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Evaluation of bioactive compounds of *aloe vera* extract using sub-critical water method

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ABSTRACT

The *Aloe vera* peel extraction of 10 minutes at 115°C gave the best effect of its chemical composition. Whilst *Aloe vera* skin using subcritical water method was obtained from extracted with water at 125°C for 60 minutes. It had following properties: mineral content of 20.654 mg/g; protein content of 120.114 mg/g; vitamin C content of 161.431 mg/g; aloin content of 10.251 mg/g; saponin content of 9.71 mg/g; total phenolic content of 39.851 mg/g; antioxidant activity of 87.651 %. The FTIR analysis showed that CH Stretch in alkane vibration at 2941.24 cm⁻¹ to 2991.39 cm⁻¹ wave was found for the methylene compound as antioxidant agent.
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KEYWORDS

Aloe vera skin;
 Bioactive compounds;
 Time and temperature.

INTRODUCTION

Aloe vera have been attributed to the polysaccharides contained in the gel and skin. Recently, *Aloe vera* gel have been diversification into cessing product mostly from gel^[1]. Although there is high interest in gel, the interest of skin into a

tection to the bioactive compound on *Aloe vera* can be conducted by subcritical water method, such as extraction process using water with temperature between boiling point and critical point. Subcritical water extraction has several advantages of being readily available, cheap and efficient^[2]. The result of subcritical water extraction, extraction water

processing is very limited information. However, the *Aloe vera* skin has a big potentio to developed into a diversification product. *Aloe vera* (L.) skin contained organic and bioactive compound that act as antioxidant^[3-2].

phenolic compound (flavonoid, phenolic acid, and phenolic diterpene) and anthraquinone compound (aloin)^[3]. *Aloe vera* also contained alkaloid and also saponin that act as antinutritive^[4].

The reduction of antinutritive compound and pro-

become short time, cheap and safe for health^[5] and subcritical water at 100 to 110°C appears to be an alternative to organic solvent due to efficiency at low polarity condition^[7]. However, there is limited information on the effect of time and temperature of extraction on subcritical water to decrease antinutritive compound and bioactive compound of *Aloe vera* keep protected.

The objectives of current study was to find out the bioactive compound from the effect of time and

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temperature of subcritical water which result from the previous study.

MATERIALS AND METHODS

acid solution which has been in the mixed indicator. Subsequently titrated with a solution of HCl 0.1 N. Blank determination is also performed.

Sample (1 g) was dissolved in 1 mL chloroform: methanol 95:5 (v/v) and 10 µL was drop on TLC

Results

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High-performance liquid chromatography analysis of plant saponins: An update 2005-2010

Aloe vera of 10 months old and weight of 3 kg were obtained from Kalimantan Barat, Indonesia. After harvesting the *Aloe vera* peel were then washed by aquades, weight, peeled and separated from the gel. Blended raw materials were extracted with water at 125°C for 60 minutes. Sample was filtered using vacuum filtration until produce filtrate and residue.

Evaporator rotary vacuum was used to disappear solvent in filtrate at 40°C for 1 h. Filtrate was centrifuged with a speed of 5500 rpm for 10 minutes. The supernatant obtained was used to concerned parameter analysis.

Total phenolic content was determined using 0.5 mg/g of gallic acid as a standard. Briefly, 1 mL sample extract in methanol solution was transferred in tubes containing 5 mL Folin-Ciocalteu's reagent and also 4 mL Na₂CO₃ (75% w/v) was transferred in tubes. The tubes were then mixed and then allowed to stand at room temperature for 30 min before absorbance at 765 nm was measured. The total phenolic content was expressed as gallic acid equivalents (GAE) in mg/100 mL of sample.

Extract of *Aloe vera* skin was weight of 2 g into the vitresile crucible overnight in an electric muffle furnace maintaining the temperature between 400-410 °C until obtain the ash. This ashing destroys all the organic material from the sample. The ash was removed from crucible and allowed to dry in desiccator. The yield of ash was approximately 5 g/100 g.

Sample was weight of 3.5 g and entered into Kjeldahl apparatus. 10 g of Na₂SO₄ anhydrate and 15-25 mL of H₂SO₄ concentrated were added and then heated on burner flame until obtain clear greenish. Cooled, then diluted and put into a 200 mL volumetric flask, matched to the dash. A total of 5 mL of solution pipetted and introduced into the distillation device, add 5 mL 45% NaOH and several drops of phenolphthalein indicator. Distilled for about 10 minutes, as the reservoir using 10 mL of 2% boric

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plat distance of 2 cm. Poured TLC plat to the glass beaker for 40 minutes. Measured the top edge and the plats need to dried for 10 minutes. Plat was heated at 90°C for 10 minutes. To visualize the spots, plat was sprayed with the solution mixed between 25 mL concentrated sulfate acid and 25 mL aquadest (1:1), then heated at 140°C for 40 minutes. The amount of spot were counted and measured by the Rf value.

The *Aloe vera* (L.) skin were analyzed for its antioxidant activity using the DPPH (2,2-diphenyl-2-picrylhydrazyl) radical scavenging assay. Sample (200 g) was dissolved in 100 mM Tris-HCl buffer (800 µL, pH 7.4) followed by the addition of 1 mL 500 µM DPPH. The solution was homogenized using a shaker and storage in dark room for 20 min. Spectrophotometry was used to determine the absorbance at 517 nm.

The *Aloe vera* (L.) skin was analyzed for its functional compounds using FTIR (Fourier Transform Infra Red). The IR spectra were recorded on FTIR-8400S (Shimadzu Deutschland GmbH) spectrophotometer in KBr and polyethylene pellets. Samples were weigh-in at 0.01 g and homogenized with 0.01 g KBr anhydrous by mortar agate. The sample and KBr mixture were pressed by vacuum hydrolic (Graseby Specac) at 1.2 psi to obtained transparency pellet. Scanned sample passed through infra red, where its continuing wave by detector connected to computer with set values of tested sample spectrum. Samples were usually scanned in the absorption area of 500-4000 cm⁻¹. The results of analysis consisted of chemical structure, molecular binding form and certain functional group of tested sample as basic of spectrum type.

RESULTS AND DISCUSSION

Mineral content

Minerals found in *Aloe vera* are calcium (118,77 ppm), zinc (0,45 ppm), chromium (0,28 ppm), po-

tassium (640,51 ppm), copper (1,28 ppm), manganese (0,36 ppm) and iron (0,23 ppm) (extract by Atomic Absorption Spectrophotometer (AAS))^[8]. They are essential for the proper functioning of various enzyme systems in different metabolic pathways and few are antioxidants^[9].

Mineral content of *Aloe vera* skin was 20.654 mg/g. Minerals are not affected significantly by chemical and physical treatment during processing as a presence of oxygen. Some minerals lead an oxidized become a higher valent. Although several components damaged as a heating process, this method was not affected to the amount of mineral and also the value of its nutrition. Heat treatment had no significant effect on the ash content^[10].

Protein content

Aloe vera gel provides 20 of the 22 necessary amino acids required by the human body, there are 7 of the 8 non-essential amino acids and 12 essential amino acids^[8]. Protein of *Aloe vera* consist of lectins

in *Aloe vera* is known as tocopherol^[17]. Vitamin C and E are two important nutrients that may reduce free radicals and a strong line of defense to slow Reactive Oxygen Species (ROS)-induced cellular damage. Vitamin C prevents the prooxidant activity of vitamin E by decreasing the activity of tocopheroxyl radical to tocopherol, thereby contributing to increased total antioxidant status and reducing oxidative stress^[18].

Vitamin C content of *Aloe vera* skin was 161.431 mg/g, which is similar to the one reported by^[19], who found vitamin C content from *Aloe vera* extracted at 80 °C for 60 min was 154.64 mg/g. Properties of vitamin C is easily changed due to oxidation yet if a stable crystalline (pure)^[15]. Degradation of vitamin C depends upon many factors such as oxygen, heat, light storage temperature and storage time^[20]. Antioxidant compound such as vitamin C may occur the degradation at 35 °C. Antioxidant such as vitamin C will lead the degradation about 38% during contact

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and lectin-like substance^[11].

Protein content of *Aloe vera* skin was 120.114 mg/g. The protein content was increased with the increase in extraction temperature up to 240 °C and showed no obvious degradation to the extracted material^[12]. Heat treatment over 125°C increases in concentrations of Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), Acid Detergent Insoluble Nitrogen (ADIN) and Non-Degradable Protein (NDP) on forages^[13]. However, the further increase in temperature can transform the molecule structure called as degradation. Damaged proteins are first recognized by molecular chaperones, which facilitate protein repairing, if the damage is too extensive or under conditions unfavorable for protein repair, damaged proteins are targeted for degradation^[14].

Vitamin C content

Vitamin C in plant is secondary metabolites as it is formed from glucose through D-glucuronic acid and L-gulonate^[15]. Vitamin C is an important antioxidant contained *Aloe vera*. At certain concentrations, vitamin C can increase bioavailability by increasing the concentration in blood cells^[16]. Vitamin C and E

with a high heat^[21] and decrease of vitamin C about 40 to 60% may occur due to heating at 82-92 °C^[22].

Vitamin C was decreased about 15% affected both by increasing temperature and duration of storage^[23] and Pi noted that the long duration of extraction may be decreased of vitamin C due to hydrolysis and degradation^[24]. Vitamin C content of orange were 232.9 mg/100g, but after an eight week storage, vitamin C contents of orange at 28, 37 and 45°C decreased to 194.9, 52.4 and 39.3 mg/100g, it was observed that vitamin C decreased with increasing temperature as expected^[20].

Aloin and saponin content

Aloin is the major anthraquinone of aloe exudates and gels and it is characterized as the C-glycoside of aloe-emodin^[25]. **Aloin and aloe-emodin are present largely as aglycones in aloe, and the contents were similar to those of aloe hydrolysate, which might be due to the cleavage of the sugar moiety by heat during processing^[26].**

Aloin content of *Aloe vera* skin was 10.251 mg/g. However, this result was less than the one reported by^[19], where they found the aloin content from *Aloe vera* skin which extracted at 80 °C for 60 min

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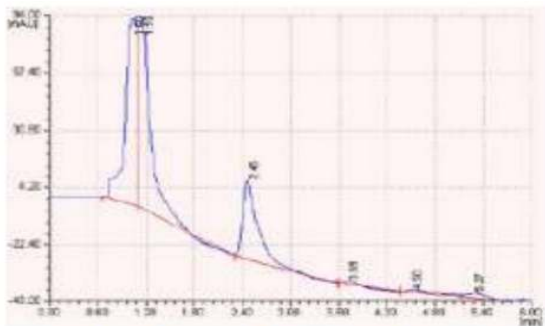


Figure 1 : HPLC chromatogram of sample

was 10.34 mg/g. This different figures might possibly be due to temperature of extraction. The aloe-emodin content decreased as the heating was extended^[26].

The aloin can be identified using High Performance Liquid Chromatography (HPLC) method. High performance liquid chromatography (HPLC) is the usual technique for the determination of individual components in *Aloe vera* leaf extracts^[27].

The HPLC separations of isolated sample from *Aloe vera* can be done with references to standard were performed on a Cyber Lab C-18 column (250 x 4.0 mm, 5µ)^[28]. Their result can be seen on Figure 1.

Saponin is the type of glycosides commonly found in plants. Saponins characterized by foam, so when treated with water and whipped it will form a foam which can last long^[29]. Saponins can have anti-nutritional effects, whereas some saponins may have beneficial health effects^[30]. *Aloe vera* contains saponins which are soapy substances form 3 % of the gel and are general cleansers, having antiseptic and anticarcinogen properties. Aloe contains campesterol, F2 sitosterol and lupeol^[8].

Saponin content of *Aloe vera* skin was 9.71 mg/g. This result was also less than the reported by^[19], where they found saponin content was 10.250 mg/g. Saponin content depends on factors such as the cultivar, the age, the physiological state and the geographical location of the plant^[31], such as between 1.5-23 g/kg in seed crops, 100 g/kg in Madhuca seeds,

up to 100-300 g/kg in quillaja bark and licorice root, respectively (Alexander et al., 2009). The heat has effect to the degradation of saponin and saponins are soluble in water, so saponins reduction can be caused also by subcritical water method^[32].

The saponin can be identified using High Performance Liquid Chromatography (HPLC) method too. **The normal- and reverse-phase HPLCs are commonly used for separation, identification, and purification of saponins. But for the best separation of saponins, RP-HPLC is normally used. HPLC is increasingly used in the separation of various compounds including saponins. This technique is rapid, selective, and highly sensitive. Separation of saponins can be affected by HPLC using variety of stationary and mobile phases^[33].**

Total phenolic content

Phenolics are mainly present in fruits, seeds, and herbs. Phenolic compounds have powerful antioxidant activities in vitro, based on their structure, hydrogen-donating potential, and ability to chelate metal ions^[32].

Total phenolic content of this research was 39.851 mg/g. The amount and type of phenolics and their conjugates differ markedly even in different tissues of the same species^[32]. Subcritical water extraction has been applied in the determination of organic pollutants in soils, sludges and sediments, and also is used for the extraction of volatiles from plant

material^[34]. Increased total phenol caused by high of extraction temperature, so resulted in the degradation of cell wall due to destruction of carbohydrate and protein by heat which facilitated the discharge of phenol from plant tissue^[35]. Total phenolic content of *Aloe vera* gel increased from 54.46 ± 7.87 to 103.95 ± 5.83 mg GA/100 g d. m. by high hydrostatic pressure. **High pressure treatment can increase the rate of mass transfer, and enhance solvent penetration into the cells by disrupting the cellular walls and hydrophobic bonds in the cell membrane, which may lead to a high permeability, the increased of total phenolic compounds could have resulted from plant cell disruption caused by high hydrostatic pressure leading to a higher extractability of these compounds**^[36].

Increase in total phenolic content may be related to an increased extractability of some of the antioxidant components following high pressure processing^[37]. The yield of phenolic compounds was increased with increasing of extraction temperature, but high yield of phenolic compounds not necessary accompanies with high antioxidant capacity, as the antioxidant activity of crude extract can also be influenced by the structure and interaction between extracted phenolic compounds^[38].

Antioxidant activity

Antioxidants are substances that neutralize free radicals or their actions, act at different stages^[39]. Antioxidants are important in the body defense system and reactive oxygen species^[40]. Antioxidants have traditionally been divided into two groups, primary (phenolics or vitamin E) and secondary (acetates, citrates, tartrates, and phosphates)^[32]. *Aloe vera* is one example of plants that contain antioxidants^[39].

Antioxidant activity of *Aloe vera* skin was 87.651 % and this result was higher than the report one by^[19], where they found *Aloe vera* skin which extracted at 80 °C for 60 min was 86.166%. The antioxidant potential of the plant extracts are influenced by various factors and largely depends on both the composition of the extract and the analytical test system^[40]. The higher level of antioxidant activity are probably due to extraction of subcritical water method.

But, antioxidant activity can decreases with increases the temperature^[41]. Drying temperature influenced on the loss of antioxidant capacity. Drying at 90° C caused antioxidant capacity losses of 25% higher than at 50° C^[42]. The heat could cause a re-

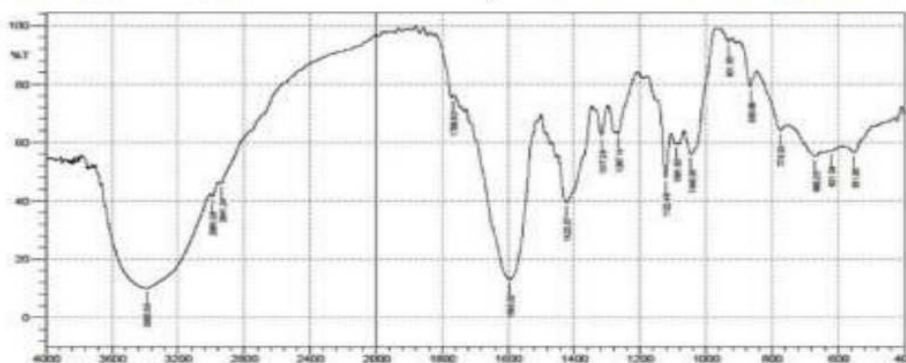


Figure 2 : Infra red spectrum of *Aloe vera* skin extract

TABLE 1 : Functional compounds of *Aloe vera* skin extracted

No	Wave lenght (cm ⁻¹)	Vibration type	Functional compound
1	551.6	Ring Roxing	Aromatic
2	621.04	C-OH bending	Fenol AR-H
3	669.25	CH Bending	Aromatic
4	775.33	CH Bending	Alkena substituted
5	865.98	C-C or C-Cl stretch	Aromatic acid halida
6	931.55	C-C or C-Cl stretch	Aliphatic acid halida
7	1045.35	C-O-C stretching aliphatic	Ether ROR
8	1091.63	C-O-C stretching aliphatic	Alkohol secondary R-OH
9	1122.49	C-O-C stretching aliphatic	Alkohol secondary R-OH
10	1267.14	C-O-C stretch alkil aril ether	Ether ROR
11	1317.29	NO ₂ stretching aromatic	Nitro NO ₂
12	1423.37	Ring aromatic stretch (4p)	Aromatic
13	1595.02	NO ₂ stretch konjugation	Keton RCOR
14	1768.6	C=O stretch α-lakton	L akton
15	2941.24	CH Stretch in alkana	Methilene-CH ₂
16	2991.39	CH Stretch in alkanena	Methilene-CH ₂
17	3392.55	OH stretching in bonded	OH

duction in the antioxidant capacities of crude extract^[38].

Identification compounds of *aloe vera* skin extract by fourier transform infra red (FTIR)

The infra red spectrum of *Aloe vera* skin extract as shown in Figure 2 was in wave length range of 551.6 cm^{-1} to 3392.55 cm^{-1} , and there were 17 functional compounds found (see TABLE 1). The presence of wave length of *Aloe vera* were aromatic, phenol, alkene substituted, aromatic acid halida, aliphatic acid halida, ether (R-O-R), alcohol secondary (R-OR), Nitro (NO_2), Keton (R-CO-R), Lactone, methilene, and OH.

The infrared spectrum of *Aloe vera* skin extract had generated one functional compound namely OH at 3392.55 cm^{-1} . While the presence wave length range at 2941.24 cm^{-1} to 2991.39 cm^{-1} can be attributed to methilene, wave length at 1768.6 cm^{-1} can be attributed to keton. The presence of strong to medium intensities bands were also observed at 1423.37 cm^{-1} ; 1317.29 cm^{-1} ; 865.98 cm^{-1} ; 669.25 cm^{-1} ; and 551.6 cm^{-1} which confirms of aromatic group.

Infrared spectrum of *Aloe vera* skin which extracted at 80 °C for 60 min had generated wave length at 330.69 cm^{-1} and 1728.1 cm^{-1} which confirms of

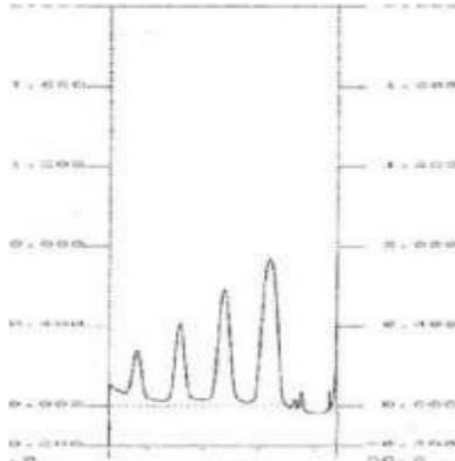


Figure 3 : α -tocopherol of best aloe vera skin extract carboxylic acid, wave length at 2981.74 cm^{-1} to 2935.46 cm^{-1} which confirms of methilene^[19]. Infrared spectrum of *Aloe vera* had generated wave length range at 611.4 cm^{-1} ; 717.5 cm^{-1} ; 1051.1 cm^{-1} ; 1398.3 cm^{-1} ; 1623.9 cm^{-1} ; 1730.0 cm^{-1} ; 2912.3 cm^{-1} ;

3155.3 cm^{-1} ; and 3398.3 cm^{-1} ^[43].

Figure 3 can be seen that the levels of α -tocopherol contained in the sample was 4, 82 532 μg . The use of maltodextrin between 5-10 % in addition to functioning as a mineral can also protect the vitamin from the effects of temperature, but it does not mean that these components can not be damaged^[44]. The maltodextrin used will be the wall of a thin polymer that serves as a wrapper or coating the core material, so more the amount of maltodextrin is added will be more bioactive compounds that are protected, including α -tocopherol, although the protection afforded is not maximum, because α -tocopherol remain damaged by heating^[45]. However, drying with the use of foaming agents still very well applied to foods that are sensitive to heat, because at least still be able to protect components that are sensitive to heat than the method without foaming.

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