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Identification of Aloin and Saponin and Chemical Composition of Volatile Constituents from *Aloe vera (L.)* Peel

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ABSTRACT

Aloe vera peel is a part from aloe vera plant which has chemical composition and ability as antioxidant agent. In the other hand, *Aloe vera* peel also contain of antinutrition compounds, namely aloin and saponin which lead negative effect. Extraction process of aloe vera peel at 80^0C for 60 minutes could decrease aloin and saponin compounds from 4.27% to 0.987% and from 5.43% to 0.728%, respectively and the product was found increase in antioxidant activity from 50.37% to 86.166%. The GCMS analysis for the major compounds identified were squalene (23,60%), 7-tetradecane (19,66%), limonene (13,86%), n-hexadecanoic acid (10,20%), campesterol (1,60%), β sitosterol (1,37%), 9-octadecanoic methyl ester (7,56%), carvone (9,44%), comaric (7,64%), lupeol (3,45%) and eicosane (1,58%). The FTIR analysis shows that the functional components in the *Aloe vera* peel were nitro $–NO_2$, aromatic, phenol Ar-OH, substituted alkene, aromatic acid halide, aliphatic acid halide, eter R-OR, secondary alcohol R-OH, carboxylic acid, RCOOH, and metylene $–CH_2$.

Keywords: Aloin, saponin, *Aloe vera (L.)* peel, active compounds and functional compounds

INTRODUCTION

Aloe Vera is a plant which many contain python-components compounds such as vitamins, nutrients and compounds anti-nutrient [1]. Currently the most widely used from *Aloe Vera (L.)* is the part a gel, whereas part of its peel not yet utilized optimally. Miladi and Damak [2] reported that the skin of *Aloe Vera* has antioxidant activity, while Lakshmi and Pa Rajalakshmi [3] also suggested that its antioxidant activity due to volatile compounds namely tetradecanoic acid, hexadecanoic methyl ester, n-Hexadecanoic acid and Squkene.

In the other hand, the peel of *Aloe vera (L.)* is also containing aloin and saponin compound. Bozzi et al. [4] noted that aloin compounds found in most parts of the skin than the *Aloe vera* gel. Singh et al. [5] also noted that aloin in aloe vera is a bitter component which is applied to the skin as first aid for burns, laxative, anti-obesity preparation and pharmaceutical formulations. Adhusan [6] reported that in aloin also can act as antioxidant compounds, but can lead negative effect for health in an excessive amount

Saponins are the complicated natural compounds and have a large molecule consisting of aglicosteroid or triterpenoid with one or more chains of sugar/ glycosides. Supardjo [7] noted that saponin can lead antinutrition and toxic effect in food.

Gulia et al. [8] reported that extracted at 50-80^0C can cover to antioxidant compounds and also can decrease antinutrition such as aloin and saponin from 10.6 to 1.7 ppm. Azman et al. [9] also reported that the amount of antioxidant increase at 45-100^0C and have decreased at 120^0C. The objectives of current study were to find out the effect of extraction process at 80^0C for 60 minutes to alteration of aloin, saponin, antioxidant activity and volatile compounds and functional groups of *Aloe vera (L.)* peel.

MATERIALS AND METHODS

Sample preparation

Aloe vera of 10 months old and weight of 1.2 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.

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Aloe vera peel were sliced thin and filled in the blender glass jar and were analyzed for its aloin, saponin and antioxidant activity as raw material.

Blended raw materials were extracted with water at 80^0C for 60 minutes. Sample was filtered using vacuum filtration until produce filtrate and residue. Evaporator rotary vacuum was used to disappearance solvent in filtrate at 40^0C for 1 h. Filtrate was centrifuged with a speed of 5500 rpm for 10 minutes. The supernatant obtained was used to concerned parameter analysis

Determination of aloin and saponin compounds using TLC (Thin Layer Cromatography) [10].

Sample (1g) was dissolved in 1 ml chloroform: methanol 95:5 (v/v) and 10 µl was drop on TLC plat with 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 90^0C 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 140^0C for 40 minutes. The amount of spot were counted and measured by the Rf value.

Determination of antioxidant using 2,2-diphenyl-2-picrylhydrazyl (DPPH) [11]

The *aloe Vera* peel extract was analyzed for its antioxidant activity using 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) and then added 1 ml 500 µM DPPH. The solution was homogenies for 20 minute in a dark room. Spectrophotometry was used to determine the absorbance at 517 nm.

Gas chromatography and mass spectroscopy (GC-MS) and Fourier Transform Infra Red (FTIR) analysis [12]

Volatile compound from the peel of *Aloe vera (L.)* was analyzed using GC-MS QP2010S-Shimadzu under the following condition: column used were Rtx-5MS, 30 m length and inner diameter of 0.25 mm and the initial column temperature was 70^oC and final temperature was 280^oC (5^o C/minute), while the injector temperature was 300^o C with split mode injector and split ratio of 72.6 and pressure of 14.0 kPa. The flow rate was 40 ml/minute and the flow within the column was 0.50 ml/minute. The detector temperature was 300^0C and using Helium as the gas carrier with EI (Electron Impact); and the samples volume injected was 1µl. Compounds were identified by comparing retention indices/comparing mass spectra of each compound with those of authentic samples and library. While for functional unit determination the Shimadzu Fourier Transform Infrared Spectrophotometer - FTIR-8400S were used.

Fourier transforms Infrared (FTIR) spectrophotometric analysis [12]

The Infra red spectra were recorded on FTIR-8400S (Shimadzu Deutchland 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 mixture of sample and KBr were pressed by vacuum hydraulic (Graseby Specac) at 1.2 psi to obtained transparency pellet. Scanned sample passed through infra red, where its continuing wave by detector that connected to computer and given described 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

Aloin on the extracted *Aloe vera (L.)* peel at 80^0C for 60 minutes was 1.034%. It was decrease compared with aloin from raw material which was 4.27%. Gulia et al. [8] noted that the decrease of aloin was due to thermal processing at 50-80^0C from 10.6 ppm to 1.7 ppm and also aloin has not the nature of heat-resistant and will be hydrolyzed. There were also similar reported by Ramachandra and Rao [13], the heating at 30-80^0C can decrease aloin compound due to the destruction of a parenkim tissue on the extracted material. Lullmann et al. [14] noted that aloin was included in anthraquinon compound which is strong in the aloe vera plant. Aloin was glycoside group which formed in the hydroxil benzene carbon and easily disengaged with electron donor.

Saponin on the extracted *Aloe vera (L.)* peel at 80^0C for 60 minutes was 1.025%. It was decrease compared with saponin from raw material which was 5.43%. Shi et al. [14] reported that the cooking medium and methods greatly influenced saponin B degradation during cooking. A similar research was carried out by Rickert et al. [15], the effects of process temperature (25^0C or 60^0C) on plant extract which contained

saponin lead an increasing number of saponins extracted. There were also similar reported by Alupului et al. [16], due to process heating at a certain temperature to occur a disorder of mechanical in the cell walls of plant, therefore saponin lead extrication and displacement of the period. The other factors which lead decrease saponin in a material were extraction time. Battal [17] reported that the extraction of saponin at 90-95^{0}C for 15 minute were 11.58-19.58%. And Vivekananda et al. [18] noted that time is influential in the process of extraction, by increasing the extraction time, quantity and quality of extracted material analyses will lead increased and degradation.

The antioxidant activity of *Aloe vera (L.)* peel extract at 80^{0}C for 60 minutes was 86.166%, which is similar to the one reported by Sultana [19], who found the antioxidant activity of their sample was 37.2-86.6%. The high of antioxidant activity from a plant was affected by the content of phenolic compounds. Anilakumar et al. [20] suggested that the antioxidant activity of *Aloe vera* was 72.2%, which was higher than synthetic antioxidant such as BHT was about 70.5% and α- tokoferol was about 65.65%.

A typical gas chromatogram of extracts of *Aloe vera (L.)* peel at 80^{0}C for 60 minutes is shown in Figure 1 and a list of eleven compounds identified appears in Table 1. The major compound identified was squalene (23.60%), followed by 7-tetradecane 19,66%, limonene 13,86%, n-hexadecanoic acid 10,20%, campesterol 1,60%, β sitosterol 1,37%, 9-octadecanoic methyl ester 7,56%, carvone 9,44%, comaric 7,64%, Lupeol 3,45% and Eicosane 1,58%. Increase dactivity the capture of free radicals due to n the solubility of the active components is possible because the cell wall materials are damaged due to warming [21]. Besides the heat factor, the high capture activity of free radicals by Rahayuni, Sutardi and Umar [22], caused by the extract on were performed using water, where in the antioxidant properties of aloe vera on the skin is hidofilik, so that the bioactive antioxidant extract edquite a lot.

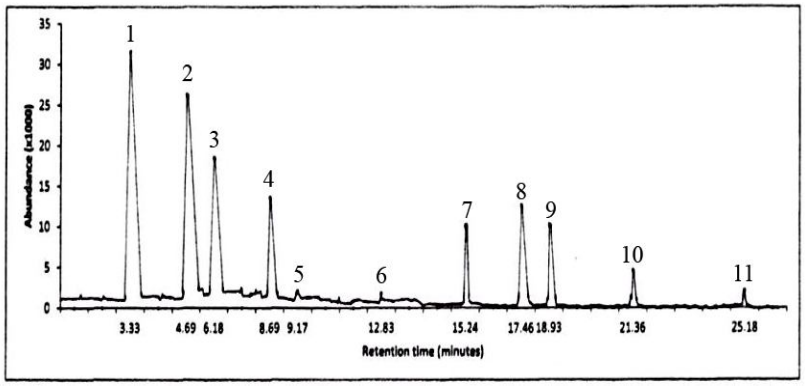


Figure 1 GC-MS chromatogram of *Aloe Vera (L.)* peel extract

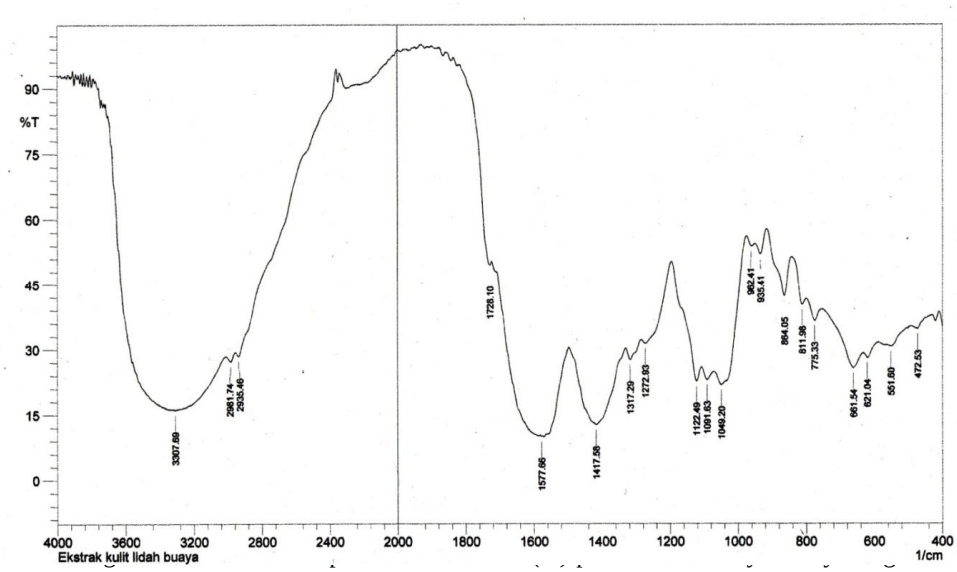
Table 1 Major pytho-components from *Aloe Vera (L.)* peel extract for 60 minutes at 80^{0}C

Peak	Compound	Composition (%)
1	Squalene	23.60
2	7-Tetradecane	19.66
3	Limonene	13.86
4	n-Hexadecanoic acid	10.20
5	Campestral	1.60
6	B-Sitosterol	1.37
7	9-octadecanoic methyl ester	7.56
8	Carvone	9.44
9	Comaric	7.64
10	Lupeol	3.45
11	Eicosane	1.58

Lakshami and Rajalakshami [3] noted that some phyto-components in *Aloe vera* which in function as antimicrobial and antioxidants were 1-heptanol 2 propyl, diazene, tetradecanoic acid methyl ester, hexadecenoic, benzendycarbocyclix acid, eicosyne, oleic acid and squalene, whereas Lisa et al. [23], found coumaric and phytosterol namely campesterol, β-sitosterol in their samples. Lachenmeier et al. [24] suggested the data about limonene in *Aloe vera* is 7.93% and Magwa et al. [25] noted that limonene included in hydrocarbon. Miller et al [26] suggested that limonene included in a class monoterpenoid as a group of compounds active which very promising in terms of health in addressing anticancer and antioxidant. The next

compounds as reported by Coopasamy [27] in which function as antioxidant was carvon, tetradecanoic acid and n-hexadecanoic acid and function as a topical treatment for skin problems and burns.

The infra red spectrum of *Aloe vera (L.)* peel extract at 80^0C for 60 minute as shown in Figure 2 was in the wave length range of 472.53 cm^{-1} to 3307.69 cm^{-1}, and there was 20 functional compounds found (Table 2), namely aromatic, nitro-NO_2, phenolic Ar-OH, substituted alkene, aromatic acid halide, aliphatic acid halide, ether R-OR, Alcohol secondary R-OH, carboxylic acid RCOOH and methylene $–CH_2$.



Aloe Vera skin extract

Figure 2 The infra red spectrum of *Aloe vera (L.)* peel extract at 80^0C for 60 minute

Table 2 Functional compounds of *Aloe Vera (L.)* peel extract analyzed by using FTIR

No.	Wave length (cm^{-1})	Type of vibration	Functional compound
1	472.53	NO2 Rock	Nitro-NO_2
2	551.6	Def Ring (2p)	Aromatic
3	621.04	COH bend	Fenol Ar-OH
4	661.54	OH bend	Fenol Ar-OH
5	775.33	CH_2 kel. Bid. Wag	Alkene substitution
6	811.98	C-C or C-Cl stretch	Aromatic acid halide
7	864.05	C-C or C-Cl stretch	Aromatic acid halide
8	935.41	C-C or C-Cl stretch	Aliphatic acid halide
9	962.41	C-C or C-Cl stretch	Aromatic acid halide
10	1049.2	C-O-C stretch aliphatic	Ether R-OR
11	1091.63	C-OH stretch	Alcohol secondary R-OH
12	1122.49	C-OH stretch	Alcohol secondary R-OH
13	1272.93	C-O-C stretch alkyl aril eater	Ether ROOR
14	1371.29	NO_2 stretch aromatic	Nitro NO_2
15	1417.58	Ring aromatic stretch	Aromatic
16	1577.66	Ring aromatic stretch (4p)	Nitro NO_2
17	1728.1	C=O stretch monomer	Carboxylic acid RCOOH
18	2935.46	CH stretch in alkane	Metylane $–CH_2$-
19	2981.74	CH stretch in alkane	Metylane $–CH_2$-
20	3307.69	OH stretch H- bonded	Carboxylic acid RCOOH

The presence of broad bands at 3307.69 cm^{-1} can be attributed to carboxylic acid stretching vibrations. The presence of strong to medium intensities bands were also observed at 1417.58 and 1577.66 cm^{-1} which confirms of ring aromatic stretch. Rajendran et al. [28] noted that functional compounds of *Aloe vera* skin by using infrared produced wave length (cm^{-1}) 611.4; 717.5; 1051.1; 1398.3; 1623.9; 1730.0; 2912.3; 3155.3 and 3398.3.

CONCLUSION

Aloin and saponin compounds obtained from *Aloe vera (L.)* peel which extracted at 80^0C for 60 minutes were 0.987% and 0.728%, respectively, whilst its antioxidant activity was 86.166%. It has been shown eleven volatile compound which were determined by GC-MS and FTIR, namely squalene (23,60%), 7-tetradecane (19,66%), limonene (13,86%), n-hexadecanoic acid (10,20%), campesterol (1,60%), β sitosterol (1,37%), 9-octadecanoic methyl ester (7,56%), carvone (9,44%), comaric (7,64%), lupeol (3,45%) and eicosane (1,58%). The FTIR analysis shows that the functional components in the *Aloe vera* peel were nitro $–NO_2$, aromatic, phenol Ar-OH, substituted alkene, aromatic acid halide, aliphatic acid halide, eter R-OR, secondary alcohol R-OH, carboxylic acid, RCOOH, and metilena $–CH_2$.

REFERENCES

1. Maenthalsong R, Chalyakunapruk N and Niruntrapon S. 2007.The Efficiency of aloe vera for burns and wound healing, *a systematic review. Burns*. 33:713-718.

2. Miladi S and Damak M. 2008. In Vitro Antioxidant Activities Of Aloe Vera Leaf Skin Extracts. *J. Soc. Chim. Tunisie*. 10 : 101-109.

3. Lakhsmi, P.T.V and Pa Rajalakshami. 2011. Identification of phtyo-components and its biological activities of aloe vera through the gas-chromatography-Spectrofotometry. *International Research Journal of Pharmacy*. 2 (5): 247-249.

4. Bozzi A, Perrin C, Austin S and Arce Vera F. 2006. Quality and authenticity of commercial aloe vera gel powders. *Food chem*. 103 (1): 22-30.

5. Singh B, Yadav R, Singh H, Singh H and Punia A. 2010. Studies on effect of Pcr-rapd conditions for molecular analysis in asparagus (Satawari) and aloe vera" medicinal plants. *Australian Journal of Basic and Applied Sciences*. 4 (12): 6570-6574.

6. Adushan, P. 2008. Synthesis and biological activity of aloin derivates. Tesis Master of Science In the School of Chemistry University of KwaZulu-Natal Pietermaritzburg.
7. Supardjo, 2010. The effect of saponin to livestock and human life. Animal husbandary faculty. Jambi University.

8. Gulia A, Sharma HK, Sarkar BC, Upadhyay A, Shitandi A, 2009. Changes in physico-chemical and functional properties during convective drying of aloe vera (*Aloe barbadensis*) leaves. *Food and Bioproducts Processing*. 88 (2-3): 161-164

9. Azman, M., Abdul, R., Jailani,S., Mashitah, M. Y., Ibrahim, A. B and Mohd, R. M. D. 2010. Effect of Temperature and Time to the Antioxidant Activity in Air 8 *Plecranthus amboinicus* Lour. *Journal American Sci Terapan.* 7 (9) : 1195-1199.

10. Irma, K and Achmad, Z. 2009. The antioxidant activities and antibacterial of ethanol extract of gambier leaves (*Uncaria gambir*). *Jurnal Littri.* 150 (4) : 145 – 151

11. Khalaf, N. A., Shakya, A. K., Al-Othman, A., El-Agbar, Z and Farah, H. 2008. Antioxidant Activity of Some Common Plants. *Turky Journal Biology.* 51 : 55

12. Wartini, N. M. 2007. The comparison between separated method and curing effect to flavor compound in laurel leave. Dissertation. Brawijaya University. Malang.

13. Ramachandra CT and Rao PS. 2008. Processing of aloe vera leaf gel: a review. *American Journal Agricultural and Biological Sciences*. 3 (2): 502-510

14. Lullmann., Heinz., Klaus, M., Lutz, H and Detlef, B. 2005. Colour Atlas of Pharmacology 3rd Edition. Theime Stuttgart, New York 2005; 178.

15. Shi J, Xue SJ, Ying M., Dong L, Kakuda Y and Lan Y. (2009). Kinetic study of saponins B stability in navy beans under different processing conditions**.** *Journal of Food Engineering*. 93: 59–65.

16. Rickert DA, Meyer MA, Hu J and Murhpy PA. 2004. Effect of extraction pH and temperature on isoflavone and saponin partitioning and profile during soy protein isolate production. *Journal Food Chemistry and Toxicology*. 69 (8): 623.

17. Alupului A, Calinescu I and Lavric V. 2007. *Ultrasonic vs. microwave extraction intensification of active principles from medicinal plants*. University Politehnica of Bucharest. Romania.

18. Vivikananda et.al., 2007

19. Sultana, 2009

20. Battal H. 2002. *A Research on The Production of Soapwort Extract. Ankara University Graduate School of Natural and Applied Sciences*. Department of Food Engineering. Master Thesis. Ankara, Turkey.

21. Anilakumar KR, Sudarshanakrishna KR, Chandramohan G, Ilaiyaraja N, Khanum F, Bawa AS. 2010. Effect of Aloe vera gel extract on antioxidant enzymes and azoxymethane-induced oxidative stress in rats. *Indian J Exp Biol*. 48 (8): 837-842.\

22. Khatun, M., Egucgi, S., Yamaguchi, T., Takamura, H and Matoba,T. 2006. Effect of Thermal Tratment on Radical Scavening Activity of Some Species. Food.Sci. Technol Res, 12(3):178-185.

23. Rahayuni, T., Sutardi dan Umar, S. 2002. Microencapsulation Extract of Aloe vera: Test of Characteristic of Encapsule and its Antioxidant Activity. Agricultural Technology Gadjah Mada University. *Agrosains* Journal 15 (3) : 391-402.

24. Lakhsmi, P.T.V and Pa Rajalakshami. 2011. Identification of phtyo-components and its biological activities of aloe vera through the gas-chromatography-Spectrofotometry. *International Research Journal of Pharmacy*. 2 (5): 247-249.

25. Lachenmeier K, Kuepper U, Musshoff F, Madea B, Reusch H and Lachenmeier DW. 2005. Quality Control Of Aloe Vera Beverages. Electron. *J. Environ. Agric. Food Chem*. 4 (4): 1033-1042.

26. Magwa ML, Gundidza M, Coopoosamy RM and Mayekiso B. 2006. Chemical composition of volatile constituents from the leaves of *Aloe ferox. African Journal of Biotechnology*. 5 (18): 1652-1654.

27. Miller JA, Thompson PA, Hakim IA, Chow HHS and Thomson CA. 2011. *d*Limonene: a bioactive food component from citrus and evidence for a potential role in breast cancer prevention and treatment. *Oncol Rev*. 5: 31-42.

28. Coopoosamy RM. 2010. Isolation of volatile compounds of Aloe excelsa (Berger) *African Journal of Biotechnology*. 9 (43): 7289-7294.