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Screening of new bioactive in lebui beans (*Cajanus* sp.) of Lombok

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Abstract

Lebui beans (*Cajanus* sp.) has been used by Sasak people of Lombok Island West Nusa Tenggara Indonesia as a traditional medicine. The potential compounds found in the lebui bean are thought beneficial mainly for curing the infectious diseases. The aim of this study is to extract and find out the type of bioactive compound from lebui bean by phytochemicals and screening test. Two stages of the study had been carried out. The first stage was to extract the bioactive compound from lebui bean by solvent extraction and the second stage was to screen the type of bioactive compound by phytochemicals test, Thin Layer Chromatography (TLC) and Gas Chromatography–Mass Spectrometry (GC-MS). The result of the first stage showed that ethanol 70% was best solvent to extract the bioactive compound in lebui bean, and the second stage showed that lebui bean contained alkaloids, flavonoids, polyphenols, terpenoids/steroids, and also 44 constituents of secondary metabolites ie. Neophytadiene, Methyl Laurate, Nonanedioic Acid-Dimethyl Ester, Methyl Myristate, Methyl Pentadecanoate, Methyl Palmitoleate, Methyl Linoleate, Heptadecanoic Acid, 8-Octadecenoic Acid, Methyl Stearate, Eicosanoic Acid, Methyl Tricosanoate, Methyl Lignocerate, 4-Methylimidazole-5-[1,1-Dimethylbutyric Acid Amide], 5,8,1,14-Eicosatetraenoic Acid Methyl Ester, Isopropyl Linoleate, and 8,11-Octadecadienoic Acid.

Introduction

Lebui bean (*Cajanus* sp.) is one of the most important legume grown throughout the year and had been found abundantly at Lombok Island West Nusa Tenggara Indonesia. The total production of crop including lebui beans amounting 70-648 tons per year (Department of Agriculture of West Lombok, 2011). Up to the present, lebui bean only used for local dishes and traditional medicine. In peak season, lebui beans are unutilized and only left in the field. Whereas, lebui bean is very beneficial as local material to produce high valuable bioactive compounds. This potential bioactive compound has an advantages to cure some infectious diseases such as skin infection, gastroenteritis, pneumonia, due to this lebui bean has secondary metabolites product and serves as antioxidant, antimicrobial, and antifungal (Akor and Anjorin, 2009; Zafar *et al*., 2014). Furthermore, bioactive compounds are generally found in *Leguminosae* such as flavonoids, proanthocyanidin, polyphenols, saponins, phytosterols, and isoflavones (Madhujith *et al*., 2004; Luthria *et al*., 2007; Fu *et al*., 2008; Bernhoft, 2010; Luo *et al*., 2010). However, these bioactive compounds present in the cell which are bound with glucose through glycosidic bond (Huang *et al*., 2013) and this linkage should be degraded by the used of solvent extraction. Extraction of the bioactive compound from lebui bean by solvent extraction method is urgently required in order to find out the type of bioactive compound by phytochemicals and screening test.

Extraction of the bioactive compound from lebui bean was carried out by n-hexane, ethyl acetate and ethanol in the first stage. The proximate contents and the total sugar of the extract were measured. The second stage of this study was to screen the type of bioactive compound by phytochemicals test, continued by TLC and GC-MS. Phytochemical screening was done to identify the presence of secondary metabolites such as flavonoids, alkaloids, terpenoids/steroids, and polyphenols. The TLC test used to separate and determine the components of secondary metabolites found in the extract of lebui bean. Furthermore, an analysis with GC-MS used to separate the component in the extract of lebui bean and producing a representative spectral output. Each component produces a specific spectral peak that recorded electronically by specific device.

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Materials and Methods

Raw materials

Lebui beans (*Cajanus* sp.) were collected from Gunung Sari village, Mataram, Lombok Island, West Nusa Tenggara Indonesia in 2015. The beans were washed thoroughly with water to remove the dirts, then they were immersed into water (ratio of 1:3). The beans were allowed to stand for six hours in 25-27°C, and the water was replaced for every 1 hour. The beans were filtered and coarsely grounded up to 1-2 mm in size, then they were dried by using cabinet dryer at 40°C for 6 hours until the moisture content reached 12-13%, then they finely grounded to 50 sieve mesh size and we sieved it to obtain the lebui beans powder. Proximate contents (protein, fat, carbohydrates, water, ash, and total sugar), color and odor, and also phytochemical screening test were analyzed to the lebui bean powder.

General experimental procedures

The extractions were measured on the rotary evaporator vacuum RE300 and the shaker was measured by Barnstead SHKE2000. The analytical balance apparatus was KERN ALJ 220-4NM. The color of the lebui bean powder and extracts were identified by colorimeter - Konica Minolta CR 10.

Proximate contents analysis

Proximate contents analysis were measured by AOAC International (2000) methods.

Water (moisture) content

Dry the empty dish and lid in the oven at 105°C, then transfer it to desiccator to cool. Weight the empty dish and the lid. About 3 g of samples to the dish, spread it to make the uniformity and dry it at 105°C for 3 h. Transfer the dish of samples to the desiccator to cool and reweight the dish and also the dried samples. Report loss in weight as moisture.

Protein content

Sample for 1 g in digestion flask was added 5g of Kjeldahl catalyst and 200 ml of conc. H_2SO_4. Place flasks in inclined position and heat gently unit frothing ceases, then boiled briskly until the solution was cleared. Cool and add 60 ml of distilled water cautiously. Connect the flask to digestion bulb on condenser and with the tip of condenser, immersed in standart acid and add 5-7 drops of mix indicator in receiver, the heated until all NH_3 is distilled. Titrate the excess standart acid distilled with standart NaOH solution.

Ash content

For about 5 g of sample in the crucible was heated by bunsen flame until the fumes are no longer produced. Crucible and lid were placed in furnace and heated at 550°C for 8 h till overnight, then cooled down in the desiccator and the ash was weighed.

Fat content

The sample (3-5 g) was wrapped into filter paper, taken into extraction thimble and transferred into soxhlet. Petroleum ether was added for about 250 ml into the bottle and was moved on the heating mantle. Sample was heated for 12-14 h. Solvent was evaporated by vacuum condenser at 80-90°C. The bottle with lid were moved to the desiccator to cool and weight it.

Total Sugar

This was measured by method of ASEAN Manual of Food Analysis (2011). Test sample containing approximately 30% total sugar in a 250 mL Erlenmeyer and added with 150 mL water (preheated to 60±5°C). Mechanically shake flask for 30 minutes in a water bath maintained at 60±5°C and stand it in water bath for 30 minutes then cool it to room temperature. Sample was transferred to a 250 mL volumetric, diluted to volume, mixed well and filterred or centrifuged then filterred. Accurately pipette 5 mL each of reagent into 250 mL erlenmeyer. Sample solution was pipetted into the erlenmeyer and dilute with distilled water to about 50 mL and pre-heated sample for 10 to 15 seconds. If blue colour persists, added working sugar standard solution for 0.5-1 mL at a time until disappearance of blue colour. 3 drops of methylene blue indicator was added and continued adding standard sugar solution at intervals of 10 seconds until the indicator is completely decolorised.

Extracts preparation for phytochemical screening test

The extracts were gained by standard maceration process with a slight modification (Shanab, 2007; Tensiska *et al*., 2007; Jahangir *et al*., 2011; Chohan and Perveen, 2015). The maceration method by solvent extraction and agitation was conducted to gain the crude extracts of lebui beans powder with no heat. The extraction method conducted through the agitation of plant materials was used to increase the mass transfer. The fresh lebui beans powder (40 g) was diluted and macerated separately in a flask by 70% and 90% of ethanol, ethyl acetate, and hexane (1:8 w/v) under agitation or stirring (142 rpm) for 24 hours in a dark room (25°C - 27°C). The filtration

was done by Whatman No. 41 filter paper under a vacuum condition.

The crude filtrate was evaporated and concentrated under pressure at 40°C by rotary evaporator and then continued by using nitrogen gas (N_2) to evaporated the solvents. The extracts were collected and stored separately in dark bottles (covered by aluminium foil). The extracts then stored at 10°C -12°C for further phytochemical analysis use (Tensiska *et al*., 2007; Ishtiaq *et al*., 2013; Rahim *et al*., 2013).

Phytochemical screening

Phytochemical screening was done for qualitative analysis to identify the presence or absence of secondary metabolites such as flavonoids, alkaloids, terpenoids/steroids, and polyphenols. This screening method was carried out with modification (Rahim *et al*., 2013; Unit Layanan Pengujian/ULP Airlangga University, 2015; Zafat *et al*., 2015). The extract was dissolved in 90% ethanol solution for 15 minutes by ultrasonication, then it was macerated for 1 day and kept cool (10°C -12°C) for the next analysis.

The extract (1 mL) was transferred to a centrifuge tube and 2% of ferric chloride solution (FeCl) with few dropped. The formation of dark or black-red color indicated the presence of flavonoids components by ferric chloride test. Under alkaline reagent test, the extract (1 mL) was vortexed with few drops of 1N sodium hidroxide (NaOH) solution, then the increase was observed in the intensity of the yellow color. 1N hidrogen chloride (HCl) solution was dropped to previous solution containing NaOH and vortexed for 5 min, then the decrease was observed in the intensity of the yellow color. The presence of flavonoids were showed by these changes.

The presence of flavonoids could be determined by TLC method. A portion of extract (25 µL) was spotted to TLC's plate. A thin layer of Kiesel Gel GF 254 as the stationary phase and the solution of chloroform-methanol (9:1) as the mobile phase. To express the stain, it was used 2% ferric trichloride ($FeCl_3$) solution and UV rays (366 nm dan 254 nm). The presence of flavonoid was showed by the emergence of dark spot under the UV rays at 254 nm and dark yellow/green/blue spot under the UV rays at 366 nm. The appearance of brown to dark or black color also indicates the presence of flavonoids by $FeCl_3$ solution.

Red or purple color was marked if the extract (dissolved in ethyl acetate solution) was treated with 3 drops of sodium acetate (CH_3COONa) solution through anthocyanin's test. The formation of blue color by $FeCl_3$ solution indicates the presence of anthocyanin. The emanation of purple/blue/green color by 3 drops of Na_2CO_3 solution was also the sign of anthocyanin compounds.

Screening for alkaloids by TLC method

The extract (1 g) was dissolved in to 96% ethanol solution (5 mL). Chloroform phase (10 µL) was spotted on TLC's plate. Kiesel gel GF 254 was as the stationary phase. The solution of ethyl acetate-methanol-aquades (4:6:0.5) acts as the mobile phase. Dragendorff's reagent was used to stain expressor. The appearance of orange color indicates a possitive test.

Screening for polyphenols by TLC method

1 g of extract was dissolved in 5 mL of 96% ethanol solution, then it was spotted for 10 µL on TLC's plate. The stationary phase used Kiesel gel GF 254 and the mobile phase used admixture of ethyl acetate-methanol-formic acid (16:4:1). $FeCl_3$ reagent was as the marker stain. The formation of black color showed a positive test extracting contain polyphenols.

Screening for terpenoids and steroids by TLC method

The sample (1 g) was extracted with 5 mL of n-hexane, then it was spotted for about 10 µL on the layer of stationary phase (Kiesel gel GF 254). This screening was used n-hexane-ethyl acetate (4:1) as the mobile phase and Anisaldehide-sulfuric acid as the marker stain. Terpenoids or steroids compounds were shown by red-purple or purple only.

Profile identification

Screening for the constituents of sample (lebui beans powder and extract in ethanol/ethyl acetate/n-hexane) used GC-MS by ULP of Airlangga University (2015). Lebui beans powder (1 mg) in a volumetric flask (10 mL) was added with 5 mL n-hexane, then it was treated with ultrasonic method, added more n-hexane until the precise volume (10 mL), shaken until homogeneous, and allowed to stand for a day (25°C-27°C). The sample was filtered with 0.2 µm nylon and the supernatant was taken. Supernatant (4 mL) in the derivatization tube then was dried by spraying with nitrogen gas. Saponification was done to the sample by adding 2 mL of NaOH-metanolate solution, tightly closed, mixed (Vortex for 5 min), heated (90°C for 5 min), then cooled. It was derivatized by adding 2 mL of boron trifluoride (BF_3) solution, tightly closed, mixed (Vortex for 5 min), heated for 30 min (90°C), and then cooled. Furthermore, it was added 4 mL of n-hexane, mixed (Vortex for 2 min), then allowed to stand until detached into 2 phases. The upper layer (n-hexane phases) was taken (appx. 1 µL) to be analyzed by GC-MS apparatus.

Table 1. Characteristic of color and odor of the lebui bean extract

Sample	Yield (%)	Color intensity			Manual description	
		L	a	b	Color	Odor
Lebui bean powder	90.98	51.1	0.15	-0.24	beige-light brown	lebui bean
70% Ethanol	90.95	31.72	4.24	0.51	brown	like lebui bean
90% Ethanol	80.27	32.43	10.30	2.92	dark reddish brown	like lebui bean, pungent
70% Ethyl acetate	90.45	36,63	14.82	9.43	reddish brown	like lebui bean
90% Ethyl acetate	6.82	29.40	0.93	-0.71	dark brown	like blowing ballon, pungent
70 % n-hexane	54.92	43.56	-7.25	-11.50	brownish yellow	slightly like lebui bean, pungent
90 % n-hexane	19.25	44.58	-9.93	-12.14	yellowish brown	like lebui bean, more pungent

Preparation for the extract in ethanol / ethyl acetate / n-hexane

The extract (40 mg) was transferred to derivatization tube and 2 mL of NaOH-methanolate was added. The solution was mixed (Vortex for 2 min), heated for 5 min (90°C), then cooled. The solution was added by 2 mL of BF_3, tightly closed, mixed (Vortex for 2 min), heated again for 30 min at 90°C and cooled. The solution was added by 4 mL of n-hexane and mixed (Vortex) for 2 minutes and cooled until 2 phases was formed. An aliquot of 1 µL of the upper layer was transferred and injected to GC-MC apparatus.

Results and Discussion

Stage 1: extraction of bioactive compound from lebui bean by solvent extraction

Extraction of the bioactive compound from lebui bean was carried out by n-hexane, ethyl acetate and ethanol. This solvent extraction method is urgently required to degrade the glycosidic bond of bioactive compound present in the cell of lebui bean and to separate the bioactive compound with other compound by dissolving it with specific solvent. Hexane, ethyl acetate, and ethanol are the type of organic solvent that is often used for the extraction of bioactive compounds. Hexane is nonpolar and capable of dissolving the bioactive compounds from the group of fatty acids and essential oils. Ethyl acetate can bind nonpolar bioactive compounds like alkaloid. Solvent extraction with ethanol can produce extracts containing phenolic compounds, steroids, terpenoids, and alkaloids. Water can dissolve the compounds of the flavonoid which is polar (Markham, 1982; Hougton and Raman, 1998).

The color, odor, and yield of extract

Different types of solvent combined with different concentrations has different effect on the yield and also the characteristic of color and odor. The extract in 70% of ethanol had a higher yield. The characteristics were showed by color reader and manual description. Lebui bean extract in 90% of ethyl acetate showed a darker color (dark brown) than extract in ethanol (red to brown) and extract in n-hexane (yellow to light brown). The differences of color and odor for each extract were showed by different solvent and concentration (Table 1).

Higher concentration (90%) produced darker color and more pungent odor for all the solvents. The color intensity of the lebui bean powder and extracts were conducted by manual description and enhanced by colorimeters. Colorimeters could detected the differences of color by human eyes and expressed through numbers of L (lightness), a (red or green value) and b (yellow or blue value) (X-Rite, 2004). Generally, the extracts in higher concentration (90%) of solvent were more pungent in odor and has darker color with sticky performance than the lower concentration (70%). This is possible because the extract was influenced by the characteristic of odor and color of each type of solvent. Based on the odor of the each extract, ethyl acetate has a distinctive odor that pungent more than any other type of solvent. The extract in n-hexane contained compounds that solved in non-polar phase such as the derivatives of fatty acids and could be determined by yellowish to light brown color. The compounds solved in ethyl acetate were analyzed to semi-polar characteristic like alkaloids, aglicons, and glicosides.

The extract of 70% of ethanol produced highest yield and 90% of ethyl acetate was the lowest. This result expressing the compounds had been extracted. It has been reported that the solubility of polar components in the extract had been well increased by adding polar components like ethanol or methanol. Naya and Imai (2013) stated the hydrophobicity of components according to their molecular structure. Different solvent has ability and selectivity to dissolve different compounds (Handa, 2008).

The proximate content

The major content of lebui bean (proteins,

carbohydrate, fat, water, ash content and total sugar i.e. 18.49%, 61.96%, 0.97%, 8.79%, 3.37% and 38.16%) were measured on dry basis. Lebui bean also contains fiber, anthocyanin and phenolic i.e. 5.83%, 192.37 ppm, and 750.31 mg EAG/g on dry basis. Lebui bean has the lowest fat content compared with pigeon pea (1.2-4.43%) and soybean (7.7-47%) (Pennington, 1994; Amarteifio *et al*., 2002; Akande *et al*., 2010; Nahashon and Kilonzo-Nthenge, 2013). Lebui bean has water content of 8.79% which is lower than some other types of bean. Presented by Sat and Keles (2002), the seker bean (*P. vulgaris* L.) from the class of *Leguminosae* growing in Turkey has 9.61% of water content. The differences of planting location, soil and climate conditions, the level of maturity, as well as the hardness of the skin bean and the differences in water content of the bean.

Generally, the proteins content in the bean of *Leguminosae* was in the rate of 7.2% to 23% (Amarteifio *et al*., 2002; Akande *et al*., 2010). The protein content of lebui bean was 18,49% and still in a high level. According to Yellavila *et al*. (2015), the variation of protein content in some types of bean from the class of *Leguminosae* caused by differences in planting and environmental conditions, genotyping, as well as the methods of analysis used. Messina (1999) stated that this parameter could describe the amount of total nitrogen by the Kjeldahl method and was related to the energy intake (20%-30%). Mostly 90 g of bean could serve nearly 7-15% of protein requirement. Therefore by consuming lebui bean, able to meet the protein requirements better.

Leguminosae bean contained proteins, carbohydrates, vitamins and minerals at high levels. Mineral components contained in plant seeds *Leguminosae* including Fe, Zn, Ca, Mg, P, Na, Se, and Mn with various concentrations. These minerals are components of ash (Adamu and Oyetunde, 2013). The ash content of lebui bean (3.37%) classified in the low range. Naivikul and D'Appolonia (1978) revealed that the ash content of *P. vulgaris* and *P. aureus* is 3.07-3.45% and 4.3%. Based on the research results of Adamu and Oyetunde (2013), ash content of pigeon pea was 9.93%. Pomeranz and Clifto (1981) states that a plant seeds can be used as food or feed if the ash content contained therein is in the range of 1.5% to 3.5%. Based on these studies, lebui bean are safe to use as raw materials in food because of the ash content of lebui bean still lower than 3.5%. Based on the result of the first stage showed that ethanol 70% was best solvent to extract the bioactive compound in lebui bean.

Stage 2: Identification of bioactive compound from lebui bean.

Phytochemical screening and KLT test of bioactive compound

The presence of bioactive compounds in lebui bean powder and the extract were identified by phytochemical screening, KLT, and GCMS. The data obtained from phytochemical screening showed the presence of flavonoids, alkaloids, polyphenols, terpenoids and steroids, both in the fresh lebui powder or in the extracts. The result in table 2 showed that all the samples were positive contained five types of bioactive compound, except for alkaloids and polyphenols in all concentration of n-hexane extract. Therefore, it can be reported that all the samples have high potential compounds of bioactive substances. Alkaloids was highly detected in the extract dissolved in 90% of ethanol and slightly in ethyl acetate. Through flavonoids tests it was presented that ethyl acetate was an appropriate solvent to collect the flavonoids compounds, then followed by ethanol. Polyphenols were detected in all extracts except in n-hexane extract. Alkaloids and polyphenols could not be detected from the extract of hexane fraction, because of the characteristic of alkaloids and polyphenols compounds are soluble in polar and semi-polar solvents (Yubin *et al*., 2015).

Table 2. Phytochemical screening for lebui bean powder and the extracts

Screening Sample	Flavonoids test				Alkaloids	Terpenoids	Steroids	Polyphenols
	Ferric chloride test	Alkaline reagent test	TLC method	Antho cyanin				
Lebui bean powder	++	++	++	+	+	+++	+++	+++
70% Ethanol	+	+	+	+	++	++	++	+++
90% Ethanol	+	+	+	++	+++	++	++	+++
70% Ethyl acetate	++	++	++	++	+	+++	+++	+++
90% Ethyl acetate	+++	+++	+++	+++	+	+++	+++	+++
70 % n-hexane	-	-	+	+	-	+	+	-
90 % n-hexane	-	-	+	+	-	+	+	-

+++ = High; ++ = Moderate; + = Slight; - = ND (undetect)

According to the first stage of this experiment and table 2 showed that ethanol 70% is the best solvent and concentration to extract the bioactive compound, not only based on the result of phytochemical screening test but also based on the chemical and physical characteristics. This is consistent with the previous study reported that pigeon pea in the class of *Cajanus* contained a large amount of polyphenols. The pigeon pea with darker color of bean coats had higher polyphenols or phenolic compounds. This large amount of phenolics related to the protection of the outer coat for the inner contents from oxidative damage, could act as scavenger of superoxide anion radicals and the phenolic compounds also had the capacity of anti-microbial (Robak and Gryglewski, 1988; Pereira *et al*., 2007; Chakraborty and Mitra, 2008; Kanatt *et al*., 2011).

GC-MS identification

Based on GC-MS identification method, 44 components were detected in lebui bean powder and the extracts. The components were secondary metabolites, fatty acids and fatty acid methyl ester that could be potentially active in human biological metabolism and good for health (Table 3).

Table 3. Profile Identification of lebui bean by GC-MS

No.	Components	Rt (min)
1	Neophytadiene	42.974
2	A-5-Norbornen-2-yl-A	51.436
3	4-Amino-6,8-dimethyl-2-quinolinol	54.868
4	1H-Pyrazolo[3,4-D]pyrimidine,7-ethyl-4,5,6,7-Tetrahydro-3-phenyl-5-(phenylmethyl)	56.364
5	2,4,4-Trimethyl-3-hydroxymethyl-5a-(3-methyl-but-2-enyl)-cyclohexene	59.417
6	4-Methylimidazole-5-[1,1-dimethylbutyric acid amide]	42.541
7	Hexadecanal	53.962
8	Cis-9-Hexadecenal	48.763
9	5,8,1,14-Eicosatetraenoic acid, methyl ester, (all-Z)	16.471
10	6,9,12-Octadecatrienoic acid, methyl ester	16.586
11	7-Hexadecenoic acid, methyl ester (Z)	6.250
12	7-Octadecenoic acid, methyl ester	11.961
13	8-Octadecenoic acid, methyl ester	12.076
14	9,12-Octadecadienoic acid	48.817
15	9,12-Octadecadienoic acid (9Z,12Z)	47.926
16	9,12-Octadecadienoic acid (E,E)	13.171
17	9-Octadecenoic acid (Z)	48.886
18	Docosanoic acid, methyl ester	23.063
19	Eicosanoic acid, methyl ester	1.903
20	Heneicosanoic acid, methyl ester	21.129
21	Heptadecadienoic acid, methyl ester	10.299
22	Hexadecanal, 2-methyl-	53.962
23	Isopropyl linoleate	54.210
24	Linoleic acid	9.648
25	Methyl-14-methyl-pentadecanoate	8.266
26	Methyl laurate	3.089
27	Methyl lignocerate	25.639
28	Methyl linoleate	11.887
29	Methyl linolenate	16.232
30	Methyl myristate	5.040
31	Methyl palmitate	6.488
32	Methyl palmitoleate	7.879
33	Methyl pentadecanoate	6.488
34	Methyl stearate	12.545
35	Methyl tricosanoate	24.487
36	Methyl-9,12,15-octadecatrienoate	13.500
37	Methyl-9-octadecenoate	12.093
38	Nonanedioic acid, dimethyl ester	3.271
39	Octadecanoic acid, methyl ester	12.562
40	Octanoic acid, 6,6-dimethoxy-,methyl ester	2.917
41	Palmitic acid	9.015
42	Pentacosanoic acid, methyl ester	26.635
43	Stearic acid	49.251
44	Tricosanoic acid, methyl ester	27.507

The diverse type of terpenoid was detected and the potential component for new bioactive compound detected in lebui bean extract was neophytadiene (Figure 1). Neophytadiene usually found in plants which have pungent and specific odor or scent, such as *Nicotiana tabacum* flower (Knudsen *et al*., 2006), *Erigeron sumatrensis* leave as an antifungal (Ragasa *et al*., 2009), and *Eupatorium odoratum* as an antibacterial agent (Raman *et al*., 2012). Neophytadiene which found in the extract of *E. sumatrensis* was reported had ability to against *Candida albicans, Aspergillus niger,* and *Escherichia coli* with different concentration (Ragasa *et al*., 2009). This component was also determined in the extract of *Bursera simaruba* (L.) as strong bactericidal and anti-inflammatory (Carretero *et al*., 2008; Mendiola *et al*., 2008). Octadecadienoic acid (9,12-Octadecadienoic acid) and linoleic acid were declaired to against fungi including *C. albicans* (Walters *et al*., 2004; Nuryanti, 2015). The compound of 4-methylimidazole was used for caramel coloring in food and beverages (NTP, 2007; Jacobson, 2011). Therefore, the secondary metabolites as the bioactive compounds, fatty acids and fatty acid methyl esters which identified in the lebui bean could be associated with antioxidant, anti-microbial, and usefull for additives in food.

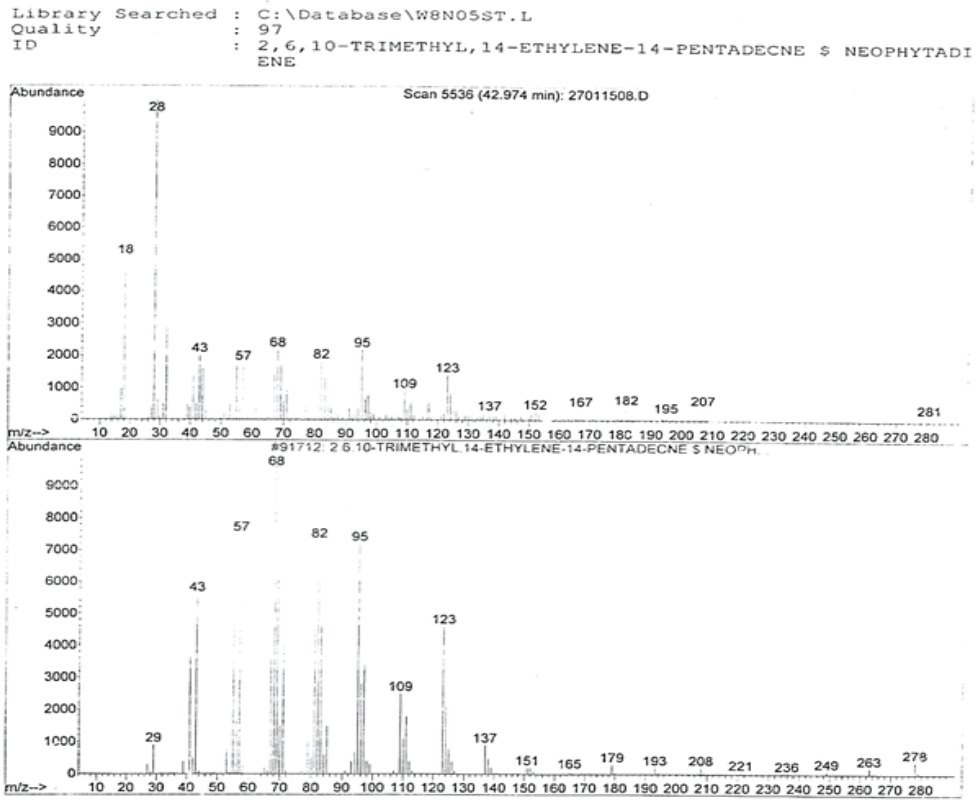

Figure 1. Mass spectrum of Neophytadiene by GC-MS

Conclusion

The results of this experiment showed ethanol 70% was the best solvent to extract bioactive compound in the lebui bean. The lebui bean powder and the extracts in ethanol and ethyl acetate contained alkaloids, flavonoids, polyphenols, terpenoids, steroids, and 44 constituents of secondary metabolites fatty acids. The largest quantities of bioactive compound were the class of terpenoids.

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