure of carpaine. Thus, we can conclude that the alkaloid isolated is carpaine.

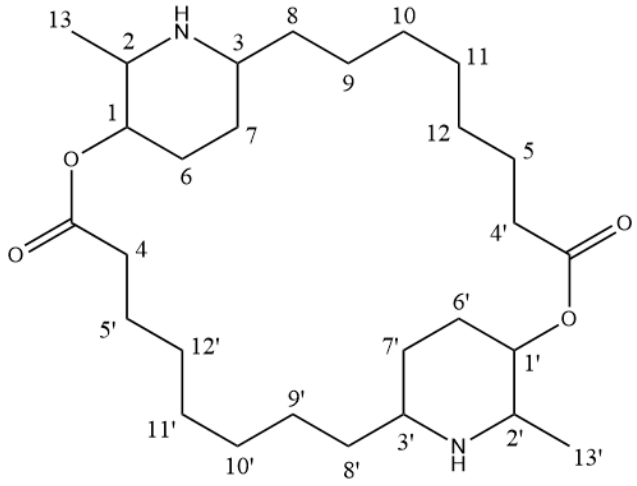

Figure 10. Structure of carpaine

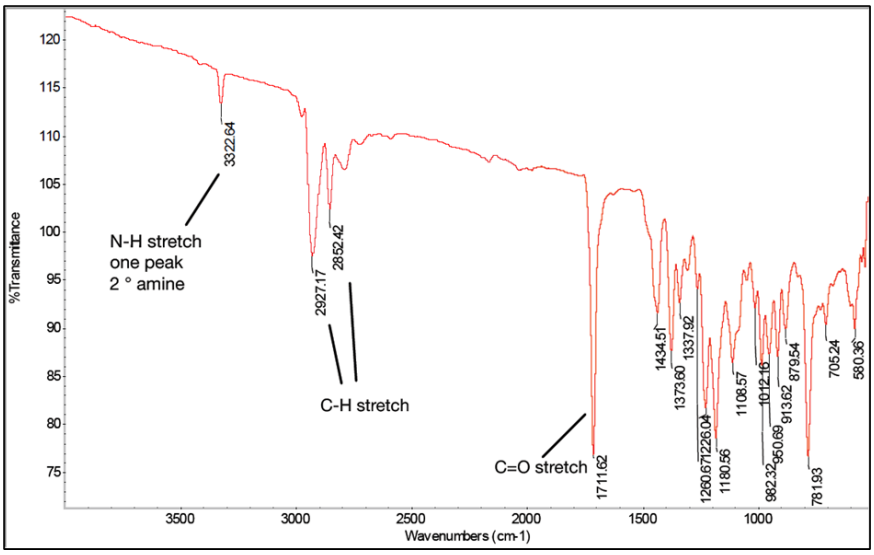

Figure 11. FTIR spectrum of carpaine.

CONCLUSION

In this study, the extraction of powdered *Carica papaya* leaves using acidified water with heat was found to be more effective in extracting alkaloid carpaine compared to alcohol extraction. The percentage yield of carpaine from acidified water extraction was 12.01%, while the yield from alcohol extraction was 2.18%. Acidified water extraction process protonates carpaine to its salt form, enhancing its solubility in water and thereby improving the overall carpaine yield. Heat and stirring further enhance the extraction process. In contrast, although ethanol can extract carpaine in both its free base and salt form, subsequent purification steps using non-polar petroleum ether that aimed to remove non-polar impurities may have removed the free base form of carpaine, resulting in a lower yield. The structure of the isolated carpaine was confirmed through NMR, FTIR and melting point analysis, and the results were consistent with the literature. An efficient extraction method is crucial for isolating substantial amounts of carpaine for the pharmaceutical industry, due to its promising anti-thrombocytopenic properties as well as its potential applications as an anti-malaria and anti-dengue drug. However, this study did not optimize the extraction parameters for acidified

water extraction, indicating the need for further research in this area.

For future studies, it is advised to explore greener extraction methods, such as acidified water extraction, which uses less solvent and is less toxic to the environment compared to alcohol extraction. Furthermore, using an advanced method for carpaine isolation, such as preparative High-Performance Liquid Chromatography (prep-HPLC), would help to achieve higher purity. To ensure the highest possible quality of isolated carpaine, the purity can be measured using analytical HPLC, which provides accurate measurements of compound purity.

ACKNOWLEDGEMENTS

The authors wish to thank Centre for Pre-University Studies and Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, for the assistance and support provided during the research. This study was made possible through PILOT Research Grant Scheme provided by Universiti Malaysia Sarawak (Grant number: UNI/C09/PILOT/85592/2023).

CONFLICT OF INTEREST

The authors have declared that no conflict of interest exists.

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