



## RESEARCH ARTICLE

# Active compound test: ethanolic extract of White Oyster Mushroom (*Pleurotus ostreatus*) Using HPLC and LC-MS

## [version 1; peer review: awaiting peer review]

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### Open Peer Review

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### Abstract

**Background:** The use of herbs as traditional medicine in Indonesia is increasing, with more than 40% of Indonesia's population utilizing them. White oyster mushroom (*Pleurotus Ostreatus*) is a fungus that has various therapeutic effects including antioxidant, anti-inflammatory, anti-bacterial, anti-cholesterol, and anti-cancer properties. This mushroom contains many active substances in its secondary metabolites which have pharmacological effects. The purpose of this study was to identify the active compounds in the ethanolic extract of white oyster mushroom that will form the extract profile and become the basis for drug development.

**Methods:** The active compound test used High-Performance Liquid Chromatography (HPLC) and Liquid Chromatography with Mass Spectrometer (LC-MS). Ethanolic extract of white oyster mushroom was processed by 70% alcohol maceration, evaporation, and thickening.

**Results:** The results of the HPLC test showed that the ethanolic extract of white oyster mushroom contained cinnamic acid and rutin, while the LC-MS test showed the presence of p-coumaric acid, Ascorbic acid, Linoleic acid, 9-Eicosene (E), Niacinamide, Veritric acid, Syringic acid, Ergosterol.

**Conclusions:** The active compounds that were detected in the ethanolic extract of white oyster mushroom showed that the extract had the potential for antioxidant and anti-inflammatory activity.

### Keywords

White oyster mushroom, ethanolic extract, HPLC, LC-MS, active compounds.



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## Introduction

White oyster mushroom (*Pleurotus ostreatus*) is a very popular food source, belonging to the Basidiomycetes class of fungi.<sup>1,2</sup> This mushroom has a very high nutritional value and various secondary metabolites that have potential for pharmacological effects.<sup>1,3</sup> Two of the most important pharmacological effects of mushrooms are the antioxidant and anti-inflammatory effects. White oyster mushroom is also widely used in the prevention of several chronic diseases such as hypertension, hypercholesterolemia, and carcinoma. Various previous studies have proven that white oyster mushrooms have strong antioxidant abilities both in vivo and in vitro.<sup>4,5</sup>

Many metabolites in white oyster mushrooms act as anti-inflammatories such as beta-glucan ( $\beta$ -glucan) or polysaccharides, phenolic components, flavonoids, terpenoids, lectins, steroids, glycoproteins and ergothioneine (ET). White oyster mushrooms, which are believed to have antioxidant effects, include vitamin C, beta-carotene, selenium, ergothioneine, and phenolic components. Phenolic components are the main components that affect its antioxidant activity.<sup>6-8</sup> Previous studies have shown the antioxidant effect of ethanolic extract of white oyster mushroom which can prevent the increase in MDA and decrease in lung surface density (S/V).<sup>5</sup>

Oyster mushrooms (of which white oyster is one type) overall when compared to winter and shitake mushrooms have antioxidant activity, reducing power, scavenging abilities, and higher total phenol content. They also have good hydroxyl and superoxide radical scavenging, and the potential to inhibit lipid peroxidase activity with an IC 50 value that is almost the same as vitamin C, which is 6-8 mg/ml.<sup>6,9</sup>

The anti-inflammatory mechanism of white oyster mushrooms varies and has not been fully elucidated, but several studies have demonstrated its anti-inflammatory potential.<sup>10-13</sup> Oyster mushroom concentrates in vitro studies can suppress the production of TNF- $\alpha$  and nitric oxide (NO) in macrophages RAW 264<sup>12</sup> induced by lipopolysaccharides (LPS) with interferon- $\gamma$  (IFN- $\gamma$ ). Other cytokines that are inhibited are interleukin-6 (IL-6), interleukin-12 (IL-12), and prostaglandin E2 (PGE2) through downregulation of cyclooxygenase-2 (COX-2) and expression of inducible nitric oxide synthase (iNOS)). in vitro activity of White oyster mushrooms can inhibit the activation of NF- $\kappa$ B through the inhibition of phosphorylation of the inhibitory protein  $\kappa$ B (I $\kappa$ B $\alpha$ ).<sup>1,7,12</sup>

The use of white oyster mushrooms as a natural antioxidant and anti-inflammatory is also very beneficial for the wider community because of its widespread availability, easy and short cultivation, and affordability. The use of herbs in medicine is expected to provide lesser side effects than the use of synthetic or chemical materials.<sup>1,2</sup>

For white oyster mushroom ethanol extract to become a standardized herbal or phytopharmaca, it is necessary to carry out several tests to strengthen the scientific data regarding the effect of ethanolic extract of white oyster mushroom. The first stage that must be carried out is the phytochemical and standard compound tests, and the compound profile analysis. The results of the phytochemical analysis in preliminary research stated that ethanolic extract of white oyster mushroom contains alkaloids, steroids, tannins, saponins, flavonoids, and phenolic compounds. Based on this data, we studied the marker compounds and analyzed the profile of ethanolic extract of white oyster mushroom, to strengthen scientific data regarding the effects of white oyster mushrooms and facilitate their development into standardized herbs.

## Methods

This research is an in vitro experimental test with the subject being the ethanolic extract of White Oyster Mushroom. Only fresh white oyster mushrooms were included. 70% ethanol maceration and phytochemical tests (alkaloids, steroids, flavonoids, quinones, saponins, and tannins) were carried out on these. The materials used in this study were: White Oyster Mushroom Extract from oyster mushroom farmers in Cisarua Village, Kec. Cisarua, 70% Ethanol, Standard Compounds for High-Performance Liquid Chromatography (HPLC): Rutin, Myricetin, Cinnamic Acid, Coumaric Acid, and Liquid Chromatography with Mass Spectrometer (LC-MS) Reagents. The tools needed are the tools for preparation and administration of ethanolic extract of white oyster mushroom, analytical balance, HPLC, and LC-MS. Each test was performed twice.

### Preparation of ethanolic extract of White Oyster Mushroom

Fresh white oyster mushrooms (50 kg) Dried taken from oyster mushroom cultivation in Kec. Cisarua were sliced and then put in an oven at 50° Celsius with a thickness of 1-2 cm, for two to three days. After drying, the simplicia was pureed using a grinding tool.<sup>14</sup>

Dried mushroom was macerated with 70% ethanol for 24 hours. Then the alcohol was filtered using filter paper. The remaining solution is called aqueous extract. The maceration was repeated five times. The dilute extract was then concentrated using a rotary evaporator until no more solvent dripped into the condenser of the rotary evaporator and concentrated again with a water bath.<sup>14</sup>

### High-performance liquid chromatography (HPLC) test for ethanolic extract of white oyster mushroom

Based on the literature, it was found that the active substances found in white oyster mushrooms are included in the form of phenolic acids also flavonoids. The phenolic acid derivatives that were studied in this study are cinnamic acid and p-coumaric acid, while the flavonoid compounds to measured were myricetin, rutin, saponins, tannins, and steroids/triterpenoids.<sup>9</sup>

The marker compounds thought to be present in white oyster mushrooms were tested using High Performance Liquid Chromatography (HPLC) by showing quantitative analysis.<sup>15,16</sup> The standard compounds tested in this study were rutin, myricetin, quercetin, cinnamic acid, and coumaric acid.

High-performance liquid chromatography (HPLC) uses the principles of chromatography to measure samples. In chromatography, analysis is done by separating molecules based on differences in structure or composition. The separation occurs when the sample moves through the stationary phase (can be solid or liquid) because it is carried away by the mobile phase (can be liquid or gas). The results were obtained, comparing the standard chromatogram with the sample chromatogram, by identifying the retention time (RT).<sup>17</sup>

The results of the measurement of the active substance in the ethanolic extract of white oyster mushroom were then compared with the results of the measurement of standard compounds, by comparing the retention time of the compounds in the ethanolic extract of white oyster mushrooms with standard compounds that have been tested first.

### Marker compound test with LC-MS

Concentrations in extracts are often very low, requiring very high selectivity and sensitivity. Detection of components with a non-targeted approach is often needed to ensure that other active substances are present in the extract and can be developed into more specific herbal medicines.

Mass spectrophotometry (MS) is an analytical method used to identify the compounds based on their molecular weight. In MS organic compound molecules are bombarded with electron beams so that the compound is ionized.<sup>18</sup> Liquid Chromatography-Mass Spectrometry (LC/MS-MS) is an analytical technique that combines the capabilities of liquid chromatography with mass spectrometry. Liquid chromatography separates the components of the herb extract and then charged ions are detected by a mass spectrometer. LC-MS data can provide information about the molecular weight, structure, identity and quantity of certain sample components. Compounds are separated based on interactions with the chemical layer of the particles (stationary phase) and solvent elution through the mobile phase. The detector then calculates the induced charge or current generated when the ions hit or pass through the surface, scan the mass and calculate the ions as mass to charge ratio (m/z). There are four mass spectrometry processes, namely ionization, acceleration, deflection, and detection.<sup>18,19</sup> The results of the LC/MS-MS data analysis yield a chromatogram in the form of a peak height groove, and the molecular weight of the compounds contained in the extract can be determined so that their number can be elucidated.

### Research setting

The research took place at Unisba Pharmacy Research Laboratory and was assisted by Aretha Medika Utama Biomolecular and Biomedical Research Center. Measurement of marker compounds with HPLC was carried out in two laboratories, namely the laboratory of the Faculty of Chemistry, Universitas Pendidikan Indonesia using HPLC (Hitachi D7000) for cinnamic acid and coumaric acid compounds and at the Poltekkes Malang Laboratory using UHPLC (ACCELLA type 1250) for rutin compounds, myricetin, and quercetin. The LC-MS test was carried out at the Areta Laboratory in collaboration with the Lab. Instrumental Analytical Chemistry, Department of Chemical Engineering, State Polytechnic of Malang.

### Ethical approval

This study received ethical approval from Medical research ethics committee of Universitas Islam Bandung Nomor: 111/KEPK-Unisba/XI/2020

### Results

The ethanolic extract of white oyster mushroom was made from 5 kg of wet simplicia of oyster mushrooms, of which only the umbrella part was then taken, so that it was reduced to 3 kg. After drying, the shrinkage was to 0.14 kg. Maceration was carried out with 70% ethanol in a ratio of 1:5 and maceration was carried out for 3 days with a daily change of 0.14 kg of ethanol: 0.7 L (1:5). The total extract obtained was 36.3111 grams in the form of a brown paste.

### Analysis of marker compounds with HPLC

The compounds to be tested in the ethanol extract of white oyster mushrooms by HPLC are five compounds, namely rutin, myricetin, quercetin, cinnamic acid, and coumaric acid. The standard compounds were validated first and then the compounds in the ethanol extract of white oyster mushroom were measured. The validation results show that the standard of cinnamic acid and p-coumaric acid has been validated and shows retention times of 18.91 for cinnamic acid and 2.21 for p-coumaric acid.

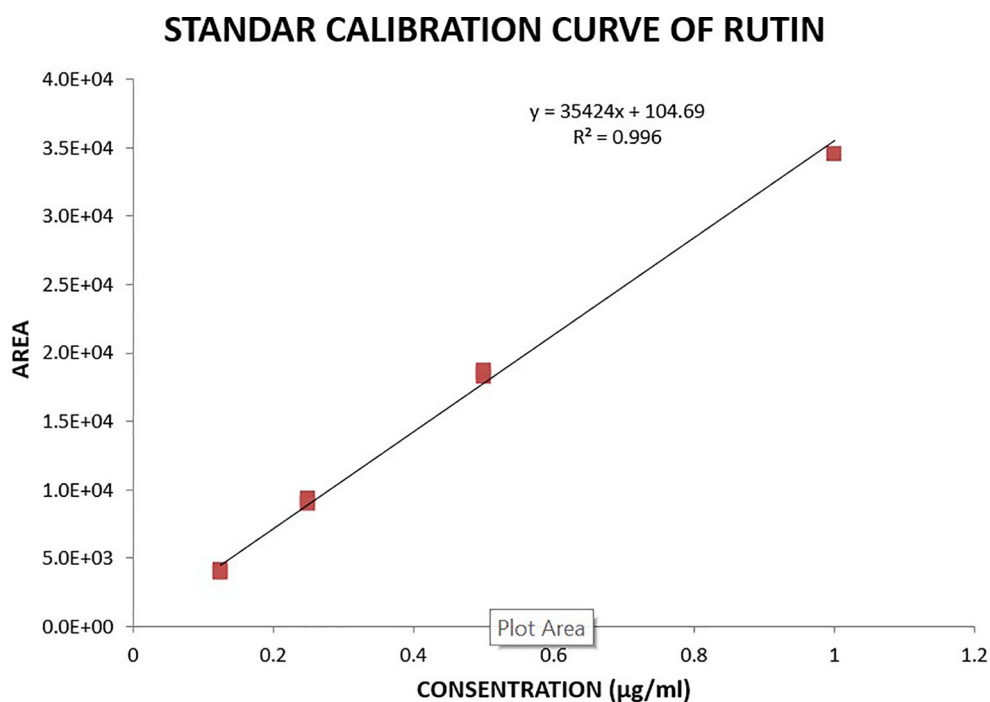
**Table 1** shows that the ethanolic extract of white oyster mushroom appears to contain cinnamic acid with a concentration of 0.073% with a retention time of 18.81 minutes. This result can be seen from the retention time which is similar to the retention time of cinnamic acid, however, for cinnamic acid and p-coumaric acid, the molecular weight of the compounds analyzed is not observed.

The test results for rutin standards with initial concentrations of 0.125, 0.250, 0.500 and 1,000 g/mL and resulted in a linear regression curve as shown in **Figure 1**. The routine standard test showed good validation.

**Table 1. HPLC results of ethanolic extract of white oyster mushroom with comparison of cinnamic acid and p-coumaric acid.**

Peaks	RT	Area	Conc. (%)
1.	1.32	5504621	71.700
2.	1.73	42152	0.549
3.	1.90	2008845	26.166
4.	2.49	50148	0.653
5.	13.32	65906	0.858
6.	18.81	5601	0.073

Keterangan: RT: *retention time (minute)*; Conc. : concentration.



**Figure 1. Rutin calibration curve.**

**Figure 1** shows the linear regression equation  $y = 35434x + 104.69$  with  $R^2 = 0.996$ . The increase in concentration shows a strong correlation with the area. This shows that an increase in the concentration of rutin will show an increase in the level of the substance detected on the curve.

**Table 2** shows the results of rutin compound measurements in the ethanolic extract of white oyster mushrooms. Ethanolic extract of white oyster mushroom samples were measured three times to get more accurate results. Based on the results of HPLC analysis, the three samples of ethanolic extract of white oyster mushroom were predicted to contain rutin, with different results. The ethanolic extract of white oyster mushroom was predicted to have a Rutin compound with a concentration of **168.45 g/g** sample.

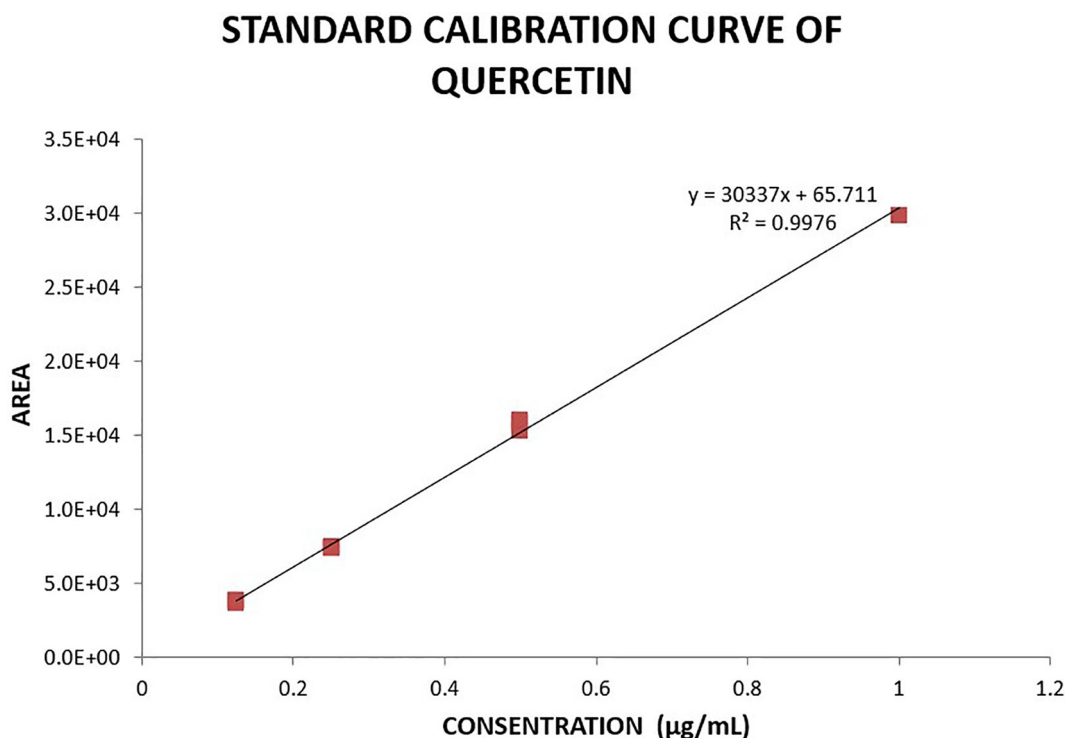
Calculations for standard quercetin with initial concentrations of 0.125, 0.250, 0.500, and 1,000 g/mL resulted in a linear regression curve as shown in **Figure 2**. The quercetin standard test showed good validation.

**Figure 2** shows the linear regression equation  $y = 30337x + 65.711$  with  $R^2 = 0.9976$ . Increased concentration shows a strong correlation with area.

**Table 2. Recapitulation of calculation of rutin samples from ethanolic extract of white oyster mushroom.**

Sample	S.W (g)	Area	M.C (µg/ml)	D.F (ml)	Weight (µg)	C.C (µg/g)	Rate (%)
EEWOM_1	0.7	387460	10.935	10	109,348	168.23	0.0168
EEWOM_2	0.7	387230	10.928	10	109,283	168.13	0.0168
EEWOM_3	0.7	389230	10.985	10	109,848	169.00	0.0169
					Average		0.016845
					STD_DEV		0.0000
					%RSD		0.0000

Description: EEWOM: Ethanolic extract of White Oyster Mushroom; S.W: Sample Weight; M.C: Measured Concentrated; D. F: Diluent Factor; C.C.: Calculated Concentration.



**Figure 2.** Quercetin calibration curve.

**Table 3** shows the measurement results of quercetin compounds in the ethanolic extract of white oyster mushrooms. Based on the results of HPLC analysis, **ethanolic extract of white Oyster Mushroom was not detected to have quercetin compounds.**

The test results for the myricetin standard showed calculations with initial concentrations of 0.5, 1.00, 2.00, and 4.00 g/mL and produced a linear regression curve as shown in **Figure 3**. The myricetin standard test showed good validation.

**Figure 3** shows the linear regression equation  $y = 1634.3x + 166.77$  with  $R^2 = 0.9986$ . The increase in concentration shows a strong correlation with the area.

**Table 4** shows the measurement results of myricetin compounds in the ethanolic extract of white oyster mushrooms. Based on the results of HPLC analysis, **the ethanolic extract of white Oyster Mushroom was not detected to have myricetin compound.**

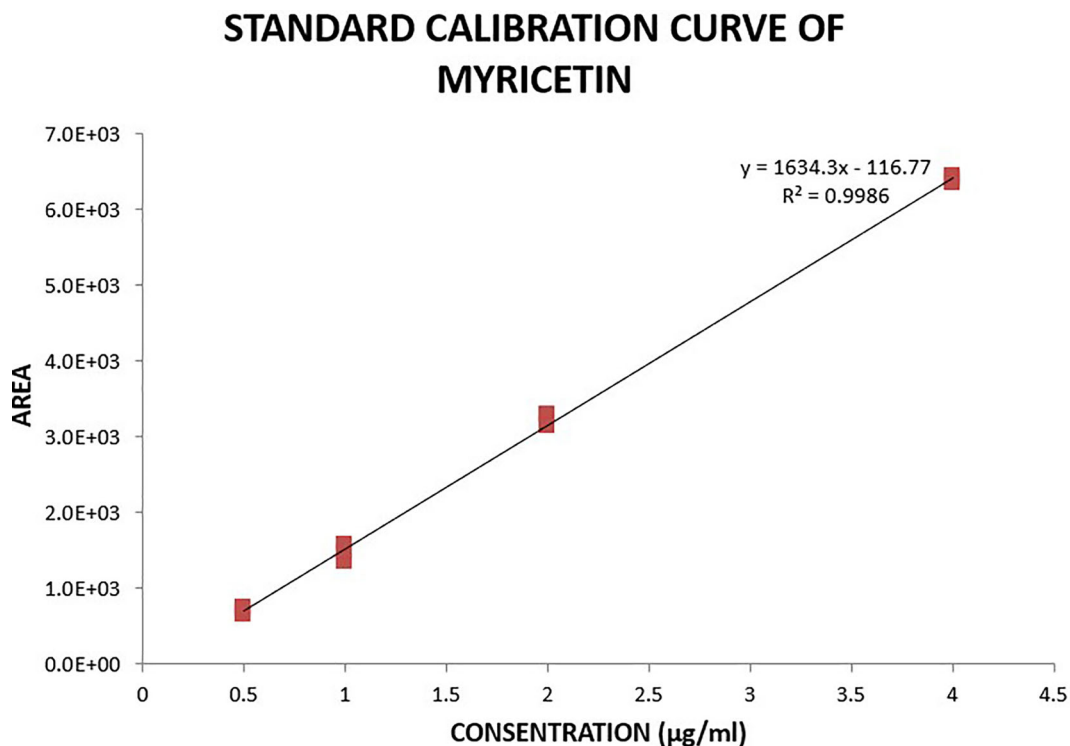
### Mass spectrophotometry analysis

The use of MS/MS Triple Q (quadrupole) TSQ QUANTUM ACCESS mass spectrometer with ESI (Electrospray Ionization) ionization source is controlled by TSQ Tune software which is operated with a positive charge. ESI-MS

**Table 3. Recapitulation of calculation of quercetin samples from ethanolic extract of white oyster mushroom.**

Sample	S.W (g)	Area	M. C (µg/ml)	D.F (ml)	Weight (µg)	C.C (µg/g)	Rate (%)
EEWOM_1	0.6524	-	< LOD	10	< LOQ	< LOQ	< LOQ
EEWOM_2	0.6524	-	< LOD	10	< LOQ	< LOQ	< LOQ
EEWOM_3	0.6524	-	< LOD	10	< LOQ	< LOQ	< LOQ

Description: EEWOM: Ethanolic extract of White Oyster Mushroom; S.W: Sample Weight; M.C: Measured Concentrated; D. F: Diluent Factor; C.C.: Calculated Concentration; M. C.: Measured Concentration; LOD: Limit of detection; LOQ: Limit of quantification.



**Figure 3. Myricetin standard curve.**

**Table 4. Recapitulation of calculation of myricetin samples from ethanolic extract of white oyster mushroom.**

Sample	S.W (g)	Area	M.C. (µg/ml)	D.F (ml)	Weight (µg)	C.C (µg/g)	Rate (%)
EEWOM_1	0.6524	-	< LOD	10	< LOQ	< LOQ	< LOQ
EEWOM_2	0.6524	-	< LOD	10	< LOQ	< LOQ	< LOQ
EEWOM_3	0.6524	-	< LOD	10	< LOQ	< LOQ	< LOQ

Description: EEWOM: Ethanolic extract of White Oyster Mushroom; S.W: Sample Weight; M.C.: Measured Concentration; D. F: Diluent Factor; C.C; Calculated Concentration; STD\_DEV: Standard of Deviation; RSD: Average of standard of deviation.

**Table 5. Identification of target compounds in the ethanolic extract of white oyster mushroom by LC-MS.**

EEWOM	MW (g/mol)	MS [M+H] <sup>+</sup> (m/z)	MS [M-H] <sup>-</sup>
p-coumaric acid	164.16		163.199
Ascorbic acid	176.12		175.094
Linoleic acid	280.44		279.900
9-Eicosene, (E)	280.5		279.900
Niaciamide	122.12	123.002	
Veratric acid	182.17	183.001	
Syringic acid	198.17	199.000	
Ergosterol	396.65	397.113	

Description: EEWOM: Ethanolic Extract of White Oyster Mushroom.

ionization conditions on direct infusion, sample flow rate of 5 l/min with MS equipment conditions are as follows: spray voltage of 3 kV; Evaporation temperature 50°C; Capillary temperature, 270°C; nitrogen as a sheath gas pressure of 5 psi.

Table 5 shows that the sample of ethanolic extract of white oyster mushroom contains p-coumaric acid, Ascorbic acid, Linoleic acid, 9-Eicosene (E), Niacinamide, Veritric acid, Syringic acid, and Ergosterol.

## Discussion

The therapeutic effects or medicinal properties of medicinal plants and fungi come from the active substances contained within them. The active substances, which are generally inert, are closely related to substances that give color and taste or affect the structure of the medicinal plant or fungi. The specificity and purification of this active substance are very important to produce a specific pharmacological effect. Standardized active substances will produce good quality herbal medicine preparations and herbal medicine standards will become more secure. The active substances contained depends on the species of the plant or fungi, the procedure of harvesting, and the manufacturing and handling of medicinal plant/fungi preparations.<sup>20,21</sup> Ethanolic extract of white oyster mushroom has become one of the medicinal preparations that is currently being developed.

The quality of herbal preparations such as ethanolic extract of white oyster mushroom is strongly influenced by the drying process, the choice of solvent used and the ratio of solvent to be dissolved. Ethanol is a universal solvent that is widely used in the manufacture of extracts because it can dissolve polar and non-polar substances. Ethanol can dissolve active substances such as tannins, phenols, saponins, proanthocyanins, reducing sugars, flavonoids, terpenoids, and glycosides. Steroids are more commonly found in water and methanol solvents.<sup>14</sup>

Phytochemical tests carried out on this white oyster mushroom extract in previous studies showed that the active substances contained in this extract were alkaloids, steroids, triterpenoids, saponins, quinones, tannins, and phenolic components.<sup>9</sup>

Alkaloids can also be found in ether, methanol, or water, but cannot be found in hexane. Alkaloids are active metabolites that have antimicrobial effects by inhibiting DNA topoisomerase. The flavonoid concentration will be reversed in 70% ethanol compared to pure ethanol because it increases the polarity. Flavonoid compounds are polyphenolic components that are widely found in plants and have various biological activities including antimutagenic and anticancer.<sup>1,13</sup> Phenols



are found in the ethanolic extract of white oyster mushrooms and are the main compounds related to the antioxidant effect of white oyster mushrooms. Its antioxidant ability is closely related to its hydroxyl content. Phenolics can act as reducing agents for hydrogen donors and singlet oxygen quenchers and have potential metal chelation effects.<sup>8,13,22</sup>

The phenolic components in *Pleurotus ostreatus* contain various types, including vanillic acid, myricetin, naringin, homogentisic acid, 5-O-caffeoylquinic acid, chrysin, rutin, gentisic acid, gallic acid, protocatechuic, caffeic acid, tannic acid, syringic acid, cinnamic acid, and p-coumaric acid. Antioxidant properties found in mushrooms are generally in the form of phenolic acids and flavonoids, followed by tocopherols, ascorbic acid, and carotenoids.<sup>2,9</sup>

The different mechanisms of the various active substances contained in herbal preparations can strengthen the biological effects caused by the herbal preparations. Based on the results of phytochemicals, two groups of compounds that are thought to play a major role in providing biological or pharmacological effects in the ethanol extract of white oyster mushrooms are phenolic compounds and flavonoids. Phenols and flavonoids are both polyphenolics. Various studies have proven their antioxidant effects and their ability to be free radical scavengers, especially against lipid peroxyl compounds, superoxide anions, and hydroxyl radicals. Polyphenols and flavonoids have been shown to have good abilities as antioxidants.<sup>8,23</sup>

Phenolic acid refers to the term phenol component which contains one functional carboxyl acid group and is a polyphenol metabolite. Phenolic acids have two different carbon groups, namely hydroxycinnamic acid which forms simple esters with glucose or hydroxy carboxylic acids. Phenolic components in plants and fungi have different molecular structures and are characterized by the presence of a hydroxylated aromatic ring. Hydroxycinnamic consists of cinnamic acid, ferulic acid, sinapic acid, and caffeic acid, while hydroxycarboxylic acids include benzoic acid, gallic acid, vanillic acid, and salicylic acid.

Flavonoids such as phenolic acid are also part of polyphenolic compounds which are very low in toxicity and are widely distributed in plants. Flavonoids are also known to have very varied structural and biological properties.

Dietary sources of flavonoids can be found in the form of flavonols, flavones, isoflavones, and flavanones. Included in the flavones class are apigenin, luteolin, and chrysin, which include flavanones such as quercetin, kaempferol, and galangin. Examples of flavonone groups are naringenin, hesperetin, while isoflavones include genistein, daidzein.<sup>12,22,24,25</sup>

Quercetin, a flavonol found in many diets, is a potent antioxidant because it has a structure suitable for free radical scavenging. Currently, phenolics, and flavonoids are considered to be strong antioxidants whose effectiveness is a better than Vitamin C, E and carotenoids. Flavones and catechins are considered the most effective flavonoids against ROS. Quercetin, kaempferol, morin, myricetin, and rutin, are also known to act as antioxidants, and have anti-inflammatory, anti-allergic, antiviral, and anticancer effects. The decrease in phenolic and flavonoid activity depends on the number of free hydroxyl groups in their chemical structure and this can be strengthened by steric hindrance.<sup>26,27</sup>

The results from HPLC showed that from the previous phytochemical results, only a few substances were detected in the white oyster mushroom extract, namely rutin, and cinnamic acid, while for coumaric acid, myricetin and quercetin had not shown significant results. The next test for the active substances in this extract was followed by LCMs and p-coumaric acid, Ascorbic acid, Linoleic acid, 9-Eicosene (E), Niaciamide, Veritric acid, Syringic acid, and Ergosterol. In LC-MS, coumaric acid was detected in the extract but cinnamic acid was not detected, while the others were not detected in HPLC because they were not examined. HPLC requires an examination standard while LCMS does not require an active substance standard as a comparison.

The advantage of LC-MS is that it can analyze a wider range of components, such as thermally labile compounds, those with high polarity or high molecular mass, and even proteins. The elution component of the chromatographic column is then transmitted to the mass spectrometer through a special interface. The principle is the separation of analytes based on their polarity, the apparatus consists of a column (as the stationary phase) and a certain solution as the mobile phase and high pressure is used to push the mobile phase. The analyte mixture will separate based on its polarity and the speed to get to the detector (retention time) will be different, this will be observed in a spectrum where the peaks are separated.<sup>15,18,19,28</sup> When compared to the results of the previous phytochemical tests and the results of HPLC and LCMS, some of them showed relevant data, but some of them showed different data. This may be influenced by several factors, including the quality of the extract, the procedure carried out, and the study of the structure of each active substance. The results of the three tests can be the basis for better understanding of the profile of the ethanol extract of white oyster mushroom.

## Conclusions

Analysis of the active substance based on the HPLC test showed that the ethanol extract of white oyster mushroom contained the active compounds rutin and cinnamic acid, while the LC-MS test showed that the ethanol extract of the white oyster mushroom contained the active compounds p-coumaric acid, ascorbic acid, linoleic acid, 9-eicosene, niaciamide, veritric acid, syringic acid, and ergosterol.

## Data availability

### Underlying data

Bhakti Rahimah, Santun (2021): UHPLC and LC-MS (ESI-MS) Test For Ethanol Extract of White Oyster Mushroom. docx. Figshare. <https://doi.org/10.6084/m9.figshare.16550910.v4>.<sup>29</sup>

This project contains the following underlying data:

- Data file 1. LC-MS (ESI-MS) Test of Ethanol Extract of White Oyster Mushroom
- Data file 2. UHPLC Test For Ethanol Extract of White Oyster Mushroom

Data are available under the terms of the [Creative Commons Zero “No rights reserved” data waiver \(CC0 1.0 Public domain dedication\)](#).

## Authors' contributions

Santun Bhakti Rahimah: supervision, conceptualization, investigation, writing- review and editing.

Agung Firmansyah: Resources, writing-review and editing.

Winni Maharani: Investigation, writing original draft, writing-review and editing.

Yuke Andriane: Methodology, conceptualization, writing original draft, writing-review and editing.

Dicky Santosa: Investigation, writing-review and editing.

Nurul Romadhona: Validation, project administration, data curation, writing-review and editing.

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