



Research Article

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Chemical Compounds' Content Determination and a Pharmacognostic Parameter of Pepaya (*Carica papaya*, Linn.) Leaves Ethanol Extract

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ABSTRACT

*The usefulness of papaya leaf is very much and people often use papaya leaves as a traditional medicine option. The thought arises to develop knowledge about papaya leaf, especially its chemical content which will be very useful in the development and utilization of these plants. The present research is aimed at studying the pharmacognostic parameter based on Indonesian Pharmacopeia of papaya (*Carica papaya* Linn.) leaves' ethanol extract and determine their compounds' content. Three different papayas from Bandung, Sumedang (West Java) and Padang (West Sumatra) were selected. The simplicia was then macerated with ethanol 95% and tested for pharmacognosy parameters, TLC, phytochemical screening and chemical content with GC-MS. It was found that the water content and the highest acid soluble from Padang were 21.69% and 98.18%, total ash content and the highest water soluble ash content in papaya from Bandung were 10,66% and 11, 5%, and water-soluble extract and soluble ethanol extract from Sumedang were 0.66% and 23%. Phytochemical screening of papaya leaf extracts from all three regions showed the presence of alkaloids, flavonoids, steroids, and saponins. The result of GC-MS showed that the highest content of the compounds in the three samples were 15, 25 and 11 chemical compounds, respectively, detected from Bandung, Sumedang, and Padang. The major phytochemicals in terms of their relative abundance was 2-ethyl-1-hexanol in papaya leaf extract of Bandung (76.78%), Sumedang (32.17%), and Padang (92.50%). It was concluded that depending on where the plant grew, it produced a different compound content and the method chosen was successful to determine the compound content of the papaya leaves and most likely to be applied to another plant samples.*

Key words: *Carica Papaya, Pharmacognostic Parameters, GC-MS, Maceration, 2-Ethyl-1-Hexanol.*

INTRODUCTION

Pepaya (Indonesian), a plant belonging to the Caricaceae family and widely cultivated everywhere, is 8 to 10 m tall. Papaya with the Latin name *Carica papaya* Linn. Coming from tropical America, can grow very fast because in the fifth or sixth month after planting, the trees are as tall as adults and have begun to bear fruit. In general, papaya plants are not older than 5 to 8 years and at that age, they a little fruit or none [1]. Papaya plants produce white latex or papaya containing papain enzymes that help digestion of albuminoid substances and have bacteriostatic properties against certain germs. Papain also is used to treat arthritis [2, 3]. Papaya leaf contains karpain, karposid, pseudokarpain, saponin, saccharose, dextrose, and levulose. It also can be used to stimulate the secretion of bile, as a laxative, fever (malaria), worm disease (*Oxyuris vermicularis*), stomach ache, treating acne, colds, menstruation, and breast milk [2, 4].

Because the usefulness of papaya leaf is very much and often people use papaya leaves as a traditional medicine option, the thought arises to develop knowledge about papaya leaf, especially its chemical content which needs to be known to obtain the completeness of information very useful in the development and utilization of these plants. This paper reports the chemical compounds' content of papaya leaf extract based on GC-MS analysis and a

pharmacognostic parameter which include water content, ash content, water-soluble ash content, acid soluble ash content, water-soluble sari content and ethanol soluble ethanol content based on Indonesian Pharmacopea IV method [5]. Zunjar et.al [6] has studied pharmacognostic, physicochemical and phytochemical on Carica papaya leaves, but they use different approaches and report only the secondary metabolite content of the extracts they studied. However, the results of this group are complementary with the results we report.

MATERIAL AND METHODS

Materials : The materials used in this research were papaya leaves (*Carica papaya* Linn.), which grew in Sumedang, Bandung, and Padang areas. The sample was determined in Laboratory of Plant Taxonomy, Department of Biology, University of Padjadjaran.

The chemicals used were Mayer, Dragendorff reagents, 10% vaniline in concentrated sulfuric acid, magnesium powder, 10% sulfuric acid in 10% ethanol, Lieberman-Burchard reagent, KOH and 95% Ethanol. If not stated otherwise, all reagents were analytical grade

Equipment : Moisture analyzer, Camag TLC tools, UV lamp, Shimadzu GC-MS QP 5050, macerator, UV/VIS spectrophotometer

Methods :

Methods include collection and processing of materials, determination of plants in laboratory of plant taxonomy, maceration extraction based on modification of standard method, phytochemical screening of extract based on modification of Farnsworth method [7]. Pharmacognocny parameter determination (Water content determination, Ash determination, Water soluble ash determination, acid soluble ash content, water-soluble solubilization, ethanol soluble concentration) refers to Indonesian Pharmacopoeia IV and *Materia Medika Indonesia* [5, 8, 9] and pharmacognosy analysis of extract by TLC method and GC-MS following procedure of structural elucidation of organic compounds by Silverstein et.al [10], and Supratman [11].

RESULTS AND DISCUSSION

Collecting and plant determination: Papaya leaves were collected from three areas namely Bandung, Sumedang (West Java), and Padang (West Sumatra). They were then determined in the Laboratory of Plant Taxonomy Department of Biology, Faculty of Mathematics and Natural Sciences, University of Padjadjaran and the resulting simplicial was *Carica papaya*. Simplicia was made by starting from wet sorting, washing, drying, chopping, and dry sorting. Wet sorting was done to remove the foreign objects from the plants. Chopping was done to speed up the drying process, easier for milling, and packing. Chopping sought not too thin to avoid the loss of active substance in the plants. Then, drying was done to stop the enzymatic or hydrolysis reaction that occurs in the cell or plant tissue so that its wrinkles are occurred and pores were formed that could be entered by the excitation solvent. After drying, the simplicia was powdered with a grinder to extend the surface in contact with the liquid of the solvent so as to obtain more extraction results [8, 12].

Extraction: Extraction was carried out using ethanol 95% by maceration [13]. Ethanol was chosen as a liquid because it could dissolve almost all secondary metabolites contained in simplicia and harmless. After extracting, ethanol extracts were concentrated using a rotary evaporator at 40°C in order to prevent the components from being damaged, especially the less stable components to high temperatures. The yield of the viscous extract of *C. papaya* Bandung, Sumedang and Padang were 17.85, 20.62 and 27.10 % w/w, respectively.

Phytochemical screening: Phytochemical screening was based on Farnsworth method [7] applied to ethanol extract of *C.papaya* (see Table 1). Juárez-Rojop et.al [14] studied phytochemical screening and hypoglycemic activity of *C.papaya* leaf in streptozotocin-induced diabetic rats. The extraction was done using chloroform, n-hexane or ethanol and they found the chloroform extract, containing steroids and quinones as major components, the presence of alkaloids and tannins in the ethanolic extract; and steroid and quinones in the three extracts. Singh et.al [15] and Nariya and Jhala [16] mentioned that they found presence of alkaloids, phenols, sugar, proteins, flavonoids in both extracts. But, methanolic extracts showed better results than the aqueous one. Presence of saponin was only detected in the methanolic extracts. As in aqueous extract, a moderate amount of terpenoids and Quinones were present. These results actually gave similarly but not the same results as ours which might be due to a different sources of plant origin.

Table 1. Phytochemical Screening of extract ethanol from C.papaya

Secondary metabolite	Bandung extract	Sumedang extract	Padang extract
Alkaloids	+	+	+
Flavonoids	+	+	+
Tannin	+	+	+
Polyphenols	-	-	-
Monoterpenoid dan Sesquiteropenoid	-	-	-
Triterpenoid	-	-	-
Steroids	+	+	+
Saponins	+	+	+
Quinone	-	-	-

Notes:(+) : detected, (-) : not detected

Pharmacognostic Parameter : Pharmacognostic parameter was determined referring to Indonesian Pharmacopoeia IV [5] and Materia Medika Indonesia [9]. Table 2 shows the result of a pharmacognostic parameter of papaya leaf extract from three regions. Determination of this extract parameter aimed to determine the standardization of papaya leaf extract parameters. The water content of the extract was high because the extract was hygroscopic so it made it easy to bind water around it. The content of water-soluble extract and the extract obtained was quite large. The content of this essence was related to the determination of the dosage calculation of an herbal preparation. Pharmacognostic parameters of Pharmacopoeia IV [5] and Materia Medika Indonesia [9] slightly different from WHO guidelines on which the WHO guidelines mentioned for the safety of herbal medicines [17].

Table 2. The pharmacognostic parameter of C.papaya from different areas

No	Pharmacognostic parameter	Bandung (%)	Sumedang (%)	Padang (%)
1	Water content	16	20	21.69
2	Total ash content	10.66	7.33	6.29
3	Water soluble ash	11.5	7.5	5.57
4	Ash content not soluble in acid	96.5	96.5	98.18
5	Water-soluble extract	0.32	0.66	0.26
6	Ethanol soluble extract	10	23	9

TLC of ethanol extract: The silica gel GF 254 was used as absorbent, and the developer was chloroform: methanol: toluene (7: 1: 2), creepage distance was 6.5 cm, spotting viewer using UV 366 nm and 10% sulfuric acid-ethanol reagent. TLC results can be seen in Table 3. Thin layer chromatography was carried out using a mixture of different polarity solvents. It was found that selected solvent system suitable for papaya leaf extract was chloroform: methanol: toluene with a ratio of 7: 1: 2. The solvent mixture was intended to obtain the desired polarity to separate well-separated components, in which the same compounds with the developer were withdrawn by the developer and leave a spot on the plate. From table 3 it could be seen that UV 366 nm obtained quite similar spots on papaya leaf extract of Bandung and Sumedang that was 6 spots, while with 10% sulfate-ethanol acid spotting effect obtained the most spots on papaya leaf extract Sumedang. This indicated a slightly different content of papaya from Java and Sumatra which was probably caused by the difference in soil structure in which the papaya was obtained. Thompson and Morgan [18], and Anjum et.al [19] reported TLC fingerprinting profile of their C.papaya exhibiting the presence of several medium polar compounds by using the mixture solvent of n-hexane: acetone (8.5:1.5) and silica gel 60 F254 TLC plate.

Table 3. TLC of extract ethanol from three different area of C. papaya

Bandung		Sumedang		Padang		Spotting viewer
Rf	Color	Rf	Color	Rf	Color	
14	green	15	green	-	-	UV 366nm
32	purple	30	purple	29	purple	10% H2SO4/EtOH
43	red	41	red	-	-	UV 366nm
-	-	43	purple	41	purple	10% H2SO4/EtOH
49	brownish-green	47	brownish-green	-	-	10% H2SO4/EtOH

50	red	50	red	50	red	UV 366nm
69	purple	67	purple	66	purple	10%H ₂ SO ₄ /EtOH
-	-	75	brownish-	-	-	10%H ₂ SO ₄ /EtOH
			green			
77	red	76	red	-	-	UV 366nm
80	green	78	green	-	-	10%H ₂ SO ₄ /EtOH
88	red	88	red	89	red	UV 366nm
93	green	95	green	-	-	10%H ₂ SO ₄ /EtOH
95	red	96	red	96	red	UV 366nm

Gas Chromatography-Mass Spectroscopy (GC-MS): Results of GC-MS analysis of papaya leaf extract of three regions can be seen in Fig. 1, 2 and Table 4, 5, 6. Fig. 1 was a sample of mass-spectrum obtained, and Fig. 2 was the result when the Mass-spectrum from Fir. 1 compared to the data-base of the GC-MS (Nist 62. Lib, Wiley 229 LIB, Pestico.LIB). The used of the data-base such as Nist 62.lib was also carried out by other researcher [20-22]. Wiley 229 LIB was mentioned by others [23-25], Pestico database was referred to Shimadzu Co. [26]. Table 4.5.6 was the conclusion of the chemical content of *C. papaya* being examined. The results of GC-MS of papaya leaf extract of Bandung, Sumedang, and Padang showed that the ethyl hexanol compound was most prevalent in all three samples, which distinguishes only the amounts. The highest 2-ethyl-1-hexanol content was found in papaya leaf extract followed by Bandung and Sumedang. This was influenced by climatic factors, soil and the degree of acidity where the papaya grew. The most influential in obtaining good results on the GC-MS was temperature setting i.e injection gate temperature, column temperature, and detector temperature. The column temperature was important to determine good retention and resolution. The column temperature setting should be as optimal as possible which should be high enough so that the analysis could be completed in a reasonable time and should be sufficiently low so that the desired separation is achieved. The temperature of the syringe gate ought to be adjusted so that the decomposition due to heat was low enough but the detector temperature should be sufficiently hot so the trailer and the stationary phase did not condense. Column size also contributes to the separation of compound components. Longer columns produce greater separation but if the size was excessive then the separation will decrease again. The choice of liquid phase should be suitable that was having good dissolving power [11, 27]. The parameter of GC-MS in this study can be seen in Fig. 3.

Other researchers also studied the chemical compounds of papaya revealing different results. Oche et.al [28] studied and evaluated the chemical constituents and nutrient composition of leaf extracts of *C. papaya* and *Vernonia amygdalina*. The chemical constituents were analyzed using GC-MS. The constituents of *C. papaya* leaf extract showed twenty constituents, dominated by oleic acid (28.98%) with a molecular weight of 282, with the least compound Trans-Geranylacetone (0.17%), with a molecular weight of 194. Ezekwe and Chikezie [29] studied unwrapped papaya and showed the presence of fifteen phytochemicals in *C. papaya*. By comparative inspection, the major phytochemicals in terms of their relative abundance were octadecanoic acid, hexadecenoic acid, Z-11 and hexadecanoic acid, methyl ester, which corresponded to 23.84%, 19.17%, and 18.25%, respectively. In the same context described above, the minor phytochemicals present within a narrow range of 0.78-5.38%. Specifically, n-hexadecanoic acid represented the photo component with the lowest concentration among the entire phytochemicals present. Setyawati et.al [30] used GC-MS analysis detecting 11 active compounds in *C. papaya* L. leaves fraction. They were assumed as the major active compound due to having the highest peak formed by molecules of hexanedioic acid, bis(2-Ethylhexyl) ester and hexanedioic acid, dioctyl ester. Igwe [31] reported that the chemical constituents of the extract of the leaves of *C. papaya* were characterized using Gas Chromatography-Mass Spectrometry (GC/MS) technique and six compounds were identified which include hexahydro-1-aH-naphtho[1,8a-b]oxiren-2(3H)-one (2.17%), 3,7- dimethyloct-7-en-1-ol (8.08%), 3-methyl-4-(phenylthio)-2-enyl-2,5-dihydrothiophene-1,1-dioxide (11.78%), cyclopentane undecanoic acid methyl ester (12.02%), 3,7,11,15-tetramethyl-2- hexadecene-1-ol (37.78%) and 9-octadecenamide (28.18%). He et.al [32] stated the major constituent was benzyl isothiocyanate (99.36%) from their seed of *c.papaya*. By using AAS and spectrophotometric instruments, some papers mentioned the papaya leaf contained protein, carbohydrate, crude fiber, Ca²⁺, Mg²⁺, Na⁺, K⁺, Cl⁻ and Li⁺ [33], and β -carotene was the most abundant vitamin [34].

Sample : PEPAYA BANDUNG
 ID : 60/2-10/320/10
 Sample Amount : 0.4
 Dilution Factor : 0
 Type : Unknown
 Operator : SURANI
 Method File Name : HOKCU175.MET
 Vial No. : 0
 Barcode :

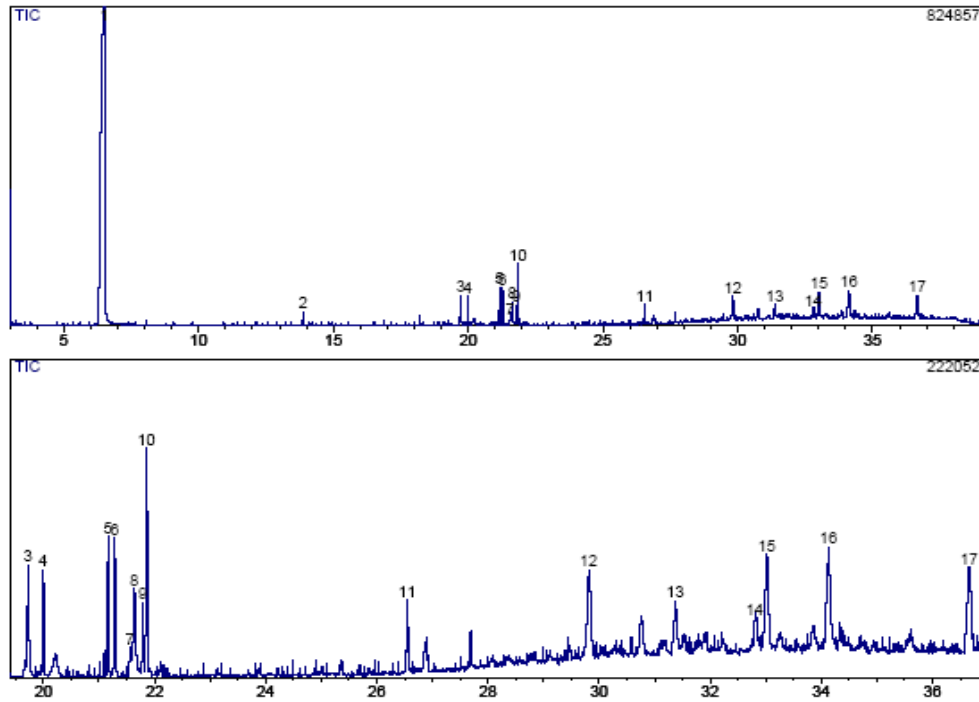


Figure 1 : GC-MS of ethanol extract of Bandung C. papaya

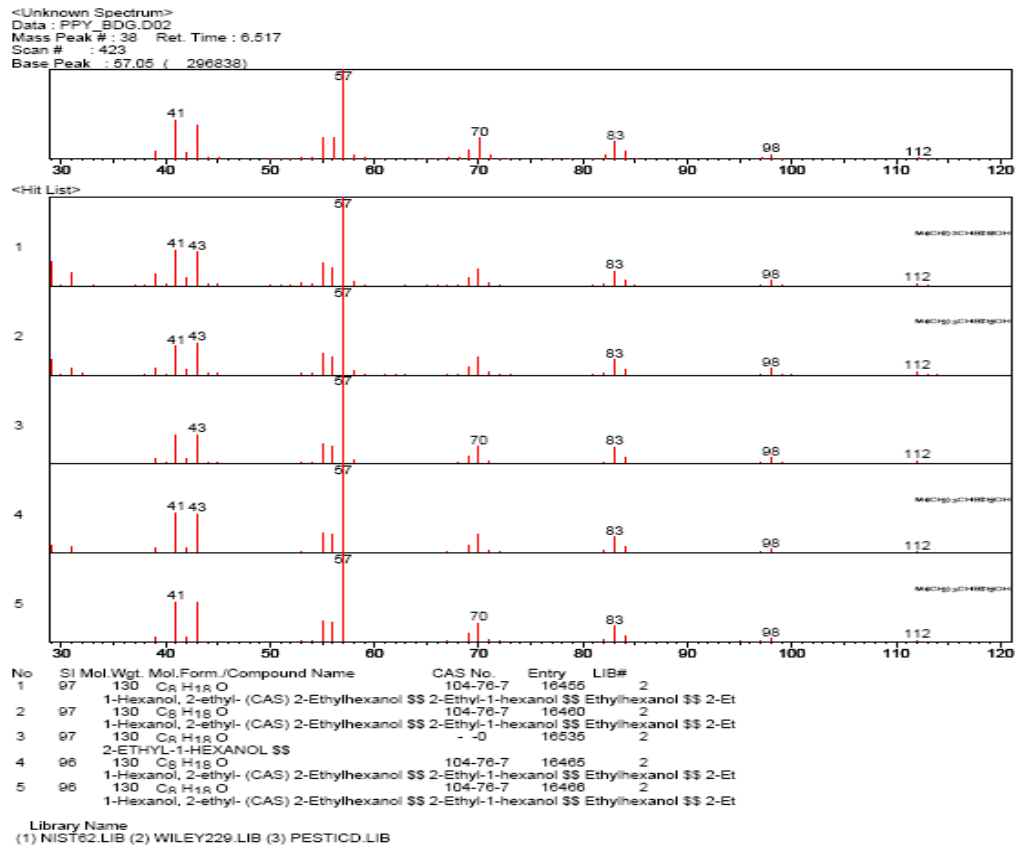


Figure 2 : Mass spectrum of ethanol extract from Bandung C.papaya

Table 4. Chemical compounds content of ethanol extract of Bandung C. Papaya

No	Compound name	Concentration (%)
1	2-Ethyl-1-Hexanol	76.78
2	Oxacyclohexadecan-2	3.68
3	Isoxazolo (2,3-Alpha) Pyridine	2.55
4	Ethyl Oleate	2.29
5	Ergost-5-en-3-ol	2.27
6	Kolestan-3,6,7-triol	2.19
7	Fitol	1.52
8	Kolestan-3	1.47
9	Hexadecanoic acid	2.38
10	9-Octadecenoic Acid	1.31
11	Anodendrosid G Monoacetate	1.12
12	Octane, 1,1-Oksibis	0.86
13	9,12-Octadekadenoat	0.66
14	2,3-Dimethylnonan	0.48
15	Linoleic acid	0.46

Table 5. Chemical compounds content of ethanol extract of Sumedang C. papaya

No	Nama Senyawa	Kadar (%)
1	2-Ethyl-1-Hexanol	32.17
2	9,12,15-Octadekatrien-1-ol	13.07
3	Hexadecanoic acid	12.94
4	Isomer Fitol	7.61
5	Isoxazolo (2,3-Alpha) Pyridine	5.37
6	9,12,15-Octadecatrienoic Acid	4.43
7	Stigmast-5-en-3-ol	3.85
8	Hexadecanoic Acid, Ethyl Ester	2.78
9	Ethyl linoleate	2.20
10	Vitamin E	1.97
11	Neofitadien	1.77
12	7-Acid Octadesenoat, Methyl Ester	1.57
13	Lanostan-3-Beta.-ol	1.56
14	Zonaron	1.31
15	Octadekanal, 2-Bromo	1.02
16	Jasmolin II	0.86
17	Spirost-8-en	0.76
18	Tetrakosaheksan	0.74
19	2-Hexadecent	0.91
20	Octane, 1,1-Oksibis	0.52
21	P-Nitrobenzoate	0.89
22	1,2-benzendicarbocyclic acid	0.47
23	Hexadecanoic Acid, Methyl Ester	0.47
24	Dikotin	0.39
25	9-octadecene	0.38

Table 6. Chemical compounds content of ethanol extract of Padang C. papaya

No	Nama Senyawa	Kadar (%)
1	2-Ethyl-1-Hexanol	92.50
2	7-Oktadesenoat	2.00
3	Ethyl Oleate	1.51
4	Octane, 1,1-Oksibis	1.28
5	9-Oktadesenal	0.47
6	Tetradekanal	0.42
7	Octadecanoic Acid	0.27
8	Linoleic acid	0.26
9	9.12-Ocadecadenoic Acid	0.26
10	5- (4-Amyloxyphenyl)	0.16
11	3-Methyl-4,6-di-tert-Butyl Phenol	0.13

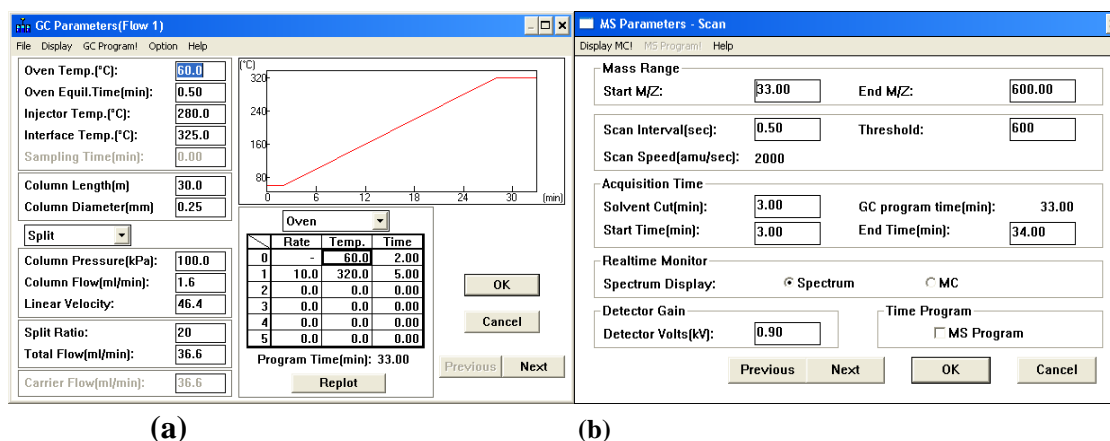


Figure 3: Parameter of GC(a) – MS(b)

CONCLUSION

From the results of phytochemical screening of papaya leaf extract (*Carica papaya* Linn.), it was showed that the metabolite compounds contained alkaloids, flavonoids, steroids, and saponins. Water content and the highest acid soluble ash content were found in papaya leaf extract of Padang (21.69% and 98.18%), total ash content and highest water soluble ash content in papaya leaf extract of Bandung (10.66% and 11.5 %), water soluble extract and the highest ethanol soluble extract content of Sumedang papaya leaf extract (0.66% and 23%). The result of GC-MS indicated that the highest content of the compounds in the three samples were 15, 25 and 11 chemical compounds, respectively, detected from Bandung, Sumedang, and Padang c.papaya. The major phytochemicals in terms of their relative abundance is 2-ethyl-1-hexanol in papaya leaf extract of Bandung (76.78%), Sumedang (32.17%), and Padang (92.50%).

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CONFLICT OF INTEREST

There is no conflict of interest between authors.

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