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Bioactivity and metabolite profile of papaya (*Carica papaya*) seed extract

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Abstract. *Alfarabi M, Siagian FE, Cing JM, Suryowati T, Turhadi, Suyono MS, Febriyamti MS, Naibaho FB. 2022. Bioactivity and metabolite profile of papaya (*Carica papaya*) seed extract. Biodiversitas 23: 4589-4600.* Papaya (*Carica papaya*) seeds are part of the papaya plant which is a source of organic waste. However, there are many scientific studies that state the metabolites found in papaya seeds have various benefits in medical aspects. Accordingly, the objective of this study was to examine the antioxidant and cytotoxic activity of California and Bangkok type papaya seeds which are two local papaya types and widely consumed by the people of Indonesia. These papaya seeds' metabolite profiling was also conducted in this study therefore the antioxidant activity and cytotoxic mechanisms can be estimated. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) method is used to investigate the antioxidant activity while 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) is used to investigate the cytotoxic effect of extract. Metabolites contained in papaya seeds were measured by Gas Chromatography-Mass Spectrometry (GC-MS) and Liquid Chromatography-Mass Spectrometry-QTOF (LC-MS/MS-QTOF). The results showed that California and Bangkok papaya seed extracts had antioxidant activity with IC_{50} being 24.4 ppm and 22.2 ppm, respectively. Both extracts were also able to inhibit the growth of cancer cells (MCF-7) and did not render any toxic effects on non-cancer cells at low concentrations. The metabolites contained in the two extracts were alkaloid, phenol hydrocarbon, flavonoid, fatty acid, and terpenoid groups. This study showed that papaya seeds have the potential to be further developed in medical field, especially as natural antioxidants and natural cancer-preventing ingredients.

Keywords: Antioxidant, cancer, *Carica papaya,* free radical, phytochemical

INTRODUCTION

Organic waste is one of the problems faced by many countries in the world today. Not only in developing countries, developed countries such as in Europe also experience the same thing. Especially organic waste from the food industry and agro-industry related to food crops are the largest contributor to the production of organic waste on this earth (FAO 2019). Flowers, leaves, stems, roots, fruit peels, seeds, and food leftovers can become wastes in industry or households food processing chain (Stenmarck et al. 2016). One example is papaya (*Carica papaya*) based food processing in Indonesia. Generally, papaya has some edible parts, such as flowers, leaves, and fruits (Cahyaningsih et al. 2022), whereas the inedible parts that can not be reused are stems, aged leaves, fruit peels, and seeds which eventually may become organic waste. Only a few Indonesian people reuse papaya seeds to be replanted.

Papaya is widely grown in tropical climates including Indonesia and is a popular plant because of its good nutritional content, relatively low price, ease of growing, and is an all seasonal plant (Bakar and Ratnawati 2017). This member of Caricaceae family originated from Central America and spread to Asia (Pesqueira and Farfan 2017). In Indonesia, papaya is consumed either as fresh fruit or processed dish (flowers, leaves, and young fruit). Not only beneficial for humans, papaya leaves can also be used as an additional feed mix to support the growth and development of livestock (Hamid et al. 2022).

There are two very well-known papaya types by Indonesian people, namely Bangkok and California. These two naming are commonly used in traditional and modern Indonesian markets. Bangkok type has large fruit with a sweet taste characteristic (Nisa et al. 2019), meanwhile the California type has a medium-sized with a sweeter taste compared to Bangkok. In fact, California type is a Calina variety papaya that comes from a plant breeding process (Ismaya et al. 2019). Papaya fruit contains quite some macro- and micro minerals (P, K, Ca, Mg, Na, Fe, Mn, Zn, Cu, and B). Further, orange color of papaya fruit indicates high carotenoid contents (Moses and Olanrewaju 2018; Laurora et al. 2021). Carotenoid is a secondary metabolite found in plants that belong to terpenoid group (Martins et al. 2016). In addition, the other parts of the papaya plant contain alkaloids, flavonoids, phytosterols, and tocopherols. Moreover, these metabolites are strongly associated with the activity of antioxidants,

antihyperglycemia, anti-inflammatory, anti-hypertensive, and anti-cancer (Dotto and Abihudi 2021).

Since papaya fruit is the most widely consumed part, organic waste production is more likely to come from unutilized papaya seeds. Based on several studies showed that papaya seed extract is effective in preventing skin inflammation caused by bacteria and parasite growth, and together with other ingredients such as chitosan may provide a better effect as an antibacterial compound (Gnanamangai et al. 2022). Alkaloid extracts from papaya seeds can be used as therapeutic agents for hepatocellular carcinoma by the mechanism of increasing the histoarchitecture of liver tissue (Barffour et al. 2021). The other property which is widely studied of papaya seeds is anti-cancer activity. In addition, Papaya seeds have an anti-cancer mechanism by increasing the regulation of the p53 and Caspase-3 genes that leads to cancer cells apoptosis induction (Anilkumar and A 2022). Accordingly, the utilization of papaya seeds especially for pharmacological purposes such as anti-bacterial, anti-parasitic, and anti-cancer should be done.

It is known that cancer patients in Indonesia are increasing annually. Breast cancer is the most common cancer suffered by the Indonesian with the number of patients as many as 65.000 cases in 2020 (Ferlay et al. 2020). Many factors can cause cancer, one of which is the high level of free radicals in the cells that might interfere metabolic processes (Alzahrani 2021). In regard to it, many studies associated with natural resources are directed to seek its antioxidant properties and cancer medicine alternatives, one of which is papaya seeds. The papaya seeds pharmacology study development has a good potential to thrive in Indonesia due to several reasons, namely its abundance and variety in the area around the community. These papaya varieties, for example Bangkok and California types, can be compared for benefits thereof. Moreover, there have not been much use of papaya seeds in the medical field, especially as an alternative cancer medicine for both papaya types.

Accordingly, the purpose of this study was to measure and compare the in vitro antioxidant, toxicity effect, and anti-cancer activity of Bangkok and California papaya seed extract. This research can increase the usefulness of papaya seeds in various fields, especially in the medical field.

MATERIALS AND METHODS

Extraction of plant materials

The papaya seeds (California and Bangkok type) were collected from fresh papaya fruit from Bogor, West Java, Indonesia (6°35′48″S 106°47′50″E). Maceration technique was used for the extraction (Alfarabi et al. 2022). The seeds from each papaya type were mashed and dissolved with 90% ethanol. Then, the mixture was filtered (Whatman filter) after soaked at room temperature for 72 h. The filtrate was evaporated at 60°C with a rotary evaporator (Buchi R-100). The result was papaya California seed extract and papaya Bangkok seed extract (hereafter, called CE and BE, respectively).

Antioxidant assay

This method refers to Yuningtyas et al. (2021) with minor modification. The free radical used in this method was DPPH (2,2-diphenyl-1-picrylhydrazyl) (Sigma). The samples with several concentrations (50, 100, 150, and 200 ppm) were mixed with 0.1 mM DPPH. These solutions were incubated at room temperature for 30 minutes. The absorbance of solutions was measured using spectrophotometer (DLAB SP-UV1100) at wavelength of 517 nm. The DPPH without sample was used as the negative control, while ascorbic acid (Sigma) as the positive control. The percentage of DPPH reduction was calculated as follows:

$$\%\ Inhibition: \frac{absrobance\ negative\ control - absorbance\ samples}{absorbance\ negative\ control} \times 100\%$$

Toxicity assay

Brine shrimp lethality test (BSLT) method was used to measure the toxicity effect of the samples. This method refers to Alfarabi and Widyadhari (2018) with minor modification. The artificial seawater (20 g sea salt in 800 ml aquadest) was used for larva (*Artemia* sp.) hatching media. After 48 h of hatching, the shrimp larvae were used in this assay. Each sample from different concentrations (75, 100, 150, and 200 ppm) was mixed with the shrimp larvae (10 larvae in each test tube) and incubated for 24 hours (under light and room temperature). Larvae in medium without samples were used as control. The percentage of dead shrimp larvae was observed and analyzed for the LC_{50} value.

Cytotoxicity assay

The 3- (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used for cytotoxicity assay. We used MCF-7 cell line for cancer cells (breast cancer) and the non-cancer cells was Chinese Hamster Ovary (CHO) cell line. The cells were obtained from Cell Culture Laboratory, Research Center of Raw Materials for Drugs and Traditional Medicine-LAPTIAB, National Research and Innovation Agency (BRIN), Puspiptek Serpong, South Tangerang, Banten, Indonesia. The cells were grown in the density 5 x 10^3 cells/well. Each sample with several concentrations (15.63; 31.25; 62.5; 125; and 250 ppm in dimethyl sulfoxide (DMSO)) was mixed on each cell culture and incubated (5% CO_2) at 37°C for 24 h. The negative control was a cell culture without samples. Furthermore, the cell culture was added 100 µL MTT (0.5 mg/mL) and incubated (5% CO_2) for 4 h before the reaction was stopped with 10% sodium dodecyl sulfate (SDS). The reduction of MTT to formazan crystal (purple) by metabolically active cells was measured using spectrophotometer (BioTek ELX800) at wavelength of 570 nm. The percentage inhibition of cell proliferation was calculated as follows:

$$\%\ Inhibition: \frac{absorbance\ negative\ control - absorbance\ samples}{absorbance\ negative\ control} \times 100$$

Metabolite identification

The metabolite composition of CE and BE were determined using Gas Chromatography-Mass Spectrometry (GC-MS) (Agilent Technologies model 7890) and Liquid Chromatography-Mass Spectrometry-QTOF (LC-MS/MS-QTOF) (Waters, United States of America). For GC-MS analysis, five µL samples were injected and the column was HP-1. The eluent was Helium (He) with flow rate of 1.2 mL/min. The temperature of the injector was 250°C, ion source was 230°C, interface was 280°C, and quadrupole was 140°C. The mass spectrum was detected in the range of 20-500 m/z. The Wiley W8N08.L database was used for metabolite identification. The used sample data had quality ≥90 % based on database.

For LC-MS/MS-QTOF analysis, the operating mode was Tof MS^E. The ionization mode was ESI (-)/ ESI (+) with acquisition range of 50-1200 Da. Ten µL samples were injected and the column was C18 at 40°C. The eluent was 0,1% formic acid in acetonitrile and 0,1% formic acid in aquabidest. The flow rate was 0,6 mL/min with gradient. Metabolite identification was performed using UNIFI software which includes a mass spectrum library from Waters database. The metabolites detection parameter used were mass error read ≤5 ppm, isotope match MZ RMS ≤6, and metabolite intensity ≥300.

RESULTS AND DISCUSSION

Antioxidant activity of papaya seed extracts

The results showed that CE and BE had antioxidant activity with various inhibitory percentages against free radicals at each sample concentration. This can be seen from the increasing inhibitory of the samples against DPPH as a free radical. Therefore, these samples extract can be role as a radical scavenger. The highest inhibitory against free radicals was found in the highest concentration of CE and BE samples, which was at 200 ppm wherein the inhibitory were 67.31% and 74.50%, respectively. Meanwhile, the lowest inhibitory of both CE and BE samples was at 10 ppm (the lowest concentration in the test) of 48.71% and 46.20%. Although each papaya seed extract uses the same concentration, the results of the T-test show antioxidant activity as inhibitory against free radicals between samples with the same concentration have statistically significantly different. Only at 10 ppm the inhibitory between CE and BE did not differ markedly, while other concentrations (50, 100, 150, and 200 ppm) had a noticeable difference (Figure 1). Based on this, the value of antioxidant activity between CE and BE were different. The IC_{50} value of CE was 24.4 ppm, while BE was 22.2 ppm. The value showed that CE can already inhibit free radicals by 50% in experiments with an extract concentration of 24.4 ppm. Meanwhile, BE was able to inhibit free radicals by 50% at a concentration of 22.2 ppm. When compared with the ascorbic acid IC_{50} value, which was 6.73 ppm, free radicals inhibitory of the samples are about 1/4 time the ascorbic acid free radicals inhibitory.

The stability of DPPH as a purple free radical at room temperature makes the molecule easy to be used as a test material in antioxidant analysis. The color of DPPH changes to yellow after receiving electrons from antioxidant molecules thus change DPPH molecules into stable molecules (Celiz et al. 2020). In addition, the DPPH method is a procedurally simple method to analyze antioxidants compared to the other methods, such as, e.g. the ferric reducing antioxidant power (FRAP) method. The sample IC_{50} value was greater than the IC_{50} value of ascorbic acid. For this result, we suggested a crude extract that still has any metabolites mixtures. Therefore, the intra metabolites interaction does not fully support the occurrence of antioxidant activity. There could be metabolites interactions that lead to other bioactivities and needs to be studied further. The sample IC_{50} value in this study was lower than the water extract of papaya seed from Nubaria, Behera, Egypt with an IC_{50} value of 907 ppm (Shaban et al. 2021). This can occur due to differences in solvents at the time the extraction is performed. Each solvent for metabolite extraction has different characteristics. Especially in the process of extraction from natural materials, metabolites contained in plants can be grouped, generally, into metabolites that are polar and non-polar in nature. Polar solvents are used to extract metabolites that are polar in nature and non-polar solvents are used to extract metabolites that are non-polar in nature (Alfarabi et al. 2022). The inhibitory range against free radicals from the two samples in this study (48-67% and 46-74.5% for CE and BE, respectively) was almost the same as the inhibitory range of papaya flower free radicals from Amarkantak, India by 7.9-64.07% (Dwivedi et al. 2020). These results suggested that the various organs of the papaya plant can be a potential natural source of antioxidants.

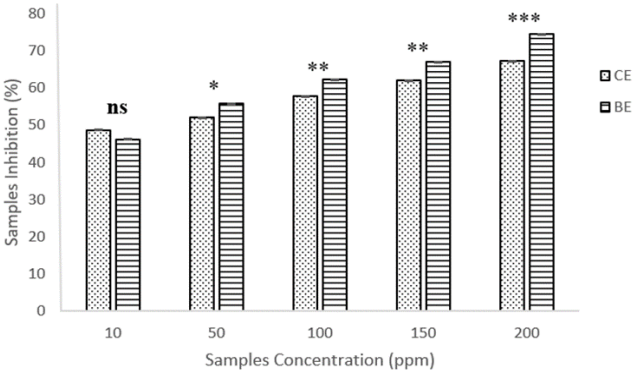

Figure 1. Antioxidant activity of papaya seed extract at different concentrations. CE: papaya California seed extract, BE: papaya Bangkok seed extract, ns: not significant, *p: 0.05; **p<0.01; ***p<0.001

Toxicity effect of papaya seed extracts

Brine shrimp lethality test (BSLT) was used to calculate the toxicity effect. The results of the toxicity test showed that both samples had a toxic effect against *Artemia* larvae. Our study showed that, the increases in the concentration of the extract were proportional to the number of larval death percentages. One hundred percent of larval mortality occurred at the highest concentration of 200 ppm. While at the smallest concentration (75 ppm), the number of larval mortalities was about 13-20% (Figure 2). In this assay, it can be confirmed that larval mortality was caused by the interaction of the larva with the sample. It is shown in the control solution that there was no larvae mortality. The LC_{50} value from CE was 184 ppm, meanwhile BE was 149 ppm. Accordingly, both samples can kill larvae by 50% in the range of 149-184 ppm.

Brine shrimp lethality test is a method at the stage of pre-elimination studies of a natural ingredients extract to determine its bioactivity and is also recommended to be conducted before the analysis enters the cytotoxicity testing stage (Laila et al. 2020). Udavant et al. (2012) reported that this method can be used to see the potential cytotoxic activity of natural ingredient extracts before in vitro cytotoxicity testing is carried out. This method is rapid and reliable for general bioassay tool. Our BLST assay showed that the lower of LC_{50} value from an extract of natural ingredients, the higher the toxicity effect of the sample. A natural ingredient extract showed to have a toxic effect when the LC_{50} value was below 1000 ppm, if the concentration is above that value, it can be assumed that the extract did not have a toxic effect (Meyer et al. 1982). The two samples in this study had LC_{50} value far below 1000 ppm so it can be said that CE and BE samples have toxic effects. The LC_{50} value of the CE samples in this study was more toxic when compared to the water extract of California papaya seed that originating from Jakarta, Indonesia which had an LC_{50} value of 302 ppm (Alfarabi and Fauziayuningtias 2017). Indeed, the solvent used in the extraction process greatly affects the content of metabolites in the extract so the use of different extraction solvents results in different toxic effects between samples.

Cytotoxic activity of papaya seed extracts

In cytotoxic assay, two types of cells were used, MCF cell line (cancer cells) and CHO cell line (normal cells). The use of the two cell types aimed to see the activity of both extracts, which are able to inhibit the proliferation of cancer cells and not inhibit the proliferation of normal cells. The results of the analysis showed that CE and BE were able to inhibit proliferation of cancer cells at each concentration. The inhibitory of cancer cells from CE was 12-20% and BE was able to inhibit by 8-13%. The resistance that occurs at each concentration of both samples did not increase by the sample concentration increment. We assumed that there were interactions between metabolites in the extract and cancer cells that were not yet known. The greatest inhibitory occurred at the highest concentration (250 ppm) of the two samples, which was 20% for CE and 13% for BE (Figure 3A).

The assay in normal cells, the highest inhibitory occurred at the highest concentration (250 ppm), which was 25% for CE and 29% for BE. Whereas at the smallest concentration (15.63 ppm), CE showed inhibit cells by 0.2%, meanwhile BE showed induce the cell growth by 3.6% compared to control (Figure 3B). This result can be assumed that at low concentrations, both extracts did not inhibit normal cell proliferation. The ability of both extracts to inhibit the cancer cell proliferation from the lowest to the highest concentrations but not inhibit normal cells at the lowest concentrations indicated that these extracts have the potential to be developed into one of the cancer alternative medicines treatments.

The difference in interaction response between cells and extracts which occurred in this study showed a structural difference between cancer cells and normal cells. This was especially seen in the cell membrane which is the most affected part of the cell when there is an interaction between the cell and metabolites from inside or outside the cell. The lipid composition in the membrane of the normal cell is different from of the cancer cell. This is one of the studies that focus on cancer drug development today in order to produce drugs that are more sensitive and selective to cancer cell membranes (Preta 2020). Cancer cells make numerous changes to the cells metabolic processes thus they can adapt to fight the body's immune system. These metabolic changes can lead to structural changes in the cell part, especially the cell membranes (Szlasa et al. 2020). Many molecular mechanisms have been hypothesized to explain the antiproliferative activity of papaya seeds and other parts against cancer cells. One of them is due to their antioxidant activity therefore the cellular oxidative stress levels can be decreased. This reduction can be one way of preventing the formation of cancer cells and additionally, increase the immune system, such as, e.g. maintaining mitochondria viability thus the cells will remain responsive to apoptosis, to eliminate cancer cells in tissue (Somanah et al. 2017).

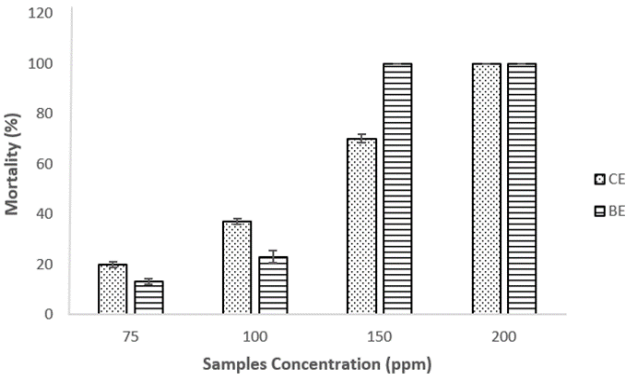

Figure 2. Toxicity effect of papaya seed extract at different concentrations. CE: papaya California seed extract, BE: papaya Bangkok seed extract

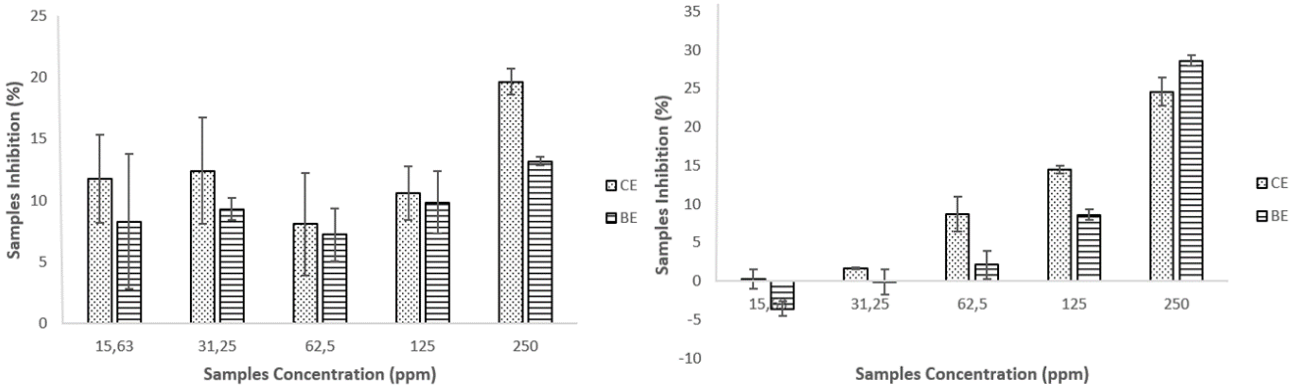

Figure 3. Cytotoxic activity of papaya seed extract at different concentrations against MCF-7 cell (A) and CHO cell (B). CE: papaya California seed extract, BE: papaya Bangkok seed extract

Metabolite profiles of papaya seed extracts

Metabolite profiling using GC-MS showed that there were 13 metabolites identified. This approach is categorized as untargeted metabolomic for initial identification of metabolite profile in both samples. From all identified metabolites, there were 4 metabolites from the sterol group, 4 metabolites from the fatty acid group, 2 metabolites from the triterpenoid group, 2 metabolites from the hydrocarbon group, and 1 metabolite from the lipid derivatives group (Figure 4A). Of the total 13 metabolites, 3 metabolites were identified only in CE, and 2 metabolites were identified only in BE, and 8 metabolites were identified in both extracts (there were 11 and 10 metabolites identified for CE and BE, respectively) (Figure 4B). The percentage of each metabolite in the CE during the analysis was about 1-14.6%, meanwhile in the BE it was about 1-22.6%. The largest percentage of metabolites identified in the CE and BE were β-sitosterol (14.63%) and oleic acid (22.67%), respectively (Table 1, Figure S1-S2, Table S1-S2).

Table 1. Identified metabolites in CE and BE extracts using GC-MS analysis

Metabolite	Group	Metabolite content (%)	
		CE	BE
Isothiocyanate	Hydrocarbon	12.92	2.86
Palmitic acid	Fatty acid	3.8	13.83
Linoleic acid	Fatty acid	4.95	3.17
Oleic acid	Fatty acid	13.32	22.67
Stearic acid	Fatty acid	1.6	nd
Squalene	Triterpenoid	1.14	1.32
Campesterol	Sterol	2.24	4.66
β-sitosterol	Sterol	14.63	19.63
Cycloartenol	Triterpenoid	2.77	nd
Stigmasterol	Sterol	9.75	6.14
Fucosterol	Sterol	1.22	nd
Octadecadienal	Hydrocarbon	nd	1.22
Pregnanediol	Lipid derivatives	nd	1.53

Note: CE: papaya California seed extract, BE: papaya Bangkok seed extract, nd: not detected

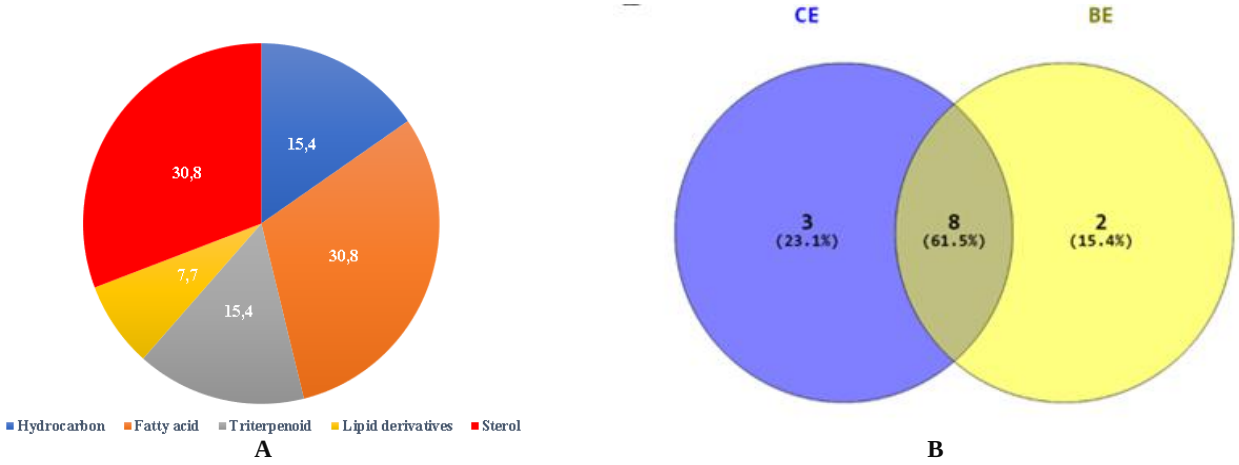

Figure 4. A. The number of metabolite groups detected in the CE extract and BE extract. B. Distribution of metabolites in the CE extract and BE extract using GC-MS analysis. CE: papaya California seed extract, BE: papaya Bangkok seed extract

Metabolite profiling using LC-MS/MS-QTOF in this study was also categorized as untargeted metabolomic to investigate the metabolite profile in our samples. Our study showed that there were 30 metabolites were identified. Overall, the metabolites identified by LC-MS/MS-QTOF showed there were 13 metabolites of alkaloids, 5 metabolites of flavonoids, 5 metabolites of hydrocarbons, 5 metabolites of phenols, and 2 metabolites of purines (Figure 5A). There were 9 metabolites identified only in CE, 11 metabolites identified only in BE, and 10 metabolites detected in both extracts. Therefore, the total metabolites detected in CE were 19 metabolites and in BE were 21 metabolites (Figure 5B). The percentage of metabolites detected at the time the analysis was conducted on CE ranged from 0.5-42.9%, with the largest percentage was norcimifugin. As in CE, the metabolite with the largest percentage at BE was norcimifugin at the time the analysis was conducted was in the range of 0.3-30.6% (Table 2, Figure S3-S6, Table S3-S6).

hromatography-mass spectrometry method is a common method for metabolites detection in natural product. Gas chromatography-mass spectrometry (GC-MS) is a separation technique for volatile and semi volatile organic molecules. This technique can be used in various fields to detect molecules such as hydrocarbons, aromatics, fatty acids and others. This technique is commonly used for molecular profiling and quality control such as food safety assay and contaminant analysis (Lisec et al. 2006). In the other method, liquid chromatography-mass spectrometry (LC-MS) is a separation technique that can be used to separate non-volatile molecules and large-sized molecules such as peptides. This technique can be applied the same manner as in the GC-MS technique, the difference is in the type of sample to be analyzed (Holcapek et al. 2012). The metabolites detected in both samples were categorized as secondary metabolites in plants (Table 1 and Table 2). Alkaloids, flavonoids, terpenoids have been scientifically proven as antioxidant molecules of plant origin and also have other bioactivity such as antibacterial and cytotoxic. These metabolites can be derived from the plant's leaves, stems, and roots (Manivannan and Johnson 2020; Larayetan et al. 2021).

Table 2. Identified metabolites in CE and BE extracts using LC-MS/MS-QTOF analysis

Metabolite	Group	Metabolite content (%)	
		CE	BE
Trigonelline	Alkaloid	17.52	0.82
Gentiatibetine	Alkaloid	nd	10.72
Norcimifugin	Flavonoid	42.9	30.60
3,5,7-Trihydroxychromone	Hydrocarbon	0.62	0.72
5-Hydroxytryptamine	Alkaloid	nd	2.27
3,7-Dihydroxy-3-(3′,4′dihydro-xybenzyl)-chroman-4-one	Hydrocarbon	nd	3.0
Methyl ophiopogonone B	Flavonoid	nd	0.47
1,2,3,5-Tetramethoxyxanthone	Phenol	0.50	4.34
1-Carbomethoxy-β-carboline	Alkaloid	nd	1.25
2′,4′-Dihydroxy-4,6′-dimethoxy-dihydrochalcone	Phenol	0.63	2.52
Lysergol	Alkaloid	nd	0.58
β-Carboline-1-propionic acid	Hydrocarbon	1.12	0.82
Carpaine	Alkaloid	nd	11.59
Bavachin	Flavonoid	nd	0.42
Peimisine	Alkaloid	5.66	13.19
6-Methoxy-2-[2-(4'-metho-xyphenyl) ethyl] chromone	Hydrocarbon	5.28	1.54
Nortropanoline	Alkaloid	nd	3.4
1-Galloyl-glucose	Hydrocarbon	2.27	9.27
Gentianal	Alkaloid	5.36	1.48
Cimifugin	Phenol	nd	0.3
Mangiferin	Phenol	nd	0.3
Candicine	Alkaloid	0.85	nd
Codonopsine	Alkaloid	0.66	nd
Kushenol	Phenol	3.6	nd
Dicentrine	Alkaloid	0.66	nd
Gentianine	Alkaloid	4.1	nd
Eupatolitin	Flavonoid	0.84	nd
Adenine	Purine	2.32	nd
Adenosine	Purine	3.9	nd
Epimedin	Flavonoid	1	nd

Note: CE: papaya California seed extract, BE: papaya Bangkok seed extract, nd: not detected

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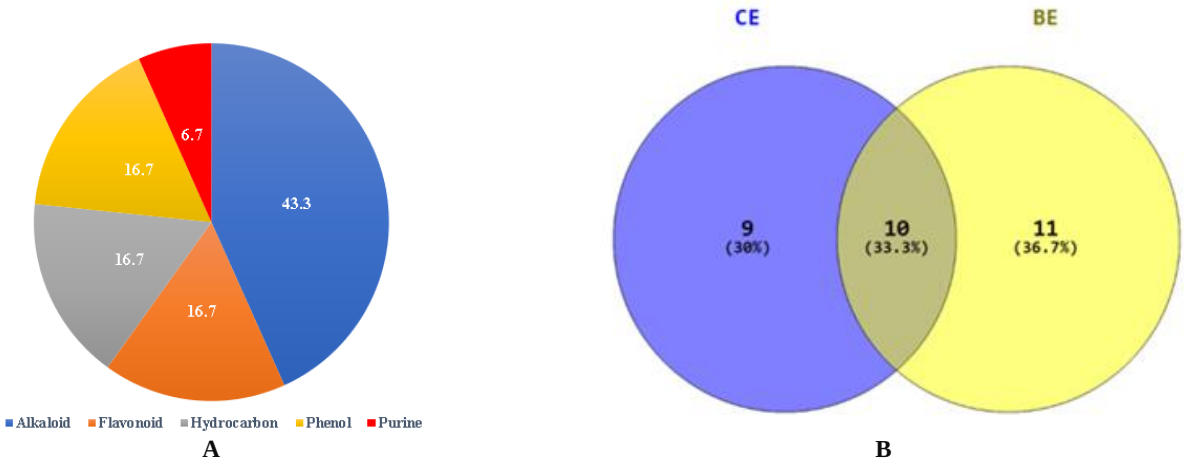

Figure 5. A. The number of metabolite groups detected in the CE extract and BE extract. B. Distribution of metabolites in the CE extract and BE extract using LC-MS/MS-QTOF analysis. CE: papaya California seed extract, BE: papaya Bangkok seed extract

Other studies that used GC-MS to detect metabolite content of papaya seeds from papaya grown in different areas showed similar metabolite composition to our study, namely fatty acid and phenolic hydrocarbon groups. The fatty acid and phenolic hydrocarbon groups have antioxidant activity as radical scavengers and inhibit the formation of lipid peroxide. In addition, it can also be an anti-hyperglycemia (inhibitor of α-amylase and α-glucosidase) (Agada et al. 2021). Likewise, to other studies that identify the metabolite content of papaya seeds using LC-MS, the metabolites contained in papaya seeds were alkaloid groups, phenolic hydrocarbon, and terpenoids (Table 2). The detected terpenoid group is a derivative group of carotenoids (Khan et al. 2022). Papaya seeds also contain many organic acids and other phenolic compounds that were not detected in this study, such as lactic acid, citric acid, malic acid, succinic acid, propionic acid, coumaric acid, and vanillic acid. Although it has bitter taste, papaya seed contains essential amino acids like leucine, valine, phenylalanine, histidine, and tryptophan. It showed that papaya seed contained good nutrition for health (Gogna et al. 2015). Therefore, we assumed that the antioxidant activity, toxic effects, and cytotoxic activity detected in this study were the result of the metabolite interaction found in both extracts.

In conclusion, this study showed that California and Bangkok papaya seeds have good potential to be antioxidants and anticancer drugs alternative sources. The content of detected metabolites such as phenols, flavonoids, terpenoids, alkaloids, and fatty acids has good interaction to indicate antioxidant activity and provide cytotoxicity effects on cancer cells in in-vitro conditions. However, the results of this study need to be supported by further in-vivo assay.

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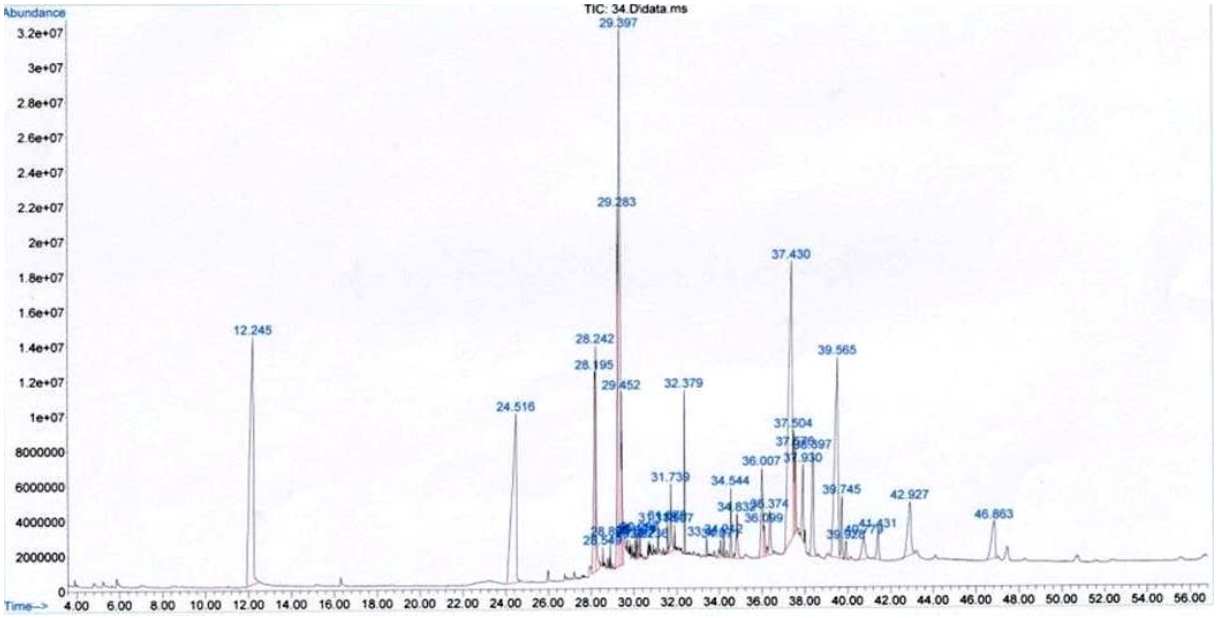

Figure S1. Chromatogram of *Carica papaya* seed (California Extract) in GC-MS analysis. The numbers indicate the retention time of metabolite peak

Table S1. Papaya seed metabolites (California Extract) detected in GC-MS analysis

Metabolite	Group	Metabolite content (%)	Retention time (minute)	Molecular Weight (g/mol)*
Isothiocyanate	Hydrocarbon	12.92	12.24	181
Palmitic acid	Fatty acid	3.8	28.19	256.4
Linoleic acid	Fatty acid	4.95	29.28	280.4
Oleic acid	Fatty acid	13.32	29.39	282.5
Stearic acid	Fatty acid	1.6	29.45	284.5
Squalene	Triterpenoid	1.14	32.38	410.7
Campesterol	Sterol	2.24	36.00	400.7
β-sitosterol	Sterol	14.63	37.42	414.7
Cycloartenol	Triterpenoid	2.77	38.39	426.7
Stigmasterol	Sterol	9.75	39.56	412.7
Fucosterol	Sterol	1.22	39.74	412.7

Note: *PubChem: https://pubchem.ncbi.nlm.nih.gov/

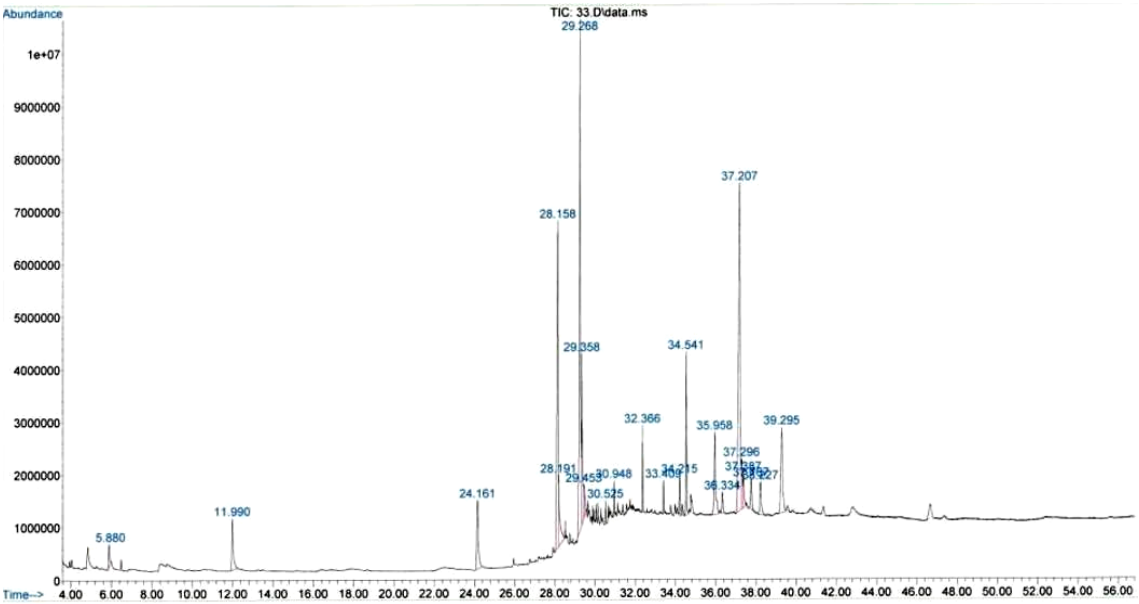

Figure S2. Chromatogram of *Carica papaya* seed (Bangkok Extract) in GC-MS analysis. The numbers indicate the retention time of metabolite peak

Table S2. Papaya seed metabolites (Bangkok Extract) detected in GC-MS analysis

Metabolite	Group	Metabolite content (%)	Retention time (minute)	Molecular Weight (g/mol)*
Isothiocyanate	Hydrocarbon	2.86	11.99	181
Palmitic acid	Fatty acid	13.83	28.15	256.4
Linoleic acid	Fatty acid	3.17	28.18	280.4
Oleic acid	Fatty acid	22.67	29.26	282.5
Octadecadienal	Hydrocarbon	1.22	29.45	264.4
Squalene	Triterpenoid	1.32	32.36	410.7
Campesterol	Sterol	4.66	35.96	400.7
β-sitosterol	Sterol	19.63	37.20	414.7
Pregnanediol	Lipid derivatives	1.53	37.38	320.5
Stigmasterol	Sterol	6.14	39.29	412.7

*PubChem: https://pubchem.ncbi.nlm.nih.gov/

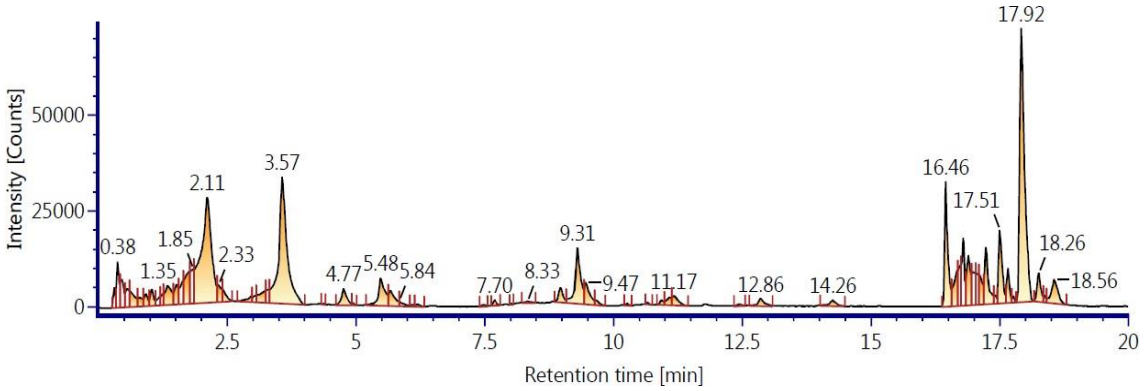

Figure S3. Chromatogram of *Carica papaya* seed (California Extract) in LC-MS/MS-QTOF (ESI +) analysis. The numbers indicate the retention time of metabolite peak

Table S3. Papaya seed metabolites (California Extract) detected in LC-MS/MS-QTOF (ESI +) analysis

Metabolite	Group	Metabolite content (%)	Retention time (minute)	Molecular weight (g/mol)*
Trigonelline	Alkaloid	17.52	0.41	137.14
Adenine	Purine	2.32	0.48	135.13
Adenosine	Purine	3.9	1.62	267.24
Norcimifugin	Flavonoid	42.9	2.33	292.28
Candicine	Alkaloid	0.85	2.84	180.27
Epimedin	Flavonoid	1.00	3.15	808.8
3,5,7-Trihydroxychromone	Hydrocarbon	0.62	3.54	194.14
Codonopsine	Alkaloid	0.66	5.32	267.32
1,2,3,5-Tetramethoxyxanthone	Phenol	0.50	8.35	364.3
Eupatolitin	Flavonoid	0.84	8.82	346.3
Kushenol	Phenol	3.6	10.27	452.5
β-Carboline-1-propionic acid	Hydrocarbon	1.12	12.25	270.28
Peimisine	Alkaloid	5.66	16.48	427.6
2′,4′-Dihydroxy-4,6′-dimethoxy-dihydrochalcone	Phenol	0.63	16.55	302.32
6-Methoxy-2-[2- (4'-methoxyphenyl) ethyl] chromone	Hydrocarbon	5.28	16.59	326.3

Note: *PubChem: https://pubchem.ncbi.nlm.nih.gov/

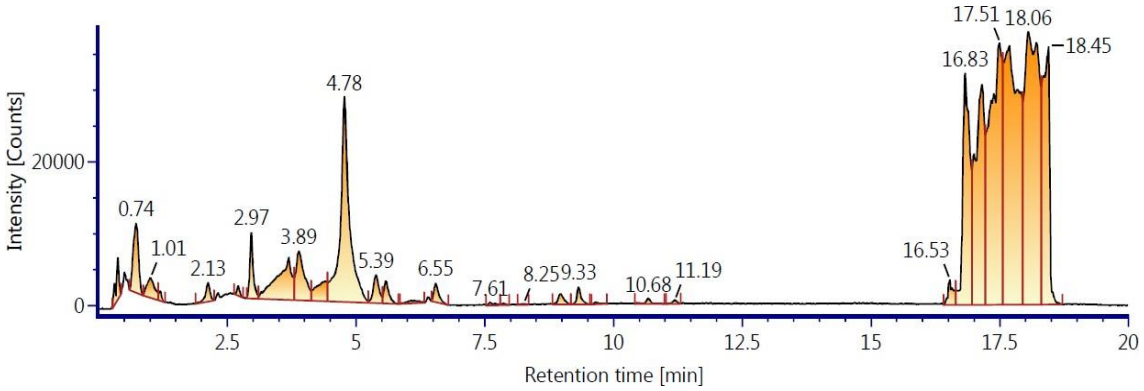

Figure S4. Chromatogram of *Carica papaya* seed (California Extract) in LC-MS/MS-QTOF (ESI -) analysis. The numbers indicate the retention time of metabolite peak

Table S4. Papaya seed metabolites (California Extract) detected in LC-MS/MS-QTOF (ESI -) analysis

Metabolite	Group	Metabolite content (%)	Retention time	Molecular weight (g/mol)*
1-Galloyl-glucose	Hydrocarbon	2.27	3.58	332.26
Gentianal	Alkaloid	5.36	4.78	193.2
Gentianine	Alkaloid	4.1	8.98	175.18
Dicentrine	Alkaloid	0.66	9.33	339.4

Note: *PubChem: https://pubchem.ncbi.nlm.nih.gov/

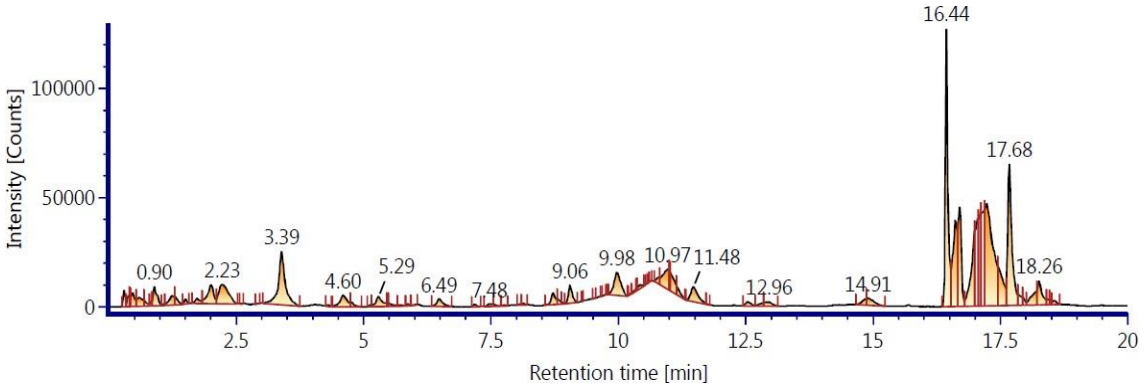

Figure S5. Chromatogram of *Carica papaya* seed (Bangkok Extract) in LC-MS/MS-QTOF (ESI +) analysis. The numbers indicate the retention time of metabolite peak

Table S5. Papaya seed metabolites (Bangkok Extract) detected in LC-MS/MS-QTOF (ESI +) analysis

Metabolite	Group	Metabolite content (%)	Retention time	Molecular weight (g/mol)*
Trigonelline	Alkaloid	0.82	0.43	137.14
Gentiatibetine	Alkaloid	10.72	2.01	165.19
Norcimifugin	Flavonoid	30.60	2.23	292.28
3,5,7-Trihydroxychromone	Hydrocarbon	0.72	3.38	194.14
5-Hydroxytryptamine	Alkaloid	2.27	4.31	176.21
3,7-Dihydroxy-3- (3′,4′-dihydroxybenzyl)-chroman-4- one	Hydrocarbon	3.0	5.88	148.16
Methyl ophiopogonone B	Flavonoid	0.47	8.07	354.3
1,2,3,5-Tetramethoxyxanthone	Phenol	4.34	8.92	364.3
1-Carbomethoxy-β-carboline	Alkaloid	1.25	9.11	240.26
2′,4′-Dihydroxy-4,6′-dimethoxy-dihydrochalcone	Phenol	2.52	10.19	302.32
Lysergol	Alkaloid	0.58	10.77	254.33
β-Carboline-1-propionic acid	Hydrocarbon	0.82	10.89	355.4
Carpaine	Alkaloid	11.59	12.93	478.7
Bavachin	Flavonoid	0.42	14.27	324.4
Peimisine	Alkaloid	13.19	16.47	427.6
6-Methoxy-2-[2- (4'- methoxyphenyl) ethyl] chromone	Hydrocarbon	1.54	16.56	310.3

Note: *PubChem: https://pubchem.ncbi.nlm.nih.gov/

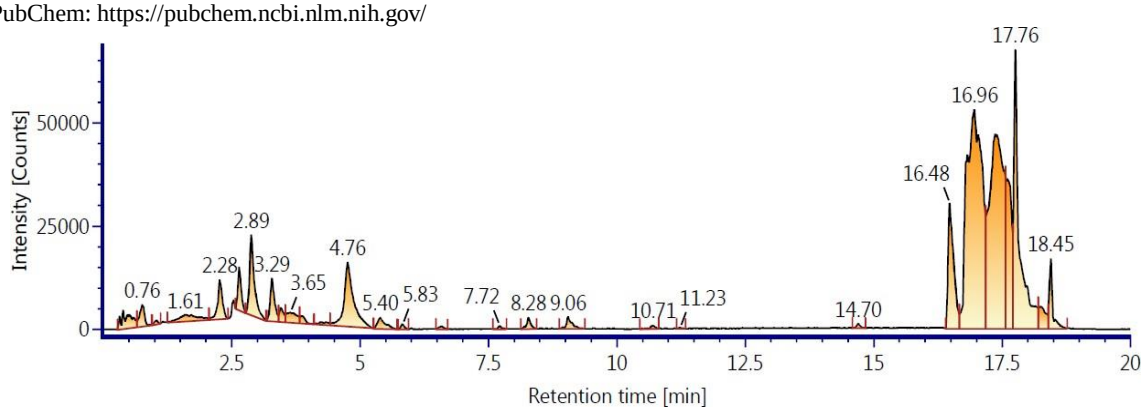

Figure S6. Chromatogram of *Carica papaya* seed (Bangkok Extract) in LC-MS/MS-QTOF (ESI -) analysis. The numbers indicate the retention time of metabolite peak

Table S6. Papaya seed metabolites (Bangkok Extract) detected in LC-MS/MS-QTOF (ESI -) analysis

Metabolite	Group	Metabolite content (%)	Retention time	Molecular weight (g/mol)*
Nortropanoline	Alkaloid	3.4	1.16	175.18
1-Galloyl-glucose	Hydrocarbon	9.27	2.21	332.26
Gentianal	Alkaloid	1.48	4.77	193.2
Cimifugin	Phenol	0.3	5.45	306.31
Mangiferin	Phenol	0.3	7.91	422.3

*PubChem: https://pubchem.ncbi.nlm.nih.gov/