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Phytochemical Screening and Antioxidant Activity of Unripe Banana Flour

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ABSTRACT

This study was to screen the phytochemical as well as total phenolic compound (TPC) and antioxidant capacity of unripe banana flour (UBF). The cultivated cultivars (*Musa abb* cv. *Kluai "namwa"*) flour was processed by sliced and dried at 50ºC, subsequently milled into the flour. The chemical composition profile of UBF was determined using the Liquid Chromatography-Mass Spectrometry-Electrospray Ion (LC-MS-ESI), while ABTS and DPPH scavenging capacity were investigated. The presence of phytochemical composition of UBF depend on the extractant type. Sugar (glucose and sucrose) and organic acid (citric acid, malic acid, lactic acid, succinic acid) were found as a generally component of UBF in all extractant. The methanolic and ethanolic extracts found the phenolic compounds such as gallic acid, glutaric acid, 2-hydroxyvaleric acid, protocatechuic acid, 1,4-Ipomeadiol, 5,6-dimethoxyflavone with exhibit the antioxidant activity.The hexane fraction found the short chain hydrocarbons, fatty acids and its derivative including caproic acid, 1-(2-Thienyl)-1-heptanone, 2-tetradecanone, azacridone A, 5-aminopentanoic acid, piperine and phytosphingosine. Various organic acids (gluconic acid, succinic acid, 3-hydroxy-cis,cis-muconic acid, trans,cis-aconitic acid dibutyl succinic acid), which was found in UBF extracted with water.

The TPC contents of methanolic and ethanolic extract were found at 1900.67 and 2000.13 mg GAE/g DW, respectively. The methanolic and ethanolic extract were greater antioxidant capacity than the hexane and distillated water. The IC50 value was found at 5.46 mg/ml and 2.54 mg/ml by DPPH assay, while the ABTS assay examined the IC50 at 6.45 mg/ml and 5.97 mg/ml of ethanolic and methanolic extract, respectively. The chemical composition and their antioxidant activity of unripe banana flour provide the valuable data to apply in healthy food products.

Keywords: *Banana, flour, bioactive compounds, antioxidant, LC-MS*

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INTRODUCTION

Banana is widely grown fruit in tropical region, especially in Thailand. It presence of a various phytochemical and nutrition which provide health benefits contribution (Sidhu and Zafar, et al., 2018; Pereira and Marachin et al., 2015; Sigh et al., 2016).The major compounds was found in bananas are phenolic compounds (tannic acid, gallic acid, catechin) (Vu et al., 2018), vitamin, carotenoid, flavonoid, fiber, resistant starch, biogenic amines, phytosterols and minerals (Singh et al., 2016; Sidhu et al., 2018). Moreover, the previous study reported that the banana contained varying of bioactive compounds type depend on the banana part. Banana rhizome rich in many polyphenolic compounds having antioxidant activity (Kandaswamy and Aradhya 2014; Russell, 2009).Banana fruit contains alpha-carotene, pro-vitamin A and trace of phenolic acid which have a strong antioxidant capacity (Anyasi et al., 2018). Banana florets are the source of anthocyanin, cyanidin-3-rutinoside, and its derivative (Pazmino-Duran et al., 2001), while pseu-do stem having dietary fiber, lignin, hemicellulose, polyphenol and flavonoids (Aziz et al., 2011). Banana peel and pulp are also rich in phenolic compounds, flavonol glycoside, phytosterol and biogenic amine. (Cook and sammon, 1996).

In Thailand, the banana field was distributed through the country. The research area in this study are called “Nongtoom Local Entroprise ”, Sukhothai, Thailand, well-known community to produce unripe sliced banana fry to export. The productivity was 11,322 ton/years contributing the income around 1,132 million THB/year. However, the small unripe banana fruit unwanted to the process, it become a waste of the factory around 2 tons/days.

This study aim to improve valuable of unripe banana fruit waste to unripe banana flour (UBF). The UBF will be extracted with different solvent, chemical composition as bioactive compounds and antioxidant capacity were determined. Previous reports found that the major compounds in unripe banana flour are gallic acid, catechin (Bennett et al., 2010; Borges et al., 2014), epicatechins, gallocatechin (Borges et al.,2014: Anyasi et al., 2018), myricetin 3-O-rhamnosyl-glucoside (Anyasi et al., 2018) and flavonoid leucocyanidin (Lewis&Shaw, 1999).The flavonoids which have been attributed to their antioxidant and chelating properties of UBF, as well as anti-mutagenic anti-tumoral effects. The flavonoids also have been reported to inhibit a variety of enzymatic activities (Pereira & Maraschin, 2015). According to the Shahidi and Ambigaipalan (2015), myricetin and rutin has been reported to protect α-tocopherol from decomposition process. In contrast, the flavonol compounds did not found in unripe banana according to the report of Regazzoni et al. (2013).

Although, various phytochemical of unripe banana has been reported, but the different cultivation area, soil, fertilizer and banana cultivars will be effect on their phytochemical content. Naczk & Shahidi, 2006 who found that the different banana cultivars provide a varying the polyphenol contents. Cultivation techniques, climatic conditions, cultivar variations, methods of extraction and degrees of ripening also effect on the phytochemical content (Naczk and Shahidi, 2006). The other factors such as storage condition, postharvest treatment, pasteurization, sterilization, fermentation, blanching and other food processing methods are also

significantly alter the phenolic concentration of these plant materials (Amarowicz et al., 2009). Therefore, the UBF which produced in the area of Sukthothai province of Thailand, subtropical climate, will be collected, extracted, and phytochemical analysis. The Liquid Chromatography-Mass Spectrometry-Electrospray Ion (LC-MS-ESI) is a powerful technique was employed to analyze and identify the phytochemical in UBF same as the previous reports. (Anyasi., 2018; Borges et al., 2014; Kandasamy et al., 2014; Fatemeh et al., 2012; Bennett., 2010) . The phytochemicals profile in UBF will provide the advantage data for food and pharmaceutical applications.

MATERIALS AND METHODS

Sample preparation

The unripe banana fruit waste was obtained from the Looktung banana frying factory, Nongtoom, Sukhothai, Thailand. Unripe banana were peeled and rondelle sliced, then soaked in the water for three times. The sliced banana were dried under oven at 50^{o}C and milled through flour milling machine at 200 mesh pore size. The unripe banana flour (UBF) will be obtained and keep in refrigerator at 4^{o}C until phytochemical compounds and antioxidant activity analysis.

Phytochemical compounds extraction

The UBF was separately mixed with ethanol, methanol, hexane and distilled water at the ratio of 1:10. The extractions were macerated with different solvent for 1 week followed by filtration using Whattman No.1 filter paper, subsequently centrifuge at 6000 rpm for 30 min. The filtrate was vacuum dried using rotary evaporator at 57^{o}C and the concentrates were stored at 4^{o}C. The residues were separately redissolved with the extraction solvent for phytochemical compounds analysis by LC-MS-ESI.

Liquid chromatography with electrospray ionization mass spectrometry analysis

The separation and identification of phytochemical in UBF was conducted using LC-MS-ESI. Briefly, the separation was conducted on the 35 °C of C18 column (Luna 4.6x150 mm,5 µm Phenomenex,USA) of HPLC (1260 infinityAgilent Technologies, Germany) with 20 µL injection volume. Eluted mobile phase was used by mixing of the solvent A (0.1%Formic acid/H_2O) and solvent B (0.1% formic acid /acetonitrile). The separation was carried out under gradient condition between mobile phase A:B which was started at the ratio of A:B at 95%:5% and then adjusted to 80%:20% within 10 min.

Mass analyzer was determined using the 6540 UHD Accurate Mass Q-TOF LC-MS (Agilent Tecnologies, Singapore) in range 50-1000 Da. Ionization was achieved with an electrospray source using a cone voltage of 10,20,40 eV using negative and positive mode or determination of phenolic compounds. Desolvation gas used was Nitrogen at a flow rate of 10 L/min, temperature of 350 °C, nebulizer 30 psig Vcap 3500V fragmentor 100V(positive mode), 250 (negative mode) skimmer1 65 V Octapole RFP 650V. The phytochemical identification of unripe banana flour was determined based on mass analyzer compared with mass spectrum database.

Total phenolic Content in unripe banana flour

The total phenolic was determined using Folin-Ciocalteu colorimetric method with slight modification (Majhenic et al, 2007). The extraction samples were separately resuspended in methanol, ethanol, hexane and water) mixed with the Folin-Ciocalteu reagent containing phosphomolybdic phosphotungstic acid reagents. The reaction products were monitored base on the reduction of MoO^{4+} to MoO^{3+}. The reaction colour change from yellow to blue and measured at 765 nm using a UV spectrophotometer microplate. Final results of total phenolics were calculated as gallic acid standard equivalent (mg GAE/100 g d.w.)

Determination of antioxidant capacity by DPPH radical scavenging

To determine the ability of UBF extract to scavenge the unstable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH radical) using the modification method proposed by Szerlauth et al, 2019. The analysis basis base on the absorption of DPPH radicals at 517 nm undergoes a decrease in absorption upon reduction by an antioxidant compounds. The DPPH radical scavenging percentage was calculated following equation;

DPPH Scavenged (%)=(AB-AA)/AB)×100, where, AB is absorbance of blank at t=0 min; AA is absorbance of the antioxidant at t=30 min. A calibration curve was plotted with %DPPH scavenged versus concentration of standard antioxidant (Trolox).

Dilution of different concentrations of 10, 20, 40, 60, 80, 100 and 120 mg/mL of the sample was used to determine the IC_{50} of the sample with final values of IC_{50} obtained by plotting the percentage disappearance of DPPH as a function of the sample concentration.

Determination of antioxidant capacity by ABTS radical scavenging assay

Free radical scavenging activity of banana flour was determined by ABTS radical cation decolorization assay by modification method of Szerlauth et al, 2019. $ABTS^{\cdot+}$ cation radical was produced by the reaction between 2,2-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid (ABTS) and potassium persulfate (1:1), stored

in the dark at room temperature for 12-16 hours before use. $ABTS^{\cdot+}$ solution was then diluted with methanol to obtain an absorbance around 0.700 at 734 nm. Addition of the extract to diluted $ABTS^{\cdot+}$ solution, the absorbance was measured at 30 min after the initial mixing. An appropriate solvent blank was run in each assay. All the measurements were carried out at least three times. Percent inhibition of absorbance at 734 nm was calculated using the formula following;

$ABTS^{\cdot+}$ scavenging percentage (%) = (AB-AA)/AB)×100, where, AB is absorbance of ABTS radical+methanol; AA is absorbance of ABTS radical+sample extract/standard. Trolox was used as standard substance.

RESULTS AND DISCUSSION

Phytochemicals content in unripe banana flour

The phytochemical component of UBF extraction were found varying with the polarity of solvent extraction under the same extraction parameters. In this work, glucose, sucrose, malic acid, citric acid, lactic acid, fructose derivative (D-1-[(3-Carboxypropyl)amino]-1-deoxyfructose), dimethylmalonic acid and the fatty acid derivative(9-hydroperoxy-12,13-epoxy-10-octadecenoic acid, 21-dimethylarsinoyl-(7Z,10Z,13Z,16Z,19Z)-heneicosapentaenoic acid, (Z)-3-Methyl-3-decenoic acid) were found as the major components in UBF of all extractant (methanol, ethanol, hexane and distillated water). Phytosphingosine,2,4-Dimethylpimelic acid, C16-Sphinganine, Piperine were obtained in the extractant including methanolic, ethanolic and hexane. Phenolic compound such as gallic acid, glutaric acid, 2-hydroxyvaleric acid, protocatechuic acid and 1,4-Ipomeadiol were only found in methanolic and ethanolic extraction. Moreover, methanolic extract was unique found choline, 5,6-Dimethoxyflavone, 9,12,13,trihode, 2-Hexyl-1,3-dioxan-5-ol. Gluconic acid, succinic acid, 3-hydroxy-cis,cis-muconic acid and cis-aconitic acid were obtained when using distillated water as extractant.

Among the UBF phytochemicals which was found in this study, we will be discussed only the main abundance compounds and their biological activity below.

Sucrose and glucose, starch degradation products, were found as the major compounds in UBF as shown in Table 1-4 and Figure 1-4 which correlated with the previous data of Menezes et al., 2011; Seymour , 1993; Kheng et al., 2012. They has been found that the sucrose is the predominant sugar at green stage of banana and it shows a decreasing pattern during ripening. In contrast, the results of Sidhu, 2018 reported that none of the sugars were detected at fully green stage. They found fructose, glucose, and sucrose in the banana flesh during ripening stage, while maltose was not detected at any stage of ripeness. This contrast result may be due to change in the variety of banana under the study and the high temperate weather. Moreover, the resistant starch and dietary fiber content in unripe banana suitable

material for the production of flour with great reduction glycemic index (GI) of diet, as in the prevention of diabetes and colon cancer (Anyasi et al., 2013).

This study also found the malic acid and citric acid as main component of all extractant. The related results reported that the malic acid and citric acid are the most naturally abundant organic acids in banana flesh-at the fully green stage, while tartaric acid and oxalic acid were detected in low amounts (Maduwanthi and Marapana, 2019). These organic acids contributing to the acidic taste and decrease along with accumulation of sugars in banana fruit flesh during fruit ripening. Pua et al., 2003 reported that malic acid accumulation in banana flesh through glyoxylate cycle, regulated by *MaMS-1* gene, which can be induced by ethylene. Furthermore, Maduwanthi and Marapana, 2019 reported that the malic acid amount will variation depend on fruit respiration rate. It increased during green stage and decline after ripening stage. The variation of malic acid somewhat corresponds with respiration rate of the fruit. It increases up to a point where ripening begins and then declines.

Interestingly, this study also found the piperine in UBF extracted with ethanol, methanol and hexane. Piperine normally abundance in pepper, it has shown many physiological functions such as antimicrobial, anti-inflammatory, anticancer, anti-infective, insecticidal, antiamoebic, antiulcer, and antidepressant (Tiwari et al., 2020). It uses for blood circulation enhancement, salivation, and stimulation of appetite (Kozukue et al., 2007), pain management, chills, rheumatism arthritis, influenza, and fever (Correa et al., 2010). Park et al., *2019* reported the piperine also inhibiting the expression of *PPAR-γ* gene of 3T3-L1 cell lines (Park et al., 2010; 2019), thus food containing piperine may use in the treatment of diseases related to obesity. Moreover, black pepper extract which containing piperine can inhibit the adipocyte differentiation of 3T3-L1 cells and also markedly inhibited the adipogenic transcription factors-(*SREBP-1c*, and *C/EBPβ*) (Park et al., 2010). Yoon, et al., 2015 reported that the ethanolic extract of sample containing piperine can induce the modulation of cAMP through signal transduction pathway, calcium levels and phosphorylation of CREB in preadipocyte 3T3-L1 cells with no toxicity. Moreover, piperine showed significant changes in the pharmacokinetic profile of simvastatin, verapamil, and secnidazole when given alone and in combination with a fixed-dose of piperine (10 mg/kg). Piperine exhibits 2.53, 1.55, and 1.08 fold increases in the bioavailability of these drugs, respectively (Auti et al., 2018;2019). Thus, plant extract containing piperine can be effectively used for the prevention and treatment of obesity because it has an effect of inhibiting the accumulation of visceral fat and weight loss

Gallic acid was found in ethanolic and methanolic extract in this study, it was classified into the phenolic compound, in which the hydroxy groups are at positions 3, 4, and 5. Various phenolics present in banana have been identified as

follows: gallic acid, catechin, epicatechin, tannins, and anthocyanins (Sidhu and Zafar, 2018). It has a role as an antioxidant, arachidonic acid 15-lipoxygenase inhibitor and an apoptosis inducer. Subramanian et al., 2016 reported that the growth of colon cancer cells (HCT-15) was inhibited after treated with gallic acid. Briefly, the flow cytometric analysis and SEM scanning method showed the evident associated with apoptosis like lipid layer breakage and fall in mitochondrial membrane. SEM images of the gallic acid-treated HCT-15 displayed membrane blebbing and shrinking characteristics of apoptosis. Cell cycle arrest was evident from the accumulation of gallic acid treated HCT-15 cells at sub-G1 phase. The methanolic and ethanolic extract were also found the phenolic compounds such as glutaric acid, 2-hydroxyvaleric acid, protocatechuic acid, 1,4-Ipomeadiol which having antioxidant activity.

Choline was also detected in methanol and ethanol fraction same as the gallic acid in this study. The previous report was found choline in cavendish banana (Sidhu and Zafar, 2018). It a precursor of acetylcholine or neurotransmitter biosynthesis, control glucose homeostasis (Korsmo et al., 2020) and lipid metabolism (Wu et al., 2012). Wu et, al., 2012 reported that mice feeding with the diet containing choline showed slowly body weight gain, decreased fat mass , plasma glucose level and improved insulin tolerance. The reduction of hepatic glucagon receptor expression in obese and reduced diabetic mice after diet containing choline mice feeding . Recently, Korsmo et al., 2020 found that the choline supplementation in high fat-fed (CSHF) mouse dams during gestation prevents fetal overgrowth and excess adiposity, improves long-term blood glucose homeostasis. Moreover, the expression of insulin receptor substrate 1 (*Irs1* gene) increase and the metabolic marker (insulin, leptin, adiponectin) decrease in serum of male and female rats offspring after 6 week post CSHF feeding.

The miscellaneous compounds which were found in this study are amino acid (glycine, asparagine, tyrosine, threonine, lysine, leucine and tryptophan) which abundance in banana flour when extracted with water. Among the amino acid, tryptophan is being interesting as the precursors for the synthesis of dopamine, possibilities of preventing neurodegenerative diseases like Parkinson. The increasing of dopamine content from unripe to the ripened stage in both the peel and pulp has been reported by many workers (Romphophak *et al.*,2005; Gonzalez-Montelongo *et al.*,2010). Moreover, the sterol and phytosphingosine, was also found in UBF of this study. The results corresponding with the lipophilic extract of ripe banana pulp from several cultivars, *M. acuminata* and *M. balbisiana,* has been found a source of ω-3 and ω-6 fatty acids, phytosterols, long-chain aliphatic alcohols, and α-tocopherol for nutritional and health benefits (Vilela *et al*., 2014)

Moreover, the phytochemicals in banana extracted with water (Table 4) did not show the any the phenolic acid composition. The main compounds in the water

faction are organic acid such as gluconic acid, malic acid, caproic acid and succinic acid. These compounds examined the biological activity as well as the other bioactive compounds. Briefly, succinic acid become a new target for the treatment of endometrium cancer by induce endometrial cancer cell lines apoptosis (Iplik et al., 2018). It also significantly reduced epididymal white adipose tissue mass and no effect on insulin, glucose, or pyruvate tolerance due to the high fat diets for 3 month (Ives, et al., 2020). Importantly, Mycielska, et al., 2019 discovered that gluconic acid, glucose derivatives which were found naturally in fruit, are a competitive and irreversible inhibitor for extracellular citrate uptake by cancer cells, and inhibiting human tumor growth in immunodeficient mice. Caproic acid was found in UBF of hexane fraction in this study, it is a short chain fatty acid containing 6 atom of carbon. Li et al., 2017 reported that the short chain fatty acid, stearidonic acid (SDA), suppress the adipocyte differentiation and lipid accumulation in 3T3-L1 cells detected by Oil red-O staining method and triglyceride (TG) quantification.

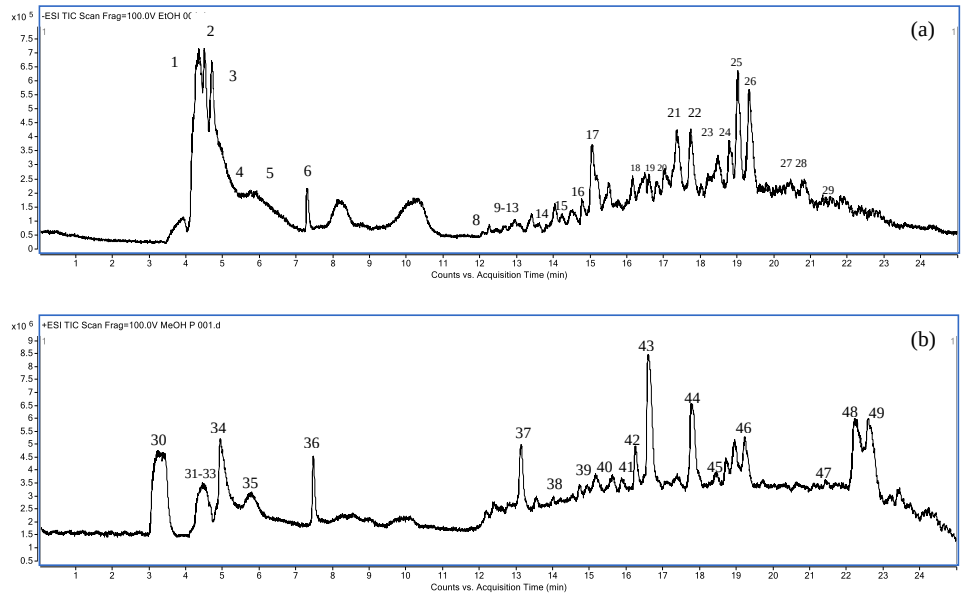


Figure 1 LC-MS Chromatograms of unripe banana flour with methanol at different MS analysis conditions: (a) negative mode (b) positive mode

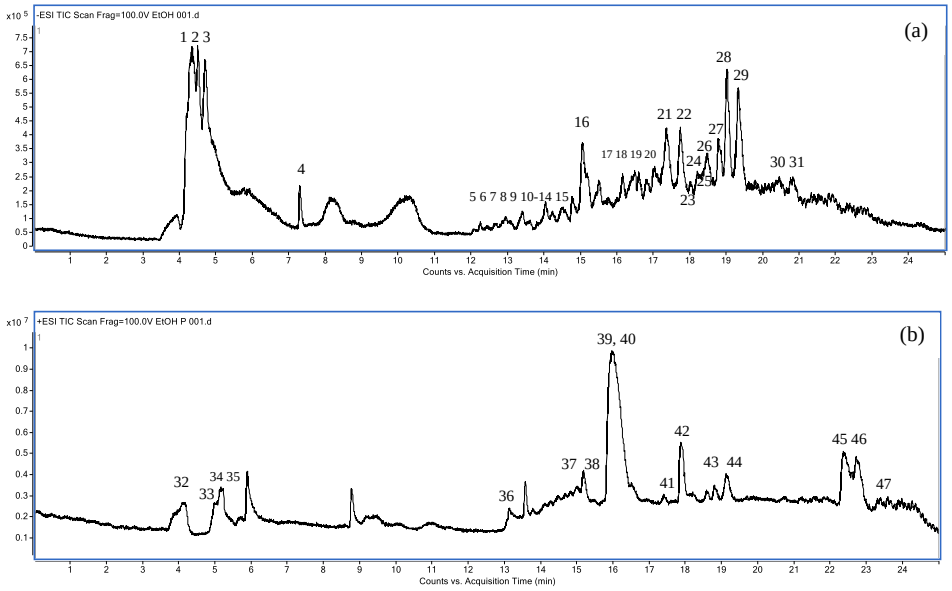


Figure 2 LC-MS Chromatograms of unripe banana extracted with ethanol at different MS analysis conditions: (a) negative mode (b) positive mode

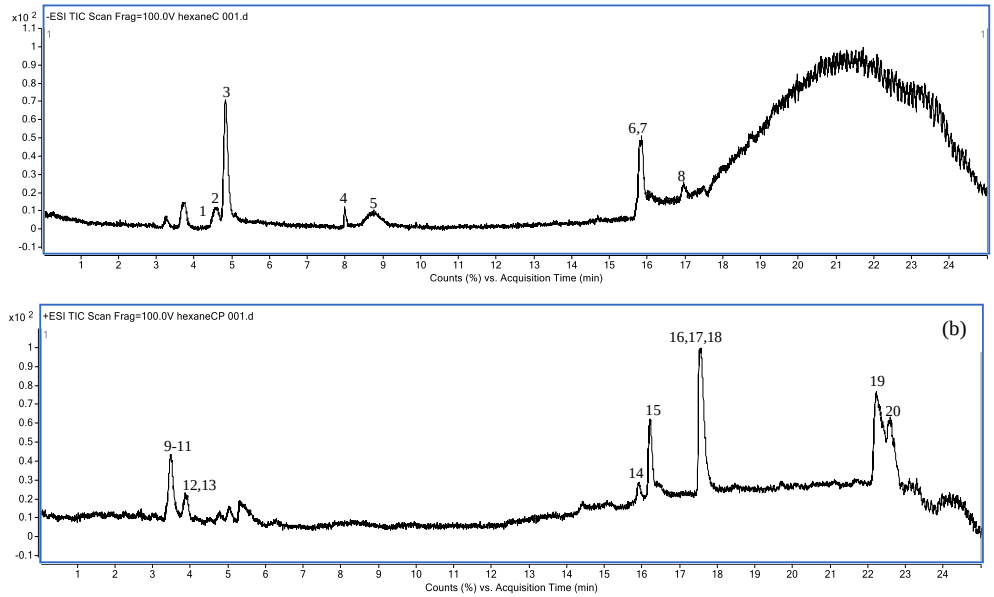


Figure 3 LC-MS Chromatograms of unripe banana flour with hexane at different MS analysis conditions: (a) negative mode (b) positive mode

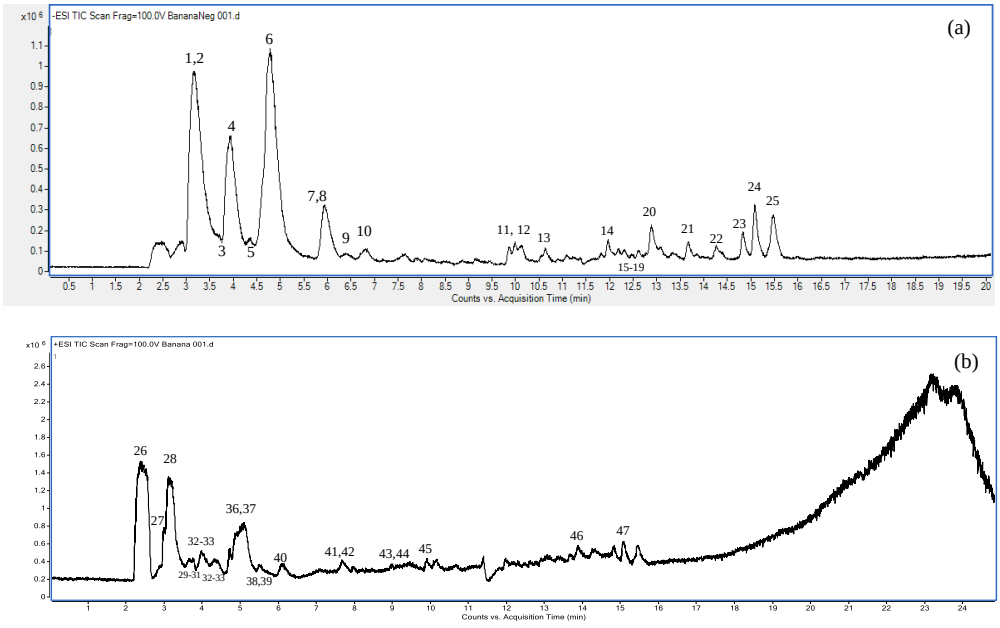


Figure 4 LC-MS Chromatograms of raw banana flour with distillated water with different MS analysis conditions: (a) negative mode (b) positive mode

Table 1 Phytochemical composition of unripe banana flour extracted with methanol

Peak number	RT (min)	M/Z	Identified name
1	4.343	215.0298	Glucose
2	4.512	377.0814	Sucrose
3	4.876	133.0117	Malic acid
4-5	5.146	191.0169	Citric acid
6	7.321	89.024	Lactic acid
8	12.812	169.0151	Gallic acid
9	13.019	131.0353	Glutaric acid
10	13.305	117.0559	2-Hydroxyvaleric acid
11	13.405	218.1051	Pantothenic acid (B5)
12	13.81	131.0353	Dimethylmalonic acid
13	13.947	153.0199	Protocatechuic acid
14	13.993	-	unidentified
15	14.425	153.0201	Protocatechuic acid
16	15.028	216.0904	1-Isothiocyanato-8-(methylthio)octane
17	15.156	175.063	2-Isopropylmalic acid
18	16.499	317.201	Menthyl pyrrolidone carboxylate
19	16.751	247.1578	2-Hexyl-1,3-dioxan-5-ol
20	16.777	341.1049	5,6-Dimethoxyflavone
21	17.324	187.1	2,4-Dimethylpimelic acid
22	17.687	444.2682	(*E*)-3-[4-[(3*E*,5*Z*)-hepta-1,3,5-trien-3-yl]phenyl]-1-[4-[(*E*)-5-phenylpent-2-en-2-yl]phenyl]prop-2-en-1-amine
23-24	18.735	327.2229	9,12,13,TriHODE
25	18.947	435.1944	21-dimethylarsinoyl-(7Z, 10Z,13Z,16Z,19Z)-heneicosapentaenoic acid
26	19.262	329.2388	11,12,13-trihydroxy-9-octadecenoic acid
27	20.348	327.2214	9R,12R,13S-Trihydroxy-10E,15Z-octadecadienoic acid
28	20.775	169.0883	1,4-Ipomeadiol
29	21.54	329.237	9S,12S,13S-trihydroxy-10E-octadecenoic acid
30	3.291	185.0666	D-Xylonic acid hydrate (1:1)
31	4.317	219.0276	Glucose
32	4.32	104.1073	Choline
33	4.454	266.1247	D-1-[(3-Carboxypropyl)amino]-1-deoxyfructose
34	5.071	381.0805	Unidentified
35	5.755	130.0489	Pyroglutamic acid

Table 1 (continued) Phytochemical composition of unripe banana flour extracted with methanol

Peak number	RT (min)	M/Z	Identified name
36	7.456	123.0539	Niacinamide
37	13.121	166.0842	Phenylalanine
38	14.704	342.1633	2-valeryl-sn-glycero-3-phosphocholine
39	15.147	207.1353	Etrogol
40-41	15.611-15.8	341.218	Unidentified
41	15.843	355.1982	Unidentified
42	16.243	679.5055	Contaminate from membrance
43	16.603	325.2242	Unidentified
44	17.776	274.2716	C16 Sphinganine
45	17.825	318.2974	Phytosphingosine
46	19.23	213.1462	Dihydrojasmonate
47	21.946	295.2241	9-hydroxy-10-Octadecen-12-ynoic acid
48	22.257	286.1412	Piperine
49	22.595	286.14	Piperine

Table 2 Phytochemical composition of unripe banana flour extracted with ethanol

Peak number	RT (min)	M/Z	Identified name
1	4.338	215.0277	Glucose
2	4.522	377.0789	Sucrose
3	4.832	133.0098	Malic acid
4	7.3	89.0239	Lactic acid
5	12.858	169.0156	Gallic acid
6	13.067	131.0355	Glutaric acid
7	13.335	117.0561	2-Hydroxyvaleric acid
8	13.408	218.1064	Pantothenic acid
9	13.97	153.0206	unidentified
10	14.019	443.2014	unidentified
11	14.448	153.0202	Protocatechuic acid
12	14.518	219.0542	unidentified
13	14.791	471.1803	unidentified
14	15.027	625.1552	unidentified
15	15.062	216.0918	1-Isothiocyanato-8-(methylthio)octane
16	15.19	175.0638	2-Isopropylmalic acid
17	16.126	289.1707	unidentified
18	16.477	261.1383	unidentified

Table 2 (continued) Phytochemical composition of unripe banana flour extracted with ethanol

Peak number	RT (min)	M/Z	Identified name
19	16.593	369.2187	unidentified
20	17.016	273.1756	unidentified
21	17.35	187.1005	2,4-Dimethylpimelic acid
22	17.729	444.2712	unidentified
23	18.006	301.2072	unidentified
24	18.167	273.1753	unidentified
25	18.205	145.088	unidentified
26	18.477	229.1483	Dipropyl hexanedioate
27	18.791	327.2254	unidentified
28	19.006	435.1979	21-dimethylarsinoyl-(7Z, 10Z,13Z,16Z,19Z)-heneicosapentaenoic acid
29	19.321	329.2414	unidentified
30	20.76	329.2392	9S,12S,13S-trihydroxy-10E-octadecenoic acid
31	20.802	169.0887	1,4-Ipomeadiol
32	5.158	219.0282	Glucose
33	5.175	455.1203	Unidentified
34	5.201	266.1256	D-1-[(3-Carboxypropyl)amino]-1-deoxyfructose
35	5.425	138.0556	3-Pyridylacetic acid
36	13.13	122.0967	N,N-Dimethylaniline
37	15.022	453.3467	Unidentified
38	15.184	679.5174	Contaminate from membrance
39	15.993	649.4527	Unidentified
40	16.024	325.2295	Unidentified
41	17.868	274.2756	C16 Sphinganine
42	18.803	454.2317	20-Oxo-leukotriene E4
43	19.106	318.3024	Phytosphingosine
44	19.151	295.2286	9-hydroxy-10-Octadecen-12-ynoic acid
45	22.389	286.1459	Piperine
46	22.721	286.1456	Piperine
47	23.319	312.1614	Piperettine

Table 3 Phytochemical composition of unripe banana flour extracted with hexane

Peak number	RT (min)	M/Z	identified name
1	4.562	215.034	Glucose
2	4.82	683.2288	Sucrose
3	5.098	133.0144	Malic acid
4	7.978	89.0247	Lactic acid
5	8.717	191.0214	Citric acid
6	15.799	723.5062	Membrane contaminated
7	15.803	713.4769	Membrane contaminated
8	16.939	187.0985	2,4-Dimethylpimelic acid
9	3.464	128.0767	Homoanserine
10	3.465	185.0676	D-Xylonic acid hydrate (1:1)
11	3.465	99.0556	1-(2-Thienyl)-1-heptanone
12	3.888	139.0741	Caproic acid
13	3.888	81.0317	Tricholomic acid
14	15.888	453.3478	unidentified
15	16.192	679.5183	Contaminate from membrance
16	17.522	274.2763	C16 Sphinganine
17	17.553	318.303	Phytosphingosine
18	17.575	230.2495	2-Tetradecanone
19	22.198	286.1475	Piperine
20	22.556	286.1465	Piperine

Table 4 Phytochemical composition of unripe banana flour extracted with water

Peak number	RT (min)	M/Z	identified name
1	3.144	195.0507	Gluconic acid
2	3.267	215.0326	Glucose
3	3.934	133.0141	Malic acid
4	3.947	191.0195	Citric acid
5	4.364	290.0882	2,7-Anhydro-alpha-N-acetylneuraminic acid
6	4.792	191.0198	Isocitric acid
7	5.95	117.0192	Succinic acid
8	5.966	157.0138	3-hydroxy-cis,cis-muconic acid
9	6.411	173.0087	cis-Aconitic acid
10	6.806	173.0085	trans-Aconitic acid
11	9.874	216.0879	(3-Methylcrotonyl)glycine methyl ester

Table 4 (continued) Phytochemical composition of unripe banana flour extracted with water

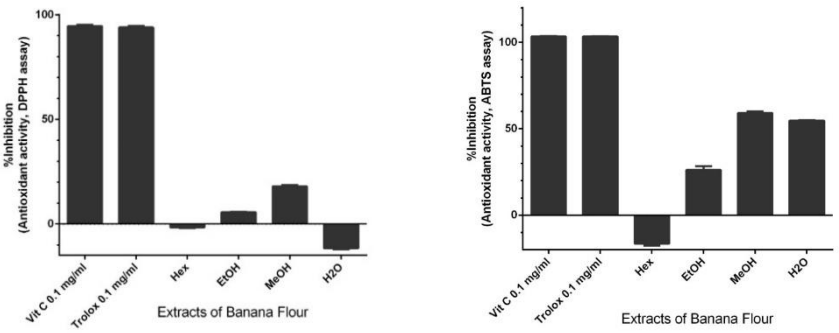
Peak number	RT (min)	M/Z	identified name
12	10.008	175.0611	2,3-Dimethyl-3-hydroxyglutaric acid
13	10.643	351.1301	Gly Asn Tyr
14	11.972	261.1343	Triethylene glycol diglycidyl ether
15	12.183	451.1818	Trp Thr Thr
16	12.32	247.155	3-Hydroxydecanoic acid
17	12.62	273.1707	3-hydroxy-tetradecanedioic acid
18	12.888	187.0975	Methyl N-(a-methylbutyryl)glycine
19	12.924	287.0772	2-Hydroxy-3-carboxy-6-oxo-7-methylocta-2,4-dienoate
20	13.098	442.2446	unidentified
21	13.67	444.2607	Lys Trp Leu
22	14.277	229.1446	Dibutyl succinate
23	14.841	327.2185	9-hydroperoxy-12,13-epoxy-10-octadecenoic acid
24	15.094	435.1881	(-)-11-hydroxy-9,10-dihydrojasmonic acid 11-beta-D-glucoside
25	15.483	329.2343	9S,10S,11R-trihydroxy-12Z-octadecenoic acid
26	2.42	185.0637	Unidentified
27	2.989	104.1061	Choline
28	3.092	309.1272	Azacridone A
29	3.159	118.0852	5-Aminopentanoic acid
30	3.646	130.0854	N-Methyl-L-proline
31	3.674	280.1372	N-(1-Deoxy-1-fructosyl)valine
32	4.013	262.0905	Unidentified
33	4.061	204.1326	Glycyl-Lysine
34	4.26	171.1479	Unidentified
35	4.413	274.0902	Unidentified
36	5.046	130.0384	Unidentified
37	5.139	268.0801	Unidentified
38	5.514	132.1008	L-Leucine
39	5.624	294.1522	N-(1-Deoxy-1-fructosyl)leucine
40	6.104	284.0961	Guanosine
41	7.667	328.1361	N-(1-Deoxy-1-fructosyl)phenylalanine
42	7.677	166.0847	L-Phenylalanine
43	9.887	234.1106	Unidentified
44	9.927	218.1005	Unidentified

45	10.094	207.136	(Z)-3-Methyl-3-decenoic acid
46	13.889	183.0763	Unidentified
47	15.447	295.2236	12-oxo-8E,10E-octadecadienoic acid

Antioxidant capacity of unripe banana with different solvent extraction

The antioxidant activities of UBF extracted with methanol, ethanol, hexane and water were compared by ABTS and DPPH assays as shown in Figure 5. The ethanolic and methanolic extraction showed the free radical inhibition capacity greater than those the other extractant. In contrast, the UBF extracted with hexane did not show the antioxidant activity. It might be due to that the ethanolic and methanolic extract containing the phenolic compounds which were exhibit free radicle scavenging activity. The phenolic compound (5,6-Dimethoxyflavone and protocatechuic acid), were found in polar solvent extract may possess antioxidant activity higher than the non-polar extractant. Therefore, the solvent polarity effect on the antioxidant capacity was found in this study.

Considering the IC_{50} value comparison, the ethanol showed the greater antioxidant activity than methanol (Figure 6). The IC_{50} value is a quantitative of bioactive compounds substance needed to 50% of oxidation process inhibition. The IC_{50} values of antioxidant compounds in ethanol were 2.54 mg/ml and 5.97 mg/ml by DPPH and ABTS assays, respectively, which less than the methanol fraction (IC_{50} = 5.46 and 6.42). Indicated that, ethanol is the most powerful extractant for antioxidant compounds in UBF in this study. This result was consistent with the higher TPC content in UBF of ethanol than methanol fraction in Table 5, may account for the better antioxidant effects in ethanol solvent. However, UBF extracted with hexane did not show the antioxidant activity, it might be due that non-polar hexane examines low ability to extract antioxidant compounds. This finding was consistent with the study of Musa et al., 2010, when increase the polarity of solvent up to 50% water will contribute the solubility of the antioxidant compounds in the polar solvent. The results indicated that the recovery of antioxidant compounds depends on the type and polarity of solvent used. Alothman et al. 2009 suggested that the polarity of solvents indirectly played a vital role in extraction process since it would increase the solubility of antioxidant compounds. Moreover, the solubility of antioxidant compounds in solvent was proven to have strong influence on the recovery of those compounds during the extraction processes.



(a) (**b**)

Figure 5 antioxidant activity of unripe banana determined by different methods (a) DPPH assay (b) ABTS assay

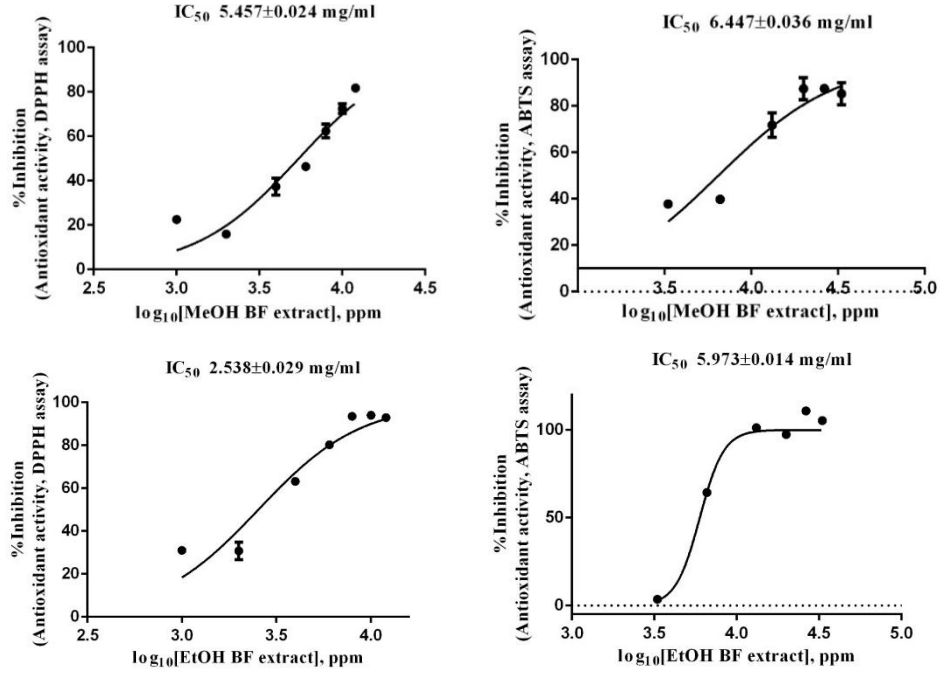


Figure 6 IC50 values of antioxidant compounds extracted with (a) methanol (b) ethanol using DPPH and ABTS methods

This observation was similar to the result reported by Yan et al. (2006) who used the ethanol for antioxidant extraction from banana tissues. Recently, Truong, et al., 2019 who identified that the polar solvent is the most effective solvent for extraction, resulting in the highest extraction yield as well as the highest content of phenolic, flavonoid, alkaloid, and terpenoids. The crude extract obtained from methanol extract showed low antioxidant capacity (IC_{50} value of 16.99 μg/mL) when compared with ethanol extraction. In contrast, Shian, et al., 2012 found that the 50% and 70% of acetone, ethanol and methanol showed the powerful extractant for antioxidant compounds extraction than the 100% of extraction solvent. It might be that the pure solvents (acetone, ethanol and water) having too polarity properties and weak extraction. Mixing the polar solvent with water will decrease the polarity index and increased antioxidant compounds solubility. However, Shian, et al., 2012 reported that the 100% methanol extraction exhibit the total phenolic content (403-1139 mg gallic acid/100 g of banana) than 100% ethanol and water of three different banana cultivars.

Furthermore, this study determined the total phenolic compounds (TPC) content in UBF as shown in Table 5. Phenolic compounds are important fruit constituents because they exhibit antioxidant activity by inactivating lipid free radicals and preventing decomposition of hydroperoxides into free radicals (Maisuthisakul et al., 2007). The ethanolic and methanolic extract in this study exhibit higher TPC content when compared with the previous reports. The TPC determined by 80% methanolic extracts of banana peel and pulp flours was 75.01 to 685.57 mg GAE/100 g, while this study found 1,900-2,000 mg GAE/100 g. However, no evidence of hexane extract containing the TPC, it might be that the non-polar of hexane does not elute the phenolic compound from the UBF. Iloki-Assanga, et al., 2015 discovered that the analysis of the hexane phase extracts revealed the presence of carotenes, triterpenes/steroids, and lactonic groups. The ethanol and aqueous extraction phases revealed the presence of a range of compounds, including tripterpenes/steroids, lactonics groups, saponins, phenols/tannins, amines and/or amino acids, and flavonoids/anthocyanins. The highest total phenolic and flavonoid content were observed at 523.886 ± 51.457 µg GAE/mg extract of methanol fractions. Pereira and Maraschin (2015) and Singh *et al.* (2016) who reported that banana is rich in many bioactive compounds such as carotenoids, flavonoids, phenolics, amines, vitamin C, and vitamin E having antioxidant activities to provide many human health benefits. Recently, Vu *et al.* (2018) reviewed the phenolic compounds and their potential health benefits prepared from banana peel. They suggested the valuable by-product from banana fruit processing industry can use in food and pharmaceutical industry (Vu *et al.*, 2018: Mathew and Negi., 2017)

Table 5 The phenolic compounds contents of banana flour extracted with different solvent using the Folin–Ciocalteau assay

Solvent	Total phenolic (mg GAE/g DW)
Water	0
Hexane	0
Ethanol	2000.13
Methanol	1900.67

CONCLUSIONS

The previous discussion will confer that the polarity of extractant will affect on bioactive compounds extraction from the UBF. Although, low polarity of ethanol and methanol (compared with water) but both extractant can eluted phenolic compounds from banana flour than those the non-polar solvents. The phenolic compounds in ethanolic and methanolic extract confer with the TPC content and antioxidant capacity. Distillated water and hexane do not exhibit the antioxidant activity may cause both solvents does not containing the phenolic compounds. However, the other chemical composition of water possesses the anti-cancer, anti-obesity and in duce cell line apoptosis. Indicated that, UBF having high content of bioactive compounds to promote health benefit. Development of many functional foods made from UBF cloud be interesting

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