



PHYTOCHEMICAL SCREENING OF *VOLVARIELLA VOLVACEA* (STRAW MUSHROOM) EXTRACT FROM ACEH'S LOCAL CULTIVATION

Sadli*

Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Syiah Kuala University, Darussalam – Banda Aceh.

*corresponding E-mail : sadli_zhafara@unsyiah.ac.id

Abstract. A research on analyzing the phytochemical content in *Volvariella volvacea* (straw mushroom) extract from a local cultivation in Aceh has been conducted. Straw mushroom is potentially a medicine ingredient. This study aims to examine the secondary metabolite compounds in the methanol extract of straw mushroom. The extraction of the secondary metabolite compounds was undertaken by using the maceration method with methanol solvent. Phytochemical test was conducted qualitatively by using the meyer's reagent, bouchardat, dragendrof, FeCl₃, HCl 0.1 M, HCl 2N and Lieberman – bouchardat. Phytochemical analysis was carried out on flavonoids, alkaloids, glycosides, saponins, tannins and terpenoids. The phytochemical analysis' result shows that the straw mushroom extract contains alkaloids, flavonoids, terpenoids, and saponins.

Keywords: straw mushroom, methanol extract, maceration method, phytochemical screening

I INTRODUCTION

Fungus (mushroom) is organism lacking chlorophyll such that it can not provide its own food through photosynthesis process like plants with chlorophyll. Therefore, fungus needs nutrients that can be absorbed for its growth. Straw mushroom has six phases of growth, which are pinhead, tiny button, button, egg, elongation, and mature. Each phase has different morphology. Straw mushroom grows in tropical and subtropical area and is planted traditionally in paddy or other straw as cultivation medium. Straw mushroom is also known as *kulat jumpung* in Acehness language [1]. Fungus needs a cultivation medium containing nutrients for its growth since it does not contain chlorophyll. Straw mushroom can grow in waste medium containing cellulose, such as oil palm waste, cotton waste, paddy straw, palm sugar waste, and other wastes. Commonly, the media used to grow straw mushroom are palm tree and paddy straw. Content difference in the cultivation medium will give different nutrients contained in the straw mushroom. Straw mushroom cultivation is still relatively new in Indonesia compared to other countries, including Aceh. The cultivation requires certain conditions, from environment factor, cultivation medium, until fertilizing process [2]. In Indonesia, straw mushroom is only made use for food and its use is not developed more as medicine. This fact happens

due to lack of research concerning the straw mushroom in Indonesia. Mushroom is rich of vitamins, minerals, protein, calorie, and low fat. Moreover, it contains many nutrients which is important to fulfill the intake nutrition [2]. Mushroom is diverse in type and highly beneficial in medical field such that it is a potential medicine ingredient [3]. Some chemical compounds contained in mushroom have been broadly analyzed [4]. Straw mushroom (*Volvariella volvacea*) is a commodity which has high economical value. Protein contained in straw mushroom varies depending on the species of the mushroom and the difference in the physical and chemical composition of the growth medium. Beside the high potassium (K) and phosphorus (P) contents, straw mushroom also contain sodium (Na), calcium (Ca), magnesium (Mg), copper (Cu), zinc (Zn) and iron (Fe) [5]. A research on secondary metabolite contents in straw mushroom is needed as a basis for its usage as medicine ingredient. Chemical compound which has biological activity is secondary metabolite compound contained in mushroom. The secondary metabolite compound in straw mushroom can be identified by using a method which can give information about the chemical compound content in mushroom. One of the methods that can be used is phytochemical screening method. A research on secondary metabolite content in straw mushroom has been reported by Shwetha et al. (2012) [6] stating

that secondary metabolites contained in straw mushroom are terpenoids, glycosides, and flavonoids. Amit *et al.*, (2014) [7] reported that there are tannins and phenolics in straw mushroom. This research used methanol solvent to extract the compound from straw mushroom. Therefore, phytochemical screening was applied to the straw mushroom methanol extract in order to examine the phytochemical content and extract characterization is carried out to determine the characters or properties of the extract.

II METHODOLOGY

The research was conducted in February until March 2017. Phytochemical test and extract characterization was carried out in Pharmacy Research Laboratory and Biology laboratory of Faculty of Mathematics and Natural Sciences, Syiah Kuala University. Instruments that were used in this research were analytical scales (sartorius), oven (memmert), furnace, maceration vessel, rotary evaporator, aluminum foil, water bath, reaction tube and shelf, analytical scales, measuring cylinders, beaker, funnel, filter paper, spatula, crucible porcelain, evaporating dish and dropper pipette. Materials that are used in this research were straw mushrooms (*Volvariella volvacea*), distilled water, technical methanol, chloroform PA, technical HCl, Zn powder (merck), Mg powder (merck), FeCl₃ (merck), anhydrous acetic acid PA, technical NaOH, NaCl, KI (merck), ethanol 95%, LPMeyer, LPdragendorf, LP bouchardat, LP lieberman-bouchardat, chloride acid 1 N and 2 N. The making of meyer reactant was undertaken by weighing 1.4 g HgCl₂ and dissolving it in distilled water up to 60 ml. In another dish, 5 g potassium iodide was dissolved in 10 ml distilled water. Then, the solutions were mixed and added in distilled water up to 100 ml [8].

The process of making of the dragendorff reactant was carried out by weighing 0.8 g bismuth (III) nitrate dissolved in 20 ml concentrated nitric acid. In another dish 27.2 g potassium iodide was dissolved in 50 ml distilled water. Then, the both solutions were mixed and set aside until a complete separation. The clear solution was taken over and diluted with distilled water up to 100 ml [8]. The making of bouchardat reactant was done by weighing 4 g potassium iodide dissolved in distilled water and adding in 2 g iodine and distilled water up to 100 ml [8]. The making of lieberman-bouchardat reactant was undertaken by mixing 1 ml acetic acid and 1 ml chloroform, then making the mixture cool and adding in 1 drop of

concentrated sulfidic acid [8]. The material used in this research was straw mushrooms (*Volvariella volvacea*) produced from a local cultivation in Darussalam, Banda Aceh city, Aceh, Indonesia. The determination was undertaken in Herbarium Bogoriense of Botanical Field, Biology Research Center - LIPI Bogor. The sample used was at age of 7-8 days when it was ready to rip with bud was in button stage. Simplicia was made by collecting the straw mushrooms as much as 8 kg, cleaning them (wet sorting), washing them through flowing water. The mushrooms were wind-dried in sunlight-free place for 3 days and dried out in the oven at temperature of 60 °C for 3 days to lower the water content and the dampness. Dry sorting was carried out to separate it from other objects and dirt that was still left. The dried simplicia then was blended until it became fine powder. Simplicia powder as much as 244.40 g was obtained and saved in dry, non-humid and sunlight-free place [9]. Observation on the simplicia's organoleptic property and water content was carried out by using gravimetric method [10].

Extracting the straw mushrooms by using methanol was done by applying maceration method to 244.40 g simplicia. The simplicia was macerated in methanol at ratio 1:10 for 5 days with occasional stir and then filtered such that the waste product and the extract were obtained. The resulting extract was combined to be evaporated by using rotary evaporator such that thick extract was obtained [11]. Examining the alkaloid type was undertaken by adding in 1 mL HCl 2 N and 9 mL water to 500 mg straw mushroom methanol extract, heating it on water steamer for 2 min, letting it cool and filtering it. It then was divided into 3 reaction tubes. Two drops of Mayer reactant was added to tube I, if white or yellow deposit is formed, then it is alkaloid positive. Two drops of Bouchardat reactant was added to tube II, if brown deposit is formed, it is alkaloid positive. Two drops of Dragendorff reactant was added to tube III, if orange deposit is formed, it is alkaloid positive [11]. Examining the flavonoid type was undertaken by adding in 10 mL water to 500 mg straw mushroom methanol extract. It then was divided into 2 different tubes. Into tube I, it was added in 500 mg Zn powder and 2 drops of HCL 2N, it was put aside for 1 min. Concentrated HCL as much as 10 ml was then added in. The existence of flavonoids is indicated by the forming of intense red color in 2-5 min; it indicates the existence of glycoside-3-flavanoid. Into tube II, Mg powder as much as 100 mg and concentrated HCL as much as 10 ml were added in. If reddish

orange color is formed then flavon, kalkan and auron exist [11].

Examining saponin type was undertaken by adding in 500 mg straw mushroom methanol extract to a reaction tube, adding in 100 mL hot water, letting it cool and intensively shaking it for 10 sec. The existence of saponins is indicated by the forming of foam as high as 1 cm to 10 cm for at least 10 min. On the addition of 1 drop of hydrochloric acid 2N, the foam will not disappear [8]. Examining tannin type was undertaken by adding in 2 mg straw mushroom methanol extract to a reaction tube, adding in 10 mL hot water, boiling it for 5 min. It was then filtered. The Filtrate was divided into two tubes with 1 mL for each. Some drops of FeCl_3 were added to the first tube. Positive reaction is indicated by the forming of purplish green color [12]. Examining the terpenoid type was undertaken by dissolving 500 mg extract into 2 mL chloroform. It then was evaporated by using the evaporating dish. Two drops of anhydrous acetic acid was added to the residue, and then 1 drop of concentrated sulfidic acid was added. Positive result is indicated by the forming of greenish red of bluish violet color [12].

Extract characterization was carried out by determination of extract's organoleptic properties including shape, color, and smell of the extract, as well as the determination of level of water, dust, water-dissolved essence, and ethanol-dissolved essence [8]. The determination of the level of the essence dissolved in water was undertaken by adding in 5 g wind-dried extract to volumetric flask, then adding in 10 mL chloroform saturated distilled water. Repeating shake was applied to it for first 6 hours, and it was then set aside for 18 h. It then was filtered and filtrates as much as 20 mL was evaporated until it dried in shallow flat-bottom dish which had been heated at temperature of 105°C and then it was flattened evenly. Thereafter, the residue was heated at temperature of 105°C until the weight is constant. The level of the water-dissolved essence was measured in percentage (%). Constant weight was obtained by heating process and measuring the evaporating dish which has weight difference at most 0.5 mg at two times of measuring consecutively [13]. The determination of the level of the essence dissolved in ethanol was undertaken by adding in 5 g wind-dried extract to volumetric flask, then adding in 100 mL methanol of 95%. Repeating shake was applied for first 6 h and it was then set aside for 18 h. It then was filtered and filtrates as much as 20 mL was evaporated until it dried in shallow flat-bottom dish which

had been heated at temperature of 105°C and then it was flattened evenly. Thereafter, the residue was heated at temperature of 105°C until the weight is constant. The level of the water-dissolved essence was measured in percentage (%). Constant weight was obtained by heating process and measuring the evaporating dish which has weight difference at most 0.5 mg at two times of measuring consecutively [13].

The determination of the level of the water was done by measuring 2 g straw mushroom methanol extract in a dish whose weight was already known. Evaporating dish containing the extract was put in the oven and it was dried out at temperature 105°C for 3 h. Thereafter, it was cooled down in desiccator. It was then weighed to calculate the water content [10]. Determination of the level of the water was done by measuring 2 g straw mushroom methanol extract in crucible porcelain which had been annealed and weighed. The annealing was gradually carried out until the charcoals ran out, then it was cooled down and weighed. If the charcoal could not be rid of then hot water was added, it then was stirred and filtered with dust-free filter paper. The filter paper and the residue were annealed in the same crucible porcelain. Filtrate was added in to crucible porcelain, evaporated and annealed until the weight became constant. The weighing was carried out until constant weight not more than 0.5 mg was obtained for two consecutive weighing [13]. Data analysis was conducted qualitatively by observing the color change and the forming of deposits after the addition of reagent.

III RESULT AND DISCUSSION

Simplicia of straw mushrooms was made from fresh straw mushrooms which were still in bud form of 8 days because the bud-formed mushrooms were generally more preferable to eat than the blossomed one. Drying method that was used in this research followed the method used in Sudha et al. (2008). Drying process of the fresh straw mushrooms was undertaken in two steps because of the high water content in the straw mushrooms which was 93.3%. The first step was through wind-drying in direct sunlight-free room for 3 days. Thereafter, the second step was continued by oven-drying at 60°C for 3 days. The obtained weight of the straw mushroom simplicia was 244.40 g. Simplicia characterization consists of the observation of simplicia's organoleptic properties and simplicia's water content. The simplicia of the straw mushrooms was in the form of chopped mushroom, dark brown colored, and straw mushroom-typically odorous.

The result showed that the water content of the simplicia was $9.34 \pm 0.26\%$. The extraction process of the simplicia was carried out through maceration which was done repeatedly. The dried simplicia of 244.40 g was macerated in the methanol solvent.



Figure 1 Simplicia of fresh straw mushrooms.



Figure 2 Simplicia powders of the straw mushrooms.

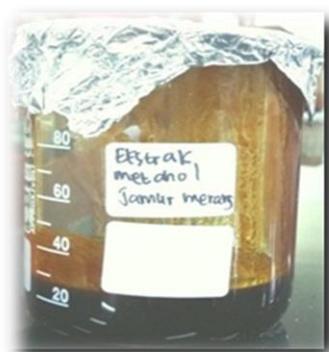


Figure 3 Straw mushroom methanol extract

Thick extract was obtained as much as 24.56 g with extract yield as much as 10.05%. The obtained straw mushroom methanol extract was dark brown colored, straw mushroom-typically odorous, and thick formed. Furthermore, phytochemical screening and characterization was applied to the straw mushroom methanol extract. Phytochemical screening was

undertaken in order to determine the secondary metabolite compound content in the extract. The results are presented in Table 1.

Table 1 Phytochemical screening results

Secondary Metabolites	Reactants	Result
Alkaloids	Meyer	-
	Bouchardat	+
	Dragendorf	+
Flavonoids	Mg and HCl powder	+
Saponins	Air dan HCl	+
Tannins	FeCl ₃	-
Terpenoids	Lieberman-Bouchardat	+
	Lieberman-Bouchardat	-

According to Table 1, phytochemical content analysis results that the straw mushrooms (*Volvariella volvacea*) contain alkaloids, terpenoids, saponins and flavonoids. The positive result of alkaloids is indicated by the positive results of the 2 reagents which are dragendrof and bouchardat. Alkaloid test with some reagents gave specific deposits against the alkaloid's structure. If an alkaloid test reagent shows positive result, it indicates that certain types of alkaloids do not exist in the plant [14]. A research conducted by Shwetha et al. (2012) suggested that the secondary metabolites contained in straw mushrooms consisted of terpenoids, glycosides and flavonoids. Meanwhile Amit et al. (2014) reported that there were tannin and phenollic contained in straw mushrooms. Contrast to the result reported by Shwetha et al. (2012) and Amit et al. (2014), Tannins and glycosides were not contained in the straw mushrooms analyzed in this research. Meanwhile the results presented by Shwetha et al. (2012) and Amit et al. (2014) did not state the saponin content as what is showed in this research. These results might be affected by different cultivation medium that were used to grow the straw mushrooms.

The straw mushroom that was examined in this research used palm tree as cultivation medium while in the research conducted by Shwetha et al., (2012) and Amit et al., (2014), the cultivation medium used was not reported. The difference in the cultivation medium might affect the nutrients contained in the straw mushrooms. As what stated in a research conducted by Adedukon et al. (2013), it was reported that straw mushrooms which were grown in three different cultivation mediums which were cotton waste, corn peel waste, and plantain leaf waste yielded different levels of protein, lipid, fiber, and carbohydrate. The different nutrient contents will cause different

secondary metabolite content in the straw mushroom because there are different biosynthesis precursors of the secondary metabolite compound [15]. Characterization of the extract served to determine the quality of the straw mushroom methanol extract. The characterization that was carried out in this research includes determination of level of water, dust, water-dissolved essence, and ethanol-dissolved essence. The results are presented in Table 2.

Table 2 Extract characterization results.

Parameters	Results \pm SD, (%)
Water level	12.94 \pm 0.25
Water-dissolved essence level	45.16 \pm 0.38
Ethanol-dissolved essence level	51.80 \pm 0.36
Total dust level	21.4 \pm 1.26

The determination of the extract's water level serves to detect the amount of water content in the extract and to define the extract's quality. According to Table 2, the water level of the straw mushroom methanol extract is 12.94 \pm 0.25%. This level surpasses the admitted water level established by Farmakope Herbal Indonesia (2008) stating that the water level in an extract is < 10%. Water level that is more than 10% can affect the stability of the extract such as speeding fungus growth which affects the extract's quality. The determination of water-dissolved essence level and ethanol-dissolved essence level serves to ascertain the percentage of the essence compound dissolved in water and organic solvent. The water-dissolved essence level and ethanol-dissolved essence level are 45.16% and 51.8% respectively. The water-dissolved essence and ethanol-dissolved essence level are indicators for active compounds that are dissolved in water and ethanol. In this research, it is shown that the ethanol-dissolved essence level is greater than the water-dissolved essence.

The determination of dust level serves to ascertain the remaining inorganic compound after dusting [16]. The extract that was used in this test was heated at high temperature such that the organic compound was destructed and the inorganic compound remained. This inorganic compound would be the dust level of the tested extract which was measured in percentage. The dust level of the straw mushroom methanol extract in this research was 21.44 \pm 1.26 %. The level indicates high mineral content. The difference in the cultivation medium also affects the amount of the dust level of the straw mushrooms. The amount of the dust level will affects the amount of mineral in the straw mushrooms. A research

conducted by Akinyele et al. (2005) suggested that the straw mushrooms which were planted in cotton waste and corncob waste gave dust level as much as 13.04% and 9.33% respectively [17]. The dust level of the straw mushrooms which are cultivated in the oil palm medium is higher than the one of the straw mushrooms cultivated in cotton and corncob waste medium because oil palm medium contains higher cellulose, hemicellulose, and lignin than the ones in cotton and corncob media [18]. The specific difference from the two researches was in the term of cultivation medium for the straw mushrooms, this difference causes the different mineral contents in the straw mushrooms.

CONCLUSION

According to the results, it can be concluded that the straw mushroom methanol extract contains secondary metabolites including alkaloids, flavonoids, saponins and terpenoids and has water level at 12.94 \pm 0.25% b/b, water-dissolved essence level at 45.16 \pm 0.38 % b/b, ethanol-dissolved essence level at 51.80 \pm 0.36% b/b, total dust level at 21.4 \pm 1.26% b/b.

REFERENCE

1. Chang, S.T. Miles, P.G 2004 Mushroom Cultivation, Nutritional Value, Medicinal Effect and Environmental Impact second edition. CRC Press Washington D.C.
2. Sinaga, M.S 2005 Straw Mushroom and its Cultivation. Penebar Swadaya Jakarta.
3. Muchoroji, Y.A.C 2008 Cultivation of Oyster Mushroom. Penebar Swadaya Jakarta
4. Donowati, T 2015 Diversity of Edible Mushroom based on the Contents and Benefits in Health. *Pros Sem Nas Masy Iodiv Indon.* **1**(6) 1520-1523
5. Wanda, S 2004 Cultivation of Straw Mushroom. PT. Agromedia Pustaka Jakarta
6. Depkes RI 1995 *Materia Medika Indonesia*. Health Department of Republic of Indonesia Jilid VI Jakarta
7. Shwetha, V.K, Sudha G.M 2012 Ameliorative effect of *Volvariella volvacea* aqueous extract (Bulliard Ex Fries) Singer on gentamicin induced renal damage. *Int. J. Pharma and Bio Sciences.* **3**(3) 105-117.
8. Amit, R. Prasad, P. Gupta, N 2014 *Volvariella volvacea*: A Macrofungus Having Nutritional and Health. *Asian J. Pharm. Tech.* 2014 **4**(2) 110-113.

9. Health Department of Republic of Indonesia 1977 *Materia Medika Indonesia*. Depkes RI Publisher Jilid IV Jakarta
10. Sudha,A. Lakshmanan, P. Kalaiselvan,B 2008 Antioxidant properties of paddy straw mushroom (*Volvariella volvacea* (Bull. ex Fr.)) Sing. *Int. J. Applied Agricultural Research* ISSN 0973-2683 **3**(1) (2008) 9-16.
11. World Health Organization 1998 *Quality Control Methods for Medicinal Plant Materials*. WHO England
12. Health Department of Republic of Indonesia Health Department of Republic of Indonesia 1989 *Materia Medika Indonesia*. Depkes RI Publisher Jilid V Jakarta
13. Sari, I.R.M 2012 Examination of Antioxidant Activity in The Extract of *Pleurotus ostreatus* Mushroom by Using DPPH Method and Identification of Chemical Compound Group from the Most Active Fraction. Bachelor Thesis University of Indonesia.
14. Health Department of Republic of Indonesia 2009 *Farmakope Herbal Indonesia*. Kemenkes RI Publisher Ed.1 Jakarta.
15. Kumar, Sunil 2014 Alkaloidal Drugs – A Review. *Asian J. Pharmaceutical Science and Technology* **4**(3) 2014, 107-119.
16. Salim, M. Yahya. Sitorus, H. Ni'mah, T. Marini 2016. The Relation between Soil Nutrition and the Production of Secondary Metabolite Compounds in *Lansium domesticum* Corr var Duku and its Potency as Larvacide. *J. Vektor Penyakit*, **10**(1) 2016 11– 18.
17. Ratnani, R.D. Hartati, I. Anas, Y. Devi, E.P dan Khilyati, D.D.D 2015 Spesific Standardization and Non-specific Extraction of Hidrotropi *Andrographolid* from *Andrographis paniculata*.
18. Akinyele, B.J. Akinyosoye, F.A 2005 Effect of *Volvariella volvacea* cultivaton on the chemical composition of agrowastes. *African J. Biotechnology* **4**(9), 979-983, September 2005. Academic Journals.
19. Apetorgbor, A.K. Apetorgbor, M.M. Derkyi, N.S.A 2015 Comparative Studies on Growth and Yield of Oil Palm Mushroom, *Volvariella Volvacea* (Bull. Ex. Fr.) Sing.on Different Substrates. *Greener J Agricultural Sciences* **5**(5) 177-189, September 2015.