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Test the Antioxidant Effectiveness of Oyster Mushroom (Pleurotus ostreatus) Ethyl Acetate Fraction Cream Formula using FRAP (Ferric Reducing Antioxidant Power) and ABTS (2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) Methods)

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Abstract: Antioxidants are compounds that can fight the influence of free radical molecules due to chemical reactions and metabolic events in the body. Phenolic compounds work to reduce the reaction of increasing the oxidation number, and are able to reduce highly reactive free radicals, lipid peroxidation processes, and metal oxides that can be obtained from oyster mushrooms. This study aims to make an antioxidant cream preparation and measure the IC50 value by conducting an antioxidant test of the ethyl acetate fraction of oyster mushroom (*Pleurotus ostreotus*) cream using FRAP (ferric reducing antioxidant power) and ABTS (2.2'-azino-bis (3-3) ethylbenzothiazoline-6-sulphonic acid) methods. The methods used are FRAP and ABTS where oyster mushrooms go through a liquid-liquid partition process and are made into cream preparations with formula concentrations of 2%, 4% and 6%, then their antioxidant activity is seen. The results of measuring three cream formulas obtained an IC50 value using the FRAP method of 110.12 bpj, and an IC50 value using the ABTS method of 84.61 bpj. The conclusion was that the IC50 value using the FRAP method (110.12 bpj) had medium category activity and the IC50 value using the ABTS method (84.61 bpj) had strong category activity.

Keywords: ABTS; Antioxidants; Frap; Free radicals; Oyster mushroom

Introduction

Free radical molecules are often found in the environment and as a result of the body's metabolism (Jan et al., 2015). The origin of free radical molecules (Aziz et al., 2019) is the result of the body's metabolism, namely oxidation, enzymatic oxidation and oxidative burst, unlike free radicals from the environment that come from exposure to cigarettes, ultraviolet light, X-rays, insecticide chemicals and air contamination (Mohite et al., 2018). Several plant species can act as antioxidants, some can come from vegetables, spices and fruit from plant species (Serafini & Peluso, 2016). Real fruits and plants have potential as antioxidants because they contain ingredients such as carotene pigments, flavonoid compounds, other phenolic compound components, vitamins C and E. The use of antioxidants is often found in topical preparations, especially cosmetic preparations. Cosmetic preparations in skin care are needed to maintain and protect the skin layer which is vulnerable to inflammatory conditions, cancer and triggers premature aging due to oxidative changes in free radical molecules (Barki et al., 2017).

Mushrooms produce a large amount of medicinal compounds, and are also an optimal source of fibres, proteins, vitamins (like groups B and D), and other

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micronutrients including potassium, magnesium, etc. Consequently, mushrooms are commonly considered to be functional foods (Parola et al., 2017). There are various sources of antioxidant compounds that can be obtained around us, for example white oyster mushrooms (*Pleurotus ostreatus*) (Jayakumar et al., 2011). Oyster mushrooms contain phenolic compounds, L-ergotien, minerals and selenium, as well as vitamin C. Phenolic compounds work to reduce the increase in oxidation numbers, and are able to reduce highly reactive free radicals, lipid peroxidation processes and metal oxides (Egra et al., 2018).

The first step to measure the antioxidant activity of plant extracts is to choose the right method. Antioxidant examination uses quantitative measurements of the ability of a component as a reducing agent (Hamad et al., 2022). The available methods for measuring antioxidant activity can be classified based on mechanism its action, where the applied compound stops the chain breaking reaction. They can be divided into two group: hydrogen atom transfer (HAT) (hydrogen atom transfer reaction) and single electron transfer (SET) (compound reduction reaction via electron transfer from antioxidants) (Chaves et al., 2020). The antioxidant test that is often used is SET (Single Electron Transfer), where measurements use a spectrophotometer to see color changes that are related to the size of the antioxidants contained in the sample. The SET method is influenced by the pH value and solvent used, apart from that the measurement is seen from the color change that is formed, if the greater the color change, the higher the reduction process (Citta, 2020). The type of test used is FRAP (Ferric Reducing Antioxidant Power) and ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid).

From the information above, it is necessary to discover the antioxidant activity of the ethyl acetate cream fraction of Oyster Mushrooms (Pleurotus ostreatus) using the FRAP and ABTS methods.

Method

This research is a type of experimental research which aims to develop new products made from natural ingredients sourced from mushrooms, with the following procedures:

Extraction

Samples of dried oyster mushroom (*Pleurotus ostreotus*) herbs were extracted using the maceration method with a filter liquid in the form of 96% ethanol for 3x24 hours. Re maceration is carried out with the same filter fluid, combined, filtered to separate the dregs and filtrate, then the extract filtrate is evaporated using a 78 °C rotary evaporator at a speed of 100 rpm until a thick extract is obtained (Leba, 2017).

Oyster Mushroom of Extract Liquid-liquid Partition

The viscous extract was separated based on different polarity levels by the liquid-liquid partition method. Initially the thick extract was dissolved in MeOH: H_2O, then partitioned again with n-hexane. The results obtained were n-hexane phase and water phase. The water phase is added with H_2O and liquid-liquid partitioned with ethyl acetate solvent. The results of the partition process will produce ethyl acetate and water fractions, ethyl acetate fraction is continued for cream formulation (Yunarto et al., 2015).

Preparation of Oyster Mushroom Ethyl Acetate Fraction Cream

The cream formulation consists of oyster mushroom ethyl acetate fraction as the active substance which is made with concentrations of 2%, 4%, 6%, and control (-), stearic acid as an emulsifier as much as 10%, cetyl alcohol as an emollient as much as 2%, Triethanolamine as emulsifier around 2%, glycerin as a humectant around 10%, sodium benzoate as a preservative around 0.1%, rose oil as a fragrance to taste and distilled water as a solvent. The cream preparations that had been made were evaluated and quantitatively tested for antioxidant activity against FRAP and ABTS (Apitalau et al., 2021).

Test of FRAP (Fe2+ standard curve from $FeSO_4.7H_2O$)

12.20 mg of Ferrous sulfate heptahydrate ($FeSO_4.7H_2O$) was dissolved in 100 ml in a volumetric flask. Total of 1,000; 0.500; 0.250; 0.125 and 0.063 ml were diluted into a 100 ml volumetric flask, until Fe^{2+} concentrations were obtained respectively 0.88; 0.44; 0.22; 0.11 and 0.05 µM. Measurements were carried out by taking 0.5 ml of the sample (concentration variations of 0, 2, 4, 8, 16 and 32 mg/l) into a test tube, adding 0.5 ml of phosphate buffer solution with a pH of 6.6 (0.2 M), added with 0.5 ml of 1% potassium hexacyanoferrate solution. The mixed solution was incubated at 50 °C ± 20 minutes, added with 0.5 ml of 10% trichloroacetic acid, if two layers formed, centrifugation was carried out. The solution mixture was incubated at 25 ˚C (5-10 minutes), the atomic absorption at λ 700 nm was measured (Yefrida et al., 2015).

Test of ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)

Aquos ABTS (5 ml 7 mM) was reacted with aquos K2S2O8 solution (88 µl 140 mM) ABTS radical cation (ABTS·+). The solution was left in a dark place for 12-16 hours at room temperature, resulting in a dark blue

ABTS·+ solution. This solution can be used after adding 99.5% ethanol until the absorbance value is 0.7 ± 0.02 at a wavelength of 734 nm. The test solution was obtained from dissolving 10 mg of the test compound in 1 ml of DMSO. Take 10 µl of the test solution and put it in a tube protected from light, add 1 mL of ABTS·+. The solution mixture was shaken with a vortex for 10 seconds. The solution was incubated at 30 °C for 4 minutes. The absorbance of the solution was measured at a wavelength of 734 nm (absorbance of the test compound). Trolox was used as a positive control. The blank solution consisted of 10 µL DMSO 99.5 % in 1 ml ABTS·+ measured at the same wavelength (blank absorbance). Antioxidant activity can be calculated by the formula: Inhibition (%) = Ab-As/As ×100% (Wulansari, 2018).

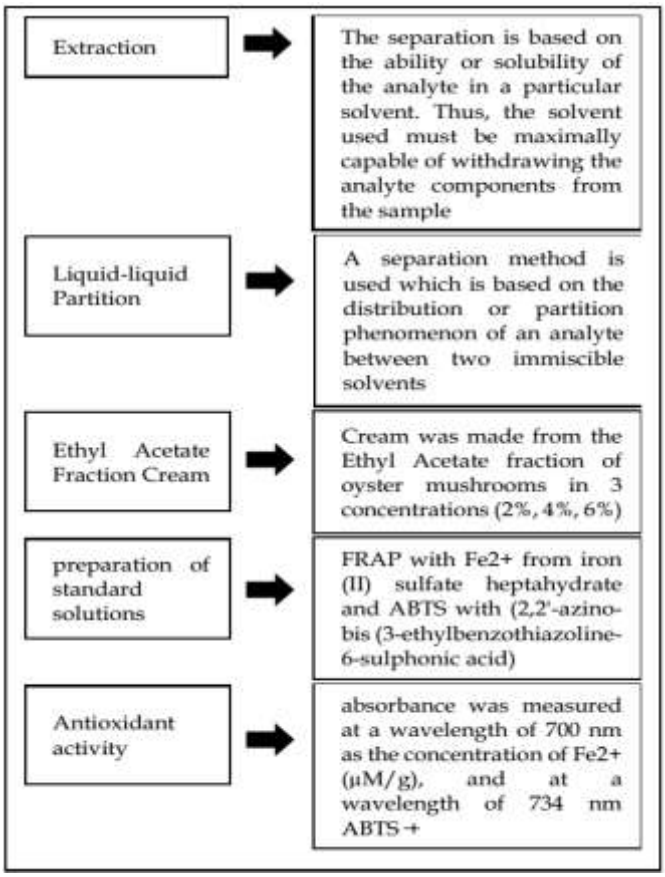

Figure 1. Graph of work procedures

Result and Discussion

Identification of Chemical Compounds in the Ethyl Acetate Fraction of Oyster Mushrooms

The results of the identification of the ethyl acetate fraction of white oyster mushrooms showed that they contained chemical compounds in the form of alkaloids, saponins and steroids (Parola et al., 2017).

Table 1. The Results of the Identification

Phytochemical test	Results	Observation	Image of test results
Alkaloids	+	White precipitate	
Flavonoids	-	An orange color forms	
Saponins	+	Foam forms	
Steroids	+	A red color forms	
Tanins	-	A blackish green color forms	

Formulation Design for Oyster Mushroom Ethyl Acetate Cream Fraction

Table 2. Formulation of Cream

Ingredient formula	Function	Concentration F1 (%)	F2 (%)	F3 (%)
Oyster mushroom fraction	Actif substance	2	4	6
Stearate acid	Emulsifier	15	15	15
Cetil alcohol	Emollien	4	4	4
Triethanolamin	Emulgator	2	2	2
Glycerin	Humectan	8	8	8
sodium benzoate	Preservative	0.1	0.1	0.1
Rose oil	Fragrance	qs	qs	qs
Aquadest	Solvent	100	100	100

Information: F1: FKK (Concentrated cream formula) 2%, F2: FKK 4%, F3: FKK 6%.

IC50 Value of Oyster Mushroom Ethyl Acetate Cream Fraction

Interpretation of the results of testing antioxidant activity in cream preparations, namely the inhibition concentration value of 50%, is expressed as a parameter for the cream concentration of the oyster mushroom ethyl acetate fraction which is able to reduce free radicals by 50% with linear regression y= bx + a. Linear regression graphs are created on sample concentration/ppm as abscissa (x) against percent inhibition as ordinate (y-axis) (Toripah, 2014). where testing the activity of antioxidant compounds using FRAP and ABTS method showed the strongest antioxidant activities, which implied that were important natural sources for preventing oxidative stress diseases (Xu et al., 2021).

Table 3. Number of IC50

Test method	IC50	
	Sample (bpj)	vitamin C (bpj)
FRAP	110.12	24.50
ABTS	84.62	2.43

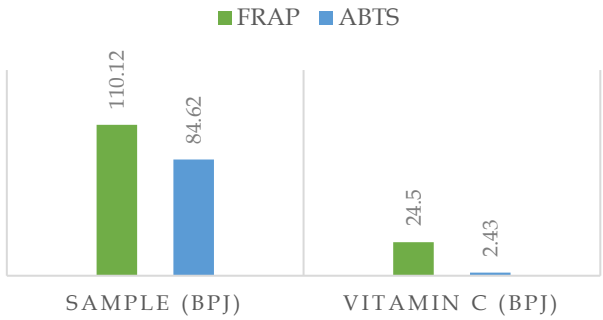

Figure 2. Graph of antioxidant activity

FRAP method shows that oyster mushroom ethyl acetate fraction cream has an IC50 value of 110.12 bpj (51-100 bpj), in terms of antioxidant strength it is in the medium category and the IC50 value of vitamin C as a comparison is 24.50 which is has strong antioxidant activity. ABTS method shows an IC50 value of 84.62 (51-100 bpj), where the level of antioxidant strength is in the strong category and the IC50 value of vitamin C is 2,430 bpj (<50 bpj) which is very strong. Measurement of antioxidant compounds using FRAP method must be made in time and used as soon as possible to be accurate because FRAP solution is less stable, and accurate if carried out if the antioxidant compound can reduce Fe^{3+} ions to Fe^{2+}. FRAP rely on a single electron transfer mechanism and ABTS relies on a more complex reaction mechanism (Rumpf et., 2023).

The results of measuring antioxidant activity values using ABTS method show better results than FRAP method. This is shown by ABTS method having flexibility in measuring in a pH range that tends to be larger, so that the IC50 value obtained is smaller or in other words has strong antioxidant activity, as in research (Mastuti et., 2015), which states that phenol compounds have a significant contribution to antioxidant activity, due to changes in oxidation (redox) numbers which enable phenol compounds to act as reducing agents and hydrogen donors.

Conclusion

The test results showed that the ethyl acetate fraction cream preparations in concentrations of F1, F2 and F3 has antioxidant activity when tested using FRAP method showed moderate IC50 activity and using ABTS method showed strong IC50 activity.

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Author Contributions

Conceptualization, M.N. and N.A.; methodology, M.N.; validation, M.N., and M.; formal analysis, Z.T.; investigation, R.A.; resources, M.N and M.; data curation, M.N. and N.A.; writing—original draft preparation, M.N and N.A.; writing—review and editing, M.N and M.; visualization, Z.T and R.A.; All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

All authors declare no conflict of interest in this manuscript.

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