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The Phytochemical Screening, Total Phenolic Contents and Antioxidant Activities *in Vitro* of White Oyster Mushroom (*Pleurotus Ostreatus*) Preparations

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Keywords: Ethanolic extract; Pleurotus ostreatus; Phytochemical; Total phenolic content; Antioxidant activity

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Abstract

BACKGROUND: The popular commercially cultivated Pleurotus ostreatus mushroom contains very high nutrients and bioactive compounds with high antioxidant activity. The ethanolic extract seems to be the most active in preparation.

AIM: This study has an aim to compare the phytochemical analysis of a fresh, dry and ethanolic extract of Pleurotus ostreatus, to measure the total phenolic content and antioxidant activities in vitro of ethanolic extracts of Pleurotus ostreatus.

METHODS: The fresh plant's materials (FPM), dry plants materials (DPM), ethanolic extracts were macerated with 70% (EE70) and 96% ethanol (EE96) of Pleurotus ostreatus which were used for phytochemical analysis, and EE96 was used for antioxidant activity in vitro. The phytochemical analysis was conducted using the Dragendorf and Meyer, FeCl3 test, Salkowsky method, Lieberman method, amyl alcohol, foam test and the NaOH reagent. The total phenol test was carried out using the Follin-Ciocalteu method. The antioxidant activity was tested using the ABTS and H2O2 essay.

RESULTS: The phytochemical screening showed that the flavonoid, phenolic compounds, tannin, saponin, alkaloids, and steroids were detected in the FPM, DPM, EE70 and also the EE96. The alkaloid, however, was not identified by the Meyer Reagent in the FPM and DPM. The DPM and EE70 seemed to have the highest amount of saponin based on the foam that was formed. Meanwhile, steroids and flavonoids were detected at a higher level in the EE96, based on the strength of visible colour. However, triterpenoid and quinones could not be identified. In the total phenol test, there was an amount of 6.67 µg phenol in a 1 mg extract sample which was equivalent to 1 mg of Gallic Acid. The EE96 has an IC50 of 108.07 µg/mL for ABTS and an IC50 reduction of 229.17 µg/mL. The process of Pleurotus ostreatus drying did not reduce the content of active substances. The polar active substances seem to be more soluble in the EE70 than the EE96.

CONCLUSION: The higher the bioactive substances in the preparation, the more significant the bio-therapeutic effects. Ethanolic extract of Pleurotus ostreatus has a phenol content and a good antioxidant action.

Introduction

Forty per cent of the population in Indonesia are using traditional medicine, and 70% of them are in rural areas. Indonesia is rich in medical plant resources, although the utilisation is estimated to be unoptimal. Research on plants that are efficacious in medicine needs to be developed as of now. The use of natural ingredients in medicine is expected to provide fewer side effects than the use of synthetic or chemical materials, without reducing the efficacy in medicine [1], [2]. One plant that has long been known to have efficacy for treatment is white oyster mushroom (Pleurotus ostreatus Jacq: Fr Kumm). The Pleurotus ostreatus is one of the commercial mushrooms that are very popular commercially, easily cultivated and is an important food source. White oyster mushrooms contain high nutrients and various other secondary metabolites that have pharmacological effects [3], [4].

One potential pharmacological effect of Pleurotus ostreatus is the antioxidant potential. Previous studies have shown that Pleurotus ostreatus

have high antioxidant abilities. White oyster mushroom extractions have protective effects on the liver, kidneys, brain and lungs [5], [6], [7]. The literature shows that Pleurotus ostreatus contains many active substances that are useful in applying therapeutic effects, including phenolic components, flavonoids, terpenoids, polysaccharides, lectins, steroids, glycoproteins, several lipid components, and ergothioneine (ET), vitamin C, beta-carotene, and selenium. The ethanol extract of Pleurotus ostreatus is expected to be an exogenous antioxidant that can prevent and inhibit oxidative stress due to free radicals in the body, while being able to synergize with the body's endogenous antioxidants [5], [6], [7], [8], [9], [10].

The preparation of the ethanolic extract of Pleurotus ostreatus is aiming to separate the active substances from other materials that are not needed by using certain solvents. Factors that can influence the quality of extract formed include the selection of plants that are used, the procedure for making extractions and the selection of solvents used. Plants used for making extractions can be fresh plants or in a dry form, but it is worried that the drying process will affect the content of the active ingredients inside. Drying techniques are often done traditionally or by using open dryers. The selection of solvents in the extraction should have the following properties. Namely, low toxicity, easily evaporated at low temperatures, increasing the speed of extractions, can be preservative, will not make the extraction complex or split. The choice of solvents in creating the extraction will affect the active substance to be dissolved. Ethanol can cause more filtered polyphenols than water extractions. This solvent is more effectively used on cell walls, or unipolar seeds and ethanol can activate the polyphenol oxidation enzyme which can degrade polyphenols. To observe the qualitative, the active substances contained in plant samples and herbal preparations should be carried out with a pre-clinical phytochemical screening [11], [12]. Measurement of total phenolic levels in the extraction will also show the estimation of phenol levels in extractions which are very important for their antioxidant potential [13], [14].

The antioxidant potential of the ethanolic extract of Pleurotus ostreatus can be measured using various methods both in vitro and in vivo. In vitro antioxidant tests are very diverse, the results of the phytochemical analysis and in vitro antioxidant tests can qualitatively be the basis for confirming the antioxidant effects of the white oyster mushroom ethanol extraction in vivo. It is hoped that the results of the vitro test will be in line with the results of the Vivo test, the substances or extracts that are thought to have antioxidant activity in vitro will also show good antioxidant potential in vivo [15].

Based on the data above, this study will compare the active content of Pleurotus ostreatus in fresh plant materials (FPM), dry plant material (DPM), ethanol extraction using 70% ethanol (EE70) and ethanol extraction using 96% ethanol (EE96). This study will also assess the antioxidant activity of the ethanolic extraction of Pleurotus ostreatus using the two in vitro methods.

Material and Method

Manufacture of White Oyster Mushroom Extraction

Pleurotus ostreatus are obtained from mushroom cultivation centres in Cisarua village, West Bandung regency, Indonesia. Fresh plants materials of Pleurotus ostreatus are brought in the morning after being harvested, then washed using running water and cleaned from contaminating material until the material is gone. The mushrooms are then dried using an oven at 50°C for 2-3 days. The dried mushrooms that are obtained are about 8% of the fresh sample. The dried Simplicia is mashed and then forms a dry powder of the white oyster mushroom (Pleurotus ostreatus). The dried oyster mushroom powder is then macerated, each with 70% ethanol and 96% ethanol, with a ratio of 1:10. The liquid extraction obtained was then filtered with a Whatman No. filter paper at 40 and then evaporated with a rotary evaporator at 50°C and put into the oven at 40°C to obtain a concentrated extraction. Fresh plant samples, dry powder, 70% ethanol extraction and 96% ethanol extractions were then subjected to phytochemical screening [1], [12], [16], [17].

Alkaloid Test

Dragendroff Test: The sample (100 mg) was dissolved in 10 mL of solvent, then filtered into a filtration. The filtration (2 mL) was added with an HCL (acid) and a few drops of the Dragendroff reagent. The formation of brownish-red deposits shows the presence of alkaloids [11], [18].

Mayer Test: Samples (100 mg) were dissolved in 10 mL of solvent, then filtered to form a filtration. The filtration (2 mL) was added with an HCL (acid) and a few drops of the Meyer reagent. The formation of brownish-yellow deposits indicates the presence of alkaloids. (Tiwari et al., 2011) [11], [18].

Triterpenoid and Steroid Tests

Liebermann-Burchard Test: 50 mg of the sample was extracted with chloroform and then filtered. 2 ml of filtrate formed which were then added with 1-2 ml of acetic anhydride and 2 drops of concentrated H2SO4 from the side of the tube. The formed colour is red, then blue and then a green color

which shows sterols [18].

Salkowski's Test: 50 mg of the sample was extracted with chloroform then filtered. The filtrate obtained was added with a few drops of concentrated sulfuric acid and then shaken. The chloroform layer shows the red and yellow colour of the acid layer indicating steroids. A brownish-red colour indicates a triterpenoid [12], [19].

Saponin Test

Foam test/Froth method: The extraction was dissolved in 20 ml in distilled water (dist. H_2O) so that all the samples were submerged, and boiled for 2-3 minutes. The solution is then cooled and shaken. The appearance of foam for ± 10-15 minutes shows a sign of saponin [11], [16].

Flavonoid Tests

Ammonium Test: The extraction is heated with 10 ml of ethyl acetate in boiling water for 3 minutes. The results are then filtered to form a filtration. The filtration is then mixed with 1 ml of dilute ammonia solution (1%), and then shaken. These two layers will separate. The absence of yellow on the ammonia layer indicates flavonoids [19].

Shinoda Tests: 2-3 ml of filtrate extraction is then added with magnesium metal. Then the HCL concentrate is added. The appearance of a magenta colour signifies the presence of flavonoids [12], [19].

Kinnon

NaOH Test: A 5 ml filtrate was added a few drops of NaOH. The reddish-blue colour that appears indicates a quinone [17].

Tanin test and phenolic compound/ polyphenolic

Fe (III) Chloride Test: Samples (100 mg) was dissolved in 10 mL of solvent, then filtered. The filtration (2 mL) is added with 1 mL FeCl3 3%. The presence of a green to slightly blackish deposits indicates the presence of tannins and polyphenols [12], [18], [19].

Gelatin Test: Extraction 0.1 g of the sample added with 10 ml of water, then boiled for several minutes. Then filtered and the filtration was added with 2 mL of a 1% gelatin solution containing NaCl. If a white precipitate is formed, it indicates the presence of tannins and phenolic components [11], [12].

Measurement of Total Phenolic Content

The total phenol test was implemented using the Follin-Ciocalteu method. The polyphenols in plant extractions will react with the Folin-Ciocalteu reagent and form a blue complex which can be quantitatively measured using visible-light spectrophotometry. The formation of a blue chromophore is caused by the forming of a phosphotungstic-phosphomolybdenum complex, and the maximum absorption depends on the alkaline solution and the phenolic concentration (the higher the phenol content, the deeper the blue colour) inside. The reactions that occur are accurate and specific for some phenolics [20], [21].

Samples of ethanolic extractions of P. ostreatus include an amount of 15 µl in each well. Then, 75 µl of Folin-Ciocalteu 10% and 7.5 µl of Sodium Carbonate amount at 60 µl. On the blank, 150 µl of DMSO sample solvent is added. Then incubated at a time of 10 minutes at 500°C. The absorbance was measured at λ = 760 nm on the spectrophotometer. The total phenol concentration will be calculated based on the calibration curve obtained using various concentrations of gallic acid and can be seen from the number of mg in gallic acid equivalents (GAEs) per gram of the dry sample [20], [21].

Measurement of Antioxidant Activity in Vitro

ABTS ({2, 2 '- azinobis- (3-ethyl-benzothiazoline-6-sulphonic acid)}) essay: The ABTS assay is a test of antioxidant potential using a spectrophotometer to measure the blue colour that appears when a sample of white oyster mushroom ethanol extraction is added to a blue-green chromophore ABTS + (2, 2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid). Herbal samples will reduce ABTS + to be oxidised ABTS and cause decolourisation. A Cation ABTS is made by adding a solid manganese dioxide (80 mg) to 5mM of a liquid stock solution of ABTS [15], [22].

A 2 µl extraction sample was inserted into the 96-well plate, then added with a 198 µl ABTS work reagent into the well containing the sample (well sample). At well blank, an amount of 200 µl sample solvent (DMSO) is added. In the well control, 200 µl of ABTS work reagent is added, then incubated in a plate for 6 minutes at 37°C. The absorbance is measured using a microplate reader at λ = 745 nm.

$$\%\ \text{Reducing activity} = \frac{\text{Control Absorption} - \text{Sample Absorption}}{\text{Control Absorption}} \times 100\%$$

The percentage of the ABTS radical inhibition (%) was determined by the absorbance ratio of ABTS • + in the sample compared to the absorbance of the control. The result is in the form of an Inhibitory Concentration (IC50); the lower IC50 value will show a stronger ABTS scavenging capacity. Measurements were made with a repetition of three times. [15], [22], [23].

Hydrogen Peroxide Scavenging (H_2O_2) Test

The examination of hydrogen peroxide scavenging (H_2O_2) assay is a method for assessing the size of the ability of the white oyster mushroom ethanol extraction in capturing hydrogen peroxides. The capture of H_2O_2 was measured with the method described by Mukhopadhyay et al. (2016) with a slight modification [24].

The trapping activity of hydrogen peroxide (H_2O_2) was measured with the ferrous ammonium sulphate and phenanthroline reaction methods with minor modifications. The ferrous ammonium sulphate which reacts with phenanthroline will form an orange Fe2+ -tri-phenanthroline complex. H_2O_2 will inhibit the formation of the complex so that antioxidants that trap H_2O_2 will cause the formation of orange Fe2+ -tri-phenanthroline complex [24].

Mixtures of control solutions, samples and blank samples that were added with H_2O_2 are included in the 96-well plate, then incubated for 5 minutes in a dark room with room temperature. Then each sample and the blank mixture was added with 1, 10-phenanthroline as much as 75 µL, and re-incubated for 10 minutes in a dark room with room temperature. The absorbance is measured using a wavelength of 510 nm [24].

The percentage of trapping actions is calculated using the formula**:**

$$\% \text{ scavenging of } H_2O_2 = \frac{\text{A Sample}}{\text{A Control}} \text{ x } 100$$

A: Absorbance

Results

A. The results of the phytochemical analysis of white oyster mushrooms

Table 1 shows that the active ingredient was contained in the preparation of fresh white oyster mushrooms, dry preparations, 70% ethanol extractions and 96% ethanol extractions are alkaloids, steroids, flavonoids, saponins, tannins and phenolic components. Quinone and triterpenoids were not found in all samples. The Meyer alkaloid method was not found in fresh samples and dry samples but was found in the extraction preparation. Flavonoids were not found by the Shinoda test method, but were found with ammonium test. Flavonoids and steroids are more commonly found in the 96% ethanol extraction than the others.

Table 1: Comparison of phytochemical preparations for white oyster mushrooms

Phytochemical analysis		Sample			
Coumpoud/ class	Methode/ Test	Fresh Plant Materials (FPM)	Dry Plant materials (DPM)	Ethanolic extraction mixed with 70% ethanol (EE70)	Ethanolic extraction mixed with 96% ethanol (EE96)
Alkaloid	Dragendroff	±	+	+	+
	Meyer	-	-	+	+
Steroid	Sawkosky	+	+	+	++
	Lieberman	+	+	+	+
Triterpenoid	Lieberman	-	-	-	-
Flavonoid	Ammonium Test	±	+	+	++
	Shinoda Test	-	-	-	-
Saponin	Foam Test	+	++	++	±
Quinone	NaOH	-	-	-	-
Tanin	Gelatin	+	+	+	+
	FeCl3	-	±	-	-
Phenolic	Gelatin test	+	+	+	+
coumpouds	FeCl3	+	+	+	+

Information: (± → weak, + → strong, ++ → very strong).

B. Total phenolic content of ethanolic extractions of Pleurotus ostreatus (EE96)

In Table 2, it can be seen that in the ethanol extraction of white oyster mushrooms were at a total phenol of 6.67 µg phenol in 1 mg extract sample which was equivalent to 1 mg Gallic Acid. (6.67 µg phenol/1 mg GAE/extraction) or equivalent to 667 mg GAE/100 gr in the sample.

Table 2: Results of the Analysis of Total Phenol of Oyster Mushroom Extractions

Final	Total Phenol			Average	Total Phenol (µg sample extraction/mg GAE)			Average	SD	RSD
Conc. (µg/ml)	1	2	3		1	2	3			
2000	10.64	10.81	10.89	10.78	5.32	5.41	5.45	5.39	0.06	1.18
1000	6.56	6.81	6.80	6.72	6.56	6.81	6.80	6.72	0.14	2.12
500	3.74	4.09	4.01	3.95	7.49	8.19	8.02	7.90	0.37	4.64

C. The antioxidant activity of white oyster mushroom extractions by the ABTS method

The concentration of the extractions of white oyster mushroom ethanol tested in the ABTS test were: 400; 200; 100; 50; 25; 12.5; 6.25 (µg/mL) and the largest reduction activity is at a dose of 400 µg/mL with an ABTS reduction activity of 90.98%. Reduction activities of various concentrations showed significantly different results with ($p < 0.05$) (Table 3).

Table 3: ABTS Reduction Activities of EE96 (Average Tukey HSD Post HocTest Results)

Concentrations (µg/mL)	Average ABTS Reduction Activity (%)
	EE96
400	90.98 ± 1.04^{g}
200	77.22 ± 0.08^{f}
100	52.13 ± 1.37^{e}
50	35.96 ± 2.14^{d}
25	22.54 ± 1.45^{c}
12.5	12.09 ± 0.31^{a}
6.25	15.43 ± 0.02^{b}

Data were presented as a mean ± standard deviation. Different letters in the same column are significant at $P < 0.05$ (Tukey HSD post hoc test).

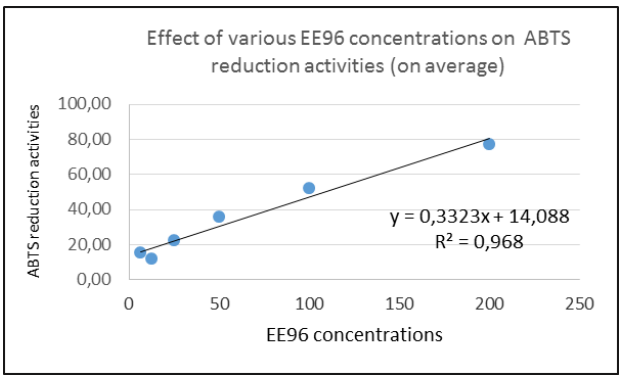

Figure 1: Dosage curve for inhibition presets of EE96 with a correlation coefficient (R2) of 0.98

The ABTS reduction activity of the white oyster mushroom ethanol extraction showed a dose-response curve with an average equation of Y = 0.3323x + 14.088 and obtained a 50% inhibitory concentration of 108.07 ± 1.28 µg/mL (Fig. 1, Table 3 and 4).

Table 3: IC50 value of ABTS reduction from EE96

Sample of EE96	Equation	R2	IC50 (µg/mL)	IC50 (µg/mL)
1 test	Y = 0.3339x + 14.084	0.97	107.57	108.07 ± 1.28
2nd test	Y = 0.3331x + 14.323	0.96	107.11	
3nd test	Y = 0.3300x + 13.857	0.98	109.32	
Average	Y = 0.3323x + 14.088	0.97	108.07	

The IC50 value of ABTS reduction from EE96 and data analysis of ABTS reduction activities from EE96 are shown in Table 3 and Table 4.

Tabel 4: Data Analysis of ABTS Reduction Activities from EE96

Sample	Final Conc. (µg/ml)	Reduction of ABTS (%) 1	2	3	Average	SD	RSD	IC50 Rata - rata - R2	IC50 Ul. 1 - R2	IC50 Ul. 2 - R2	IC50 Ul. 3 - R2	Rata-rata IC50
EE96	400	92.10	90.05	90.78	90.98	1.04	1.14	108.07	107.57	107.11	109.52	108.07
	200	77.28	77.13	77.26	77.22	0.08	0.10					
	100	52.99	52.84	50.55	52.13	1.37	2.63					108.07 ± 1.28
	50	35.78	38.19	33.92	35.96	2.14	5.96					
	25	22.13	21.35	24.16	22.54	1.45	6.43					
	12.5	12.37	12.13	11.75	12.09	0.31	2.58					
	6.25	15.41	15.46	15.43	15.43	0.02	0.14	0.97	0.97	0.96	0.98	1.28

D. The antioxidant activity of the ethanol extraction of Pleurotus ostreatus (EE96) by the H_2O_2 essay method

The concentrations of the extraction of white oyster mushroom ethanol tested in the H2O2 test were: 400; 200; 100; 50; 25; 12.5; 6.25 (µg/mL) and the greatest reduction activity is at a dose of 400 µg/mL with a combustion activity of 73.01% H_2O_2 (Table 5). Reduction activities of various concentrations showed significantly different results with a $p < 0.05$.

Table 5: H_2O_2 Capture of Ethanolic extractions of Pleurotus ostreatus (EE96)

Concentrations (µg/mL)	Average Scavenging activity of H_2O_2 (%) EE96
400	73.01 ± 2.58^{f}
200	47.88 ± 1.03^{e}
100	34.54 ± 2.59^{d}
50	26.25 ± 1.70^{c}
25	18.96 ± 2.37^{b}
12.5	16.26 ± 0.10^{ab}
6.25	13.61 ± 0.20^{a}

*) Data is presented in the form of a mean ± standard deviation; Lowercase differences in the same column show the significance of the data of P < 0.05 (Tukey HSD Post hoc Test).

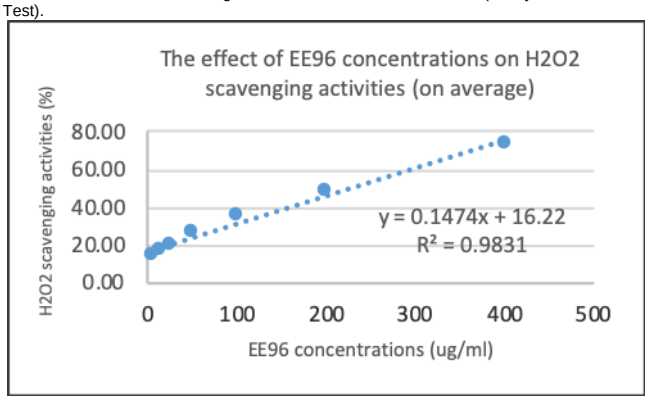

Figure 2: The dose-response curve for the percentage of the EE96 roaming activity with a correlation coefficient (R2) of 0.98

The H_2O_2 roaming activity of the ethanolic extract of Pleurotus ostreatus showed a dosage response curve with an average equation of Y = 0.1474x + 16.220 and obtained an inhibitory value of 50 concentrations at 229.31 ± 3.38 µg/mL (Fig. 2, Table 6 and 7).

Table 6: Analysis of H_2O_2 Roaming Activities for EE96

Sample	Final Conc. (µg/ml)	Roaming activities of H2O2 (%) 1	2	3	Average	SD	RSD	IC50 Rata -rata - R2	IC50 Ul. 1 - R2	IC50 Ul. 2 - R2	IC50 Ul. 3 - R2	Rata-rata IC50
EE96	400	70.55	72.79	75.69	73.01	2.58	3.53	229.17	231.40	231.12	225.41	229.31
	200	48.20	48.71	46.73	47.88	1.03	2.16					
	100	37.45	32.48	33.68	34.54	2.59	7.51					229.31 ± 3.38
	50	27.64	26.75	24.36	26.25	1.70	6.47					
	25	21.25	16.51	19.11	18.96	2.37	12.52					
	12.5	16.31	16.33	16.15	16.26	0.10	0.59					
	6.25	13.68	13.38	13.76	13.61	0.20	1.49	0.98	0.96	0.98	0.99	3.38

Analysis of H_2O_2 roaming activities for EE96 and IC50 value of capturing of H_2O_2 from EE96 are shown in Table 6 and Table 7.

Table 7: IC50 Value of Capturing of H_2O_2 from EE96

Sample of EE96	Equation	R^2	IC_{50} (µg/mL)	IC_{50} (µg/mL)
1 Test	Y = 0.1391x + 17.812	0.96	231.40	229.31±3.38
2nd Test	Y = 0.1493x + 15.494	0.98	231.12	
3nd Test	Y = 0.1537x + 15.354	0.99	225.41	
Average	Y = 0.1474x + 16.220	0.98	229.17	

Discussion

Medicinal plants contain various chemical compounds or active substances that have therapeutic properties. The active substances that are inside medicinal plants depend on the plant species, the time and method of harvesting, the drying process and the production of medicinal plants [25], [26], [27]. Pleurotus ostreatus is a medicinal plant that has many active metabolites which can be used as antioxidants, anti-inflammatory, anti-cholesterol and developed into an anti-cancer. One of the medicinal preparations of white oyster mushrooms which are currently being

developed is the ethanolic extract of Pleurotus ostreatus [4], [5], [6], [7].

The quality of herbal preparations such as the ethanol extraction of P. ostreatus is strongly influenced by the drying process, the selection of solvents used and the ratio of solvents and this solved ones. Ethanol is a universal solvent that is widely used in making extractions because it can dissolve polar and nonpolar substances. To improve the ability of ethanol in drawing polar active substances, it can be done by adding the composition of the water in the substances so that the percentage of ethanol drops, so in this study, there is a comparison between the use of 70% ethanol and 96%. Ethanol can dissolve active substances such as tannins, phenols, saponins, proanthocyanidins, reduce sugars, flavonoids, terpenoids, glycosides. More steroids are found in water and methanol solvents [11].

The result of the phytochemical screening of P. ostreatus herbal preparations showed that the drying process performance did not have much effect on the content of the active ingredient in white oyster mushrooms. Temperatures that are too high when drying can damage the structure of the active substance [26], [27]. In flavonoids and phenolics the levels of fresh preparations appear to be less strong compared to the dry preparations or in extractions, this is probably due to higher levels of freshwater in the preparation so that the levels of the active ingredients appear less.

The active ingredient contained in the preparation of FPM, DPM, EE70 and EE96 are alkaloids, steroids, flavonoids, saponins, tannins and phenolic components. Alkaloids can also be found in ether, methanol or water solvents but cannot be found in hexane. Alkaloids are active metabolic which have antimicrobial effects by inhibiting DNA topoisomerase. More flavonoids observed in the Ammonium test method were found in (EE96), but the literature mentioned that this active substance would have appeared more in water solvents and could appear in methanol solvents. In this study, the Shinoda test gave negative results for flavonoids, and this is probably since there are currently more than 4000 flavonoid compounds identified and the possibility of each test will identify different compounds. The concentration of flavonoids will be reversed more in the 70% ethanol compared to pure ethanol because it increases the polarity. Flavonoid compounds are polyphenolic components that are widely found in plants and have a variety of biological activities including antimutagenic and anticancer effects [11], [26], [27].

Phenol is found in the ethanol extractions of white oyster mushrooms; this is because phenol will show better positive results in alcohol than in water. Phenol is the main compound that is thought to be related to the antioxidant effects of white oyster mushrooms. Its antioxidant ability is strongly related to the hydroxyl content contained in it. Previous research has shown that phenol is proven to prevent damage to the liver, lungs and kidneys. The phenolic component is the main component that affects its antioxidant activity [3], [4], [5], [6], [7]. Phenol can act as a reducing agent for hydrogen donors and singlet oxygen quenchers and has a potential metal chelation effect [7], [28].

The phenolic component in Pleurotus ostreatus contains various types, including vanillic acid, myricetin, naringin, homogentic acid, 5-O-caffeoylquinic acid, chrysin, routine, gentisic acid, gallic acid, protocatechuic acid. caffeic acid, tannic acid, syringic acid, cinnamic acid and p-coumaric acid. Antioxidant properties found in many fungi are generally in the form of phenolic acids and flavonoids, followed by tocopherols, ascorbic acid and carotenoids. Phenolic compounds or polyphenols are thought to be able to inhibit TNF-α gene expression and inhibit their production [29], [30]. The tannin in this study shows positive results. Flavonoids and tannins are part of the polyphenolic component. Tests for tannins show positive results from all four samples. A high tannin intake of phenolpropyranoids can reduce the risk of coronary heart disease [18].

The results of this study showed that in the ethanol extract of 70% ethanolic extraction of P. ostreatus (EE70) there was a total phenol of 6.67 µg phenol in 1 mg of the extraction sample which was equivalent to 1 mg Gallic Acid. (6.67 µg phenol/1 mg GAE/extraction) or equivalent to 667 mg GAE/100 gr sample. This result when compared with the total phenol content of chloroform extraction from the clary sage (Salvia sclarea), an aromatic plant originating from southern Europe, does look smaller, which is the 28.91 µg in the 1 mg extraction. In the extraction of the acetone clary sage (Salvia sclarea), there is a total phenol of 35.24 µg. But in the same study also showed that it turned out that the total phenol level did not illustrate the correlation with the antioxidant strength calculated using the thiocyanate method [20].

In the research of Settharaksa, 2014, the comparison of the total phenol levels saw differences in the total phenol of the Trigasornmas formula extractions in several solvents. Formula trigasornmas are of the coral plant (Jatropha multifida L.), stamen lotus (Nelumbo nucifera Gaertn), and bael fruit (Aegle marmelos (L.) Corr). All ingredients were bought from traditional pharmacies in Bangkok, Thailand. The results showed that water extractions had a higher total phenol content than other compounds at around 1.955.23 ± 60.87 mg GAE/100 g samples. These results differed significantly ($P < 0.05$) with ethyl acetate, methanol, dichloromethane, ethanol and hexane. This research confirms that in this study water seems to be a better solvent than ethanol or acetone. This is probably due to the majority of the polyphenols dissolving in water. Solvents with different polarity can affect dissolved phenol levels [13].

Other studies have shown that the total phenol of P. citrinopileatus can be cultivated with 100% Castanea media. The Sativa is quite high at 2.529 ± 0.010 mg GAE/g, whereas Ostreatus which is bred at 50% C. Sativa + 50% P. Orientalis sawdust shows a lower total of phenol levels at 1.232 ± 0.060 mg GAE/g. This result was lower than some wild herbs containing a total phenol of 2.83-25.38mg GAE/g [14]. Other studies using Ostreatus P. samples developed in Malaysia showed water extractions of Ostreatus P. contained a total phenol of 798.55 mg GAE/100g. In this study, the antioxidant extraction of water from P. ostreatus correlated with the total phenolic content [31]. When compared with the research on the previous status, there are indeed few differences, and the results are relatively smaller, this can be due to the differences in the substrate for logs when cultivated, the harvest, characteristics of the species itself and herbal preparations made.

The ABTS reduction activity of the white oyster mushroom ethanol extraction in this study showed a dosage response curve with an average equation of Y = 0.3323x + 14,088 and obtained a 50% inhibitory concentration of 108.07 ± 1.28 µg/mL. Other studies have shown that ethanolic extractions from the aerial parts of the medicinal plant Coronopus didy have the potential of obtaining a strong ABTS with an IC50 (4.32 × 102 µg/mL) and this value is higher than the DPPH scavenging activity with an IC50 7.80 × 102 µg/mL]. This shows that the components taken with an ethanol solvent have a strong ABTS of radical-scavengers [23].

The ABTS reduction activity of Ethanol extractions of Sativa, vanillin, and the coumaric acid oriza showed that the IC50 sativa oriza extraction was the highest (145.67 µg/mL) when compared to vanillin (4.96 µg/mL) and the coumaric acid (1.67 µg/mL). These results show that the oriza sativa ethanol extraction showed a weak antioxidant activity compared to the other two pure components [32]. The ethanol extract of white oyster mushrooms measured using the same method showed lower IC 50 results than the sativa oriza, so the antioxidant potential seemed to be more this is higher compared to the sativa oriza extraction, but it is indeed lower when compared the to vanillin or asan coumarik [32].

Cultivated antioxidant Pleurotus ostreatus studies in Malaysia showed the potential of ABTS•+ scavenging from P. ostreatus (87.29 ± 0.54%) and this result was significantly higher when compared to the asm ascorbate at the concentrations of 1000 and 1500 µM (49.88 ± 0.17% and 73.11 ± 0.22%), also with the BHA in 1000 and 1500 µM concentrations (48.64 ± 0.36% and 65.84 ± 0.17%, respectively). The correlation coefficient (r) between the antioxidant activity, DPPH scavenging ability (%), and TPC was 0.915, the correlation coefficient between ABTS + scavenging ability (%) and TPC was 0.767, and the correlation coefficient between r FRAP and TPC was 0.981. This shows that the phenol content in this study contributes to the antioxidant potential of P. ostreatus extractions from Malaysia [14]. In this study the P. ostreatus showed a reduction potential of 90.98% at a concentration of 400 µg/L, this shows that the ability of antioxidants is quite well when compared to similar species originating from Malaysia.

In this study, the antioxidant activity was also assessed using an H2O2 essay. Hydrogen peroxide is an important biological compound produced in the process of aerobic metabolism, and its production is increased in the conditions of infection, exercise, stress, radiation and other inductions. Humans can be exposed to H2o2 from the environment at around 0.28 mg/kg body weight/day. Hydrogen peroxide can be inhaled or absorbed through the eyes or skin. This compound is not toxic but can turn into toxic compounds and produce more toxic compounds, such as hydroxyl through Fenton reactions, or become hypochlorous acid because of the enzyme myeloperoxidase (MPO). Increased production of H2O2 will ultimately increase ROS and cause redox imbalances resulting in oxidative stress. This oxidative stress can be inhibited by antioxidants, and the antioxidant ability to scavenge ROS can vary [24], [25], [26], [27], [28], [29], [30], [31], [32].

In the test of antioxidant activity, oyster mushroom extractions have an antioxidant activity by combining H2O2. The oyster mushroom extraction has an IC50 229.17 µg/mL. In the previous study P. florida and C. indica are at 200-1000 µg/ml with the results of the radical hydroxyl radical scavenging with IC50 values for P. florida and C. indica were 220.70 ± 6.0 µg/ml and 148.23 ± 1.01 µg/ml [34]. These results show that the ability of Pleurotus ostreatus in the capture of H2O2 is almost the same as P fluoride, both of which are in the oyster mushroom group.

In the research of Mukhopadhyay, 2016 there is a comparison of some of the abilities of H2O2 extractions with the same method for several antioxidants, which showed the scavenging activity of H202 (IC Value) as the following. Malicidal acid 53.68 ± 2.91 µg/mL, pyrogallol 47.35 ± 3.01 µg/mL, Ascorbic acid 426.80 ± 24.82 µg/mL, Sodium pyruvate > 1000 µg/mL, Mannitol Dimethyl Sulphoxide (DMSO) No effect, Sodium azide no effect, Uric acid Peroxynitrite scavenger no effect, Etodolac2699 ± 479.50 mM, Indomethacin > 5000 mM. Galic acid, pyrogallol and ascorbic acid are known as common antioxidants, while sodium pyruvate is a specific antioxidant as an H2O2 scavenger. Sodium azide is a single oxygen scavenger, and mannitol and DMSO are OH scavengers. Based on these studies, the ability of the white oyster mushroom in scavenging is very well, even when compared with the ascorbic acid [24].

Antioxidant tests for H_2O_2 activity used by Fernando used an L-Ascorbic acid 10.00.14, C. sinensis (black tea) 91.96-2.51, Gallic acid 7.82-0.19, Tannic acid 8.17 0.10 mg/ml). The H2O2 activity test used both colourimetric but different reagents.

Fernando's research used H2O2, phenol and 4-aminoantipyrine which reacted with the horseradish peroxidase (HRP). This reaction uses a chromogen quinone imine which was assessed using a wavelength of 504 nm [35]. In this study, the antioxidant activity of the white oyster mushroom ethanol extraction was seen using the TEAC and H2O2 essay methods because these two methods were used less frequently than the in vitro antioxidant activity test method. The most commonly used methods are the a-diphenyl-bpicrylhydrazyl DPPH, hydroxyl and Superoxide dismutase (SOD) and b-carotene linolate [35].

Ethanol extractions are one of the most frequently used solvents for extracting, which is thought to have an antioxidant activity which is other than water and methanol solvents. All three are good polarity solvents which can dissolve polar active substances such as phenols and flavonoids. The use of water is rather limited because it requires special techniques when evaporating, while the use of methanol is limited due to its toxic effects [33].

Plants have excellent antioxidant potential because they contain enzymatic antioxidants as well as non-antioxidant enzymes. Antioxidant enzymes, for example, are SOD, catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase. Non-enzyme antioxidants contain antioxidants with small molecular weights such as ascorbic acid, phenol, glutathione, carotenoids, flavonoids or large, heavy molecules such as tannins. This is probably due to two reasons; the first is genetically indeed are the basic ability for plants to produce phytochemicals that can reduce the physiological processes, or as protection against oxidative stress environments such as plant parasites and microbes [33].

Some secondary metabolites are routinely formed, but some are formed in response to biytic and abiotic stress. Accumulation of phenolic compounds is because changes in the phenylpropanoid metabolism have been widely observed in several stressful conditions. In plants, phenolics can act as antioxidants by donating electrocution to guaiacol-type peroxidases to detoxify the production of H_2O_2 under stressful conditions. Phenolics also have protection capabilities against UV radiation through its ability to scavenge [36].

Biotic stress, such as wounds in plants, can induce phenolic metabolism and increase phenolic synthesis. Tanin is also thought to have a protective effect resembling phenol. Alkaloids can also generally protect plants against antimicrobials or attack herbivores and UV radiation. In the in vitro test, the alkaloid antioxidant activity was reported to be moderate to undetermined. Terpenoids are a secondary metabolite whose group is broad, consisting of 40,000 components. Monoterpenes, sesquiterpenes and diterpenes have been shown to have antioxidant activity. Tetraterpenes and carotenoids are also reported to have an antioxidant activity both in vitro and in vivo, some types of carotenoids such as beta-carotene show a prooxidant effect at high concentrations and high oxygen pressure [36].

So far phenols are known as the most important secondary metabolite based on the assessment of antioxidant activity, both in vitro and in vivo. Plant phenolic components are generally classified into five groups, namely phenolic acids, flavonoids, lignans, stilbenes and tannins. Phenolic compounds generally have one or more aromatic rings with one or more hydroxyl groups. Allegedly phenolic antioxidant capacity will increase by the addition of free hydroxyl groups and conjugations from the side chains of their aromatic rings. Flavonoids and phenolic acids are the largest groups of plants that are biosynthesized from the derivatives of acetate and shikimate pathways, as well as the shikimate pathway of phenylalanine or tyrosine [36].

Phytochemicals from these two groups were found to have an excellent antioxidant activity both in vitro and in vivo. It is also known that these metabolites will interact with physiological antioxidants such as ascorbic acid or tocopherol and will synergise the biological effects of both. Flavonoids and phenylpropanoids will cause oxidation with the help of the peroxidase enzyme and can act as H2O2 scavengers. In various studies, it was seen that the antioxidant potential of plants containing phenol, generally related to electron donation, reduced the ability in the metal ion chelating [36].

In conclusion, the process of drying Pleurotus Ostreatus was not reduced in the content of active substances. The phytochemical screening is obtained from all the samples. The polar active substances seem to be more soluble in the EE70 than in the EE96. The higher the bio-substances in preparation, the more significant the bio-therapeutic effects. White oyster mushrooms contain high levels of total phenol and high antioxidant activity of ABTS and a promising capture of H2O2, because of that this plant is very convincing to develop as a source of a medicinal plant for therapy.

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