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THE EFFECT OF LINGZHI (GANODERMA LUCIDUM) EXTRACT ADDITION ON ANTIOXIDANT ACTIVITY OF HAND AND BODY LOTION FORMULATIONS

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ABSTRACT

Ganoderma lucidum, lingzhi or reishi mushroom is a plant containing secondary metabolites that are of wide interest due to their biological activities. Among them the most important is its Antioxidant activity. Free radicals are potentially damaging byproducts of metabolism. Antioxidants neutralize free radicals in the body and thus help to protect against disease (source) The study was conducted to determine the antioxidant effect, as measured by DPPH radicals scavenging capability of hand and body lotions with lingzhi mushroom extract included. Maceration was used to extract lingzhi and the yield of extraction is 14.17% The results of the preliminary phytochemical analysis showed that lingzhi has contained compounds related to flavonoid, phenolat, triterpenoid and steroid. The antioxidant activity assay further confirmed the results as lingzhi extract showed very high antioxidant (46.9 ppm) potential when tested against DPPH scavenging by abrogating red purple color at 517nm table(Table.). In addition, the incorporation of lingzhi extract with hand and body lotion also increased antioxidant activity, where 0.75% lingzhi extract was found have to produce most effective DPPH inhibition [IC50 = 28.47 ppm].

Keywords: *Lingzhi mushroom, Antioxidant,* DPPH *, IC_{50}, Hand and Body Lotion.*

INTRODUCTION

Lingzhi extract (Ganoderma lucidum) has been used for thousands of years in traditional Chinese medicine due to its potential health benefits (Bishop et al., 2015). Similarly, the strong type of the mushroom is thought to help in enhancing your immunity and might offer cure for many health issues such as swelling, fatigue along with tension (X. Liu et al., 2014). Some recent research has also indicated that extract of Lingzhi may possess antioxidant properties which could protect cells against the oxidative service by free radicals. Furthermore, research on the anti-cancer properties of Lingzhi extract led to believe that it is a valuable natural supplement for support in achieving optimal holistic health (Lam et al., 2020).

Past reports on Lingzhi extract antoxidant potential efficacies and their histological repercussions were encouraging. A study by Kozarski and others Lingzhi extract was found to have significant antioxidant activity, with potential benefits in preventing skin oxidative stress and UV damage. Using Lingzhi extract on the skin can increase skin hydration and its elasticity, resulting in a youthful glow. These observations propose a practical natural-origin extended skincare alternative such as Lingzhi extract in possible skin care remedies (Kozarski et al., 2019).

Skincare ingredients play an important role in skincare and antioxidants help to protect the skin from environmental stressors like pollution or UV rays. Antioxidants can block free radicals before they damage skin cells which means antioxidants fight against premature aging and degradation of the skin. Utilizing skincare with extremely bioactive antioxidants ie: Lingzhi extract and others can boost skin health on the whole while promoting a youthful, lustrous appearance (J.-K. Liu et al., 2022).

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In the cosmetics industry, antioxidants are increasingly crucial in the production of skincare products for their role in shielding the skin from environmental damage, thereby mitigating the risk of early aging and other skin conditions. Some plant extracts have been found to contain high levels of antioxidants, thus serving as potential natural sources of antioxidants in body lotions. (Hoang et al., 2021).For example, green tea extract, abundant in antioxidants like catechins, may help diminish inflammation and shield the skin from UV harm. By incorporating green tea extract into hand and body lotions, it can enhance their capacity to protect against environmental stress, reducing oxidative stress on the skin (Natarajan et al., 2019).

Research on the effects of natural ingredients in sunscreens is ongoing, with a growing interest in utilizing botanical extracts like Lingzhi for their skin-protective properties (Li et al., 2019). Studies have shown that natural ingredients can provide additional benefits beyond traditional sunscreen ingredients, such as antioxidant protection and hydration (Ngoc et al., 2019). Additionally, exploring the synergistic effects of combining natural ingredients with hand and body lotion may lead to more advanced and effective skincare products in the future. In the present study, we investigate the influence of adding Lingzhi extract to hand and body lotion formulations on their antioxidant activity.

RESEARCH METHOD

1. Extraction

The dried lingzhi mushroom parts were first ground into a powder using an electric mill. Then, 50 grams of the powder were mixed with ethanol solvent in a maceration vessel. After four days, the extract was filtered through filter paper, and the ethanol solvent was evaporated to obtain the concentrated lingzhi extract. Finally, the resulting lingzhi mushroom extract was stored in a cool, dark place until further use (Dominica & Handayani, 2019).

2. Phytochemical Screening

Phytochemical screening is conducted to ascertain the compound content of a substance. This screening typically includes the identification of compounds such as phenolics, flavonoids, triterpenoids, and steroids.

a) Phenolat hidroquinon

Dissolve 1 mg of the extract in hot Aqua DM. Then, transfer 1 mL of this extract into a reaction tube. Proceed by adding 3-4 drops of $FeCl_3$. A color change from blue to concentrated black signifies the presence of phenol content. The solution is positively identified as containing phenolic compounds if the test solution turns green to black (Abbas et al., 2022).

b) Flavonoid

Dissolve 1 mg of the extract in hot, deionized water. Next, transfer 1 mL of this solution into a reaction tube. Add 3–4 drops of concentrated hydrochloric acid and 1 gram of magnesium powder. The appearance of orange, red or faint black coloration signifies a positive result for flavonoids (Khafid et al., 2023).

c) Triterpenoid and Steroid

To the reaction tube, add 1 mg of lingzhi mushroom extract and 2 mL of chloroform. Mix the extracts until dissolved, soak for a period, and then filter using filter paper. Next, add 3 drops of anhydrous acetic acid, followed by 1 drop of concentrated H_2SO_4. The presence of triterpenoid compounds is indicated by a color change to red, while steroids are indicated by a color change to green (Nugrahani et al., 2016).

3. Lotion Preparation

To prepare the lotion, ingredients are measured as per Table 1 and divided into the oil phase and the water phase. The oil phase ingredients (VCO, paraffin, stearic acid, and cetyl alcohol) are dissolved at 80°C. The water phase ingredients (TEA, glycerin, methyl paraben, and

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demineralized water) are then dissolved. Once both phases reach approximately 70°C, they are combined and homogenized. Next, lingzhi mushroom ethanol extract is added at the specified concentration, mixed until homogeneous, transferred to a container, and stored at room temperature. This hand and body lotion formulation incorporates lingzhi mushroom extract, referencing and modifying the research by (Vinaeni et al., 2022).

Table 1. Formulation of Hand and Body Lotion

Ingredient	Formula (%) (b/v)				Usage
	I	II	III	IV	
Lingzhi Extract	0	0.25	0.5	0.75	Active Substance
Stearic Acid	4	4	4	4	Emulsifier
Cetyl Alcohol	2	2	2	2	Softener
Paraffin	1	1	1	1	Moisturizer
VCO	3	3	3	3	Emulsifier
TEA	0.5	0.5	0.5	0.5	Emulsifier
Glycerin	5	5	5	5	Thickener
Methyl Paraben	0.1	0.1	0.1	0.1	Preservative
Perfume	0.1	0.1	0.1	0.1	Fragrance
Dye	0.05	0.05	0.05	0.05	Dye

4. DPPH Inhibition Activity

The antioxidant activity was assessed using the DPPH inhibition method, following the procedures established by Hidayati & Fatmawati, (2019). A 10 mg test sample was dissolved in 1 mL of methanol. Subsequently, 1 mL of this solution was added to 1 mL of a DPPH test solution (0.12 mM) and incubated for 30 minutes at room temperature in the dark. The absorbance was then measured at a wavelength of 515 nm using UV-Vis spectroscopy (Hidayat & Fatmawati, 2019). Below is the formula to determine the DPPH scavenging activity:

$$DPPHscaveringactivity(\%) = \frac{controlabsorbance - sampleabsorbance}{controlabsorbance} x100\%$$

RESULTS AND DISCUSSION

1. Extraction Results

The Lingzhi mushroom (*G. lucidum*), sourced from the Jombang District in East Java, Indonesia, is dried and pulverized using a blender. It is then extracted through the maceration method over a period of 96 hours. The extraction process, utilizing a 96% ethanol solvent, yields a 14.17% concentration of Lingzhi fungal extract.

2. Phytochemical Screening

Phytochemical screening is conducted to qualitatively identify the secondary metabolite compounds present in the Lingzhi fungus extract. The outcomes of the phytochemical screening for the Lingzhi mushroom extract are presented in the table below.

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Table 2. The Results of Phytochemical Screening Test

Screening phytochemical	Result	Annotation
Flavonoids	+	Transparent yellow color changes to orange
Phenolics	+	Transparent yellow color changes to gray and black precipitate is formed
Steroids	+	Color changes from yellow to green at the top
Tritherpenoids	+	The color changes from yellow to red-brown at the bottom

Flavonoids are compounds abundant in OH-groups, exhibiting high electronegativity differences that render them polar, as noted by Ikalinus *et al*. in 2015. These substances are a category of phenolic compounds prevalent in plant tissues and known for their antioxidant properties (Ikalinus et al., 2015). The antioxidant function of flavonoids is attributed to their capacity to donate hydrogen atoms or chelate metals, as described by Marsella *et al*. In 2016 (Marsella et al., 2016). Phytochemical assays on lingzhi mushroom extracts have yielded positive outcomes, indicated by an orange hue.

The analysis of hydroquinone phenolates involves a colorimetric reaction with $FeCl_3$ solution, leading to the formation of a complex compound. This compound evidences phenolic oxidation by a mild oxidizing agent, Fe^{3+}, resulting in carbonyl compounds. The Fe ion serves as the central atom in this complex, which is fundamental to its structure. The complex's formation is a consequence of the oxygen in the phenolate reacting with $FeCl_3$, triggering a reaction that turns the solution from olive green to black under acidic conditions (Waras Nurcholis et al., 2022).

Positive steroid test results are indicated by a green-blue coloration upon reaction with acetic anhydride, which is due to the oxidation reaction in the steroid groups caused by conjugated bonds (Fajriaty et al., 2018). Research on lingzhi mushroom extract samples showed positive results, as evidenced by the solution's color change from yellow to a concentrated green at the top. The test involved a reaction between the steroid and acetic anhydride, specifically an acetylation reaction of the hydroxyl group in the steroid. The addition of acetic anhydride is to form an acetyl derivative, while H_2SO_4 acts as a water hydrolyzer that reacts with acyl derivatives to produce a color change (Nugrahani et al., 2016).

Triterpenoids, secondary metabolites derived from terpenoids, have a carbon skeleton formed from six isoprene units (2-methylbuta-1,3-diene), resulting in a C_{30} hydrocarbon frame known as squalene (Balafif et al., 2013). The detection of triterpenoid compounds was signified by a red color change at the solution's bottom. The triterpenoid test involves a condensation reaction and fusion with a carbocation. It starts with the acetylation of hydroxyl groups by acetic anhydride, followed by hydrolysis, which releases a hydrogen cluster and electrons, leading to trap displacement. The compound then resonates as an electrophilic carbocation, undergoing electrophilic addition, resulting in extended conjugation and the appearance of a red-colored ring (Nugrahani et al., 2016). The results of phytochemical screening of steroids and triterpenoids can be seen in Figure 2 below.

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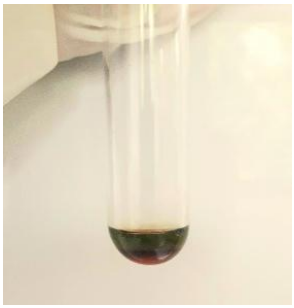

Figure 1. The Results of Phytochemical Screening of Steroids and Triterpenoids

3. Antioxidant Activity

This study evaluated the antioxidant activity of Lingzhi mushroom extract and lotion with varying concentrations of Lingzhi extract. The incorporation of Lingzhi extract into the lotion is anticipated to enhance its antioxidant properties, thereby bolstering skin protection against free radical exposure. The antioxidant testing of the Lingzhi mushroom extract, conducted using the DPPH method, yielded an IC_{50} value of 46.9 ppm, indicating very strong antioxidant activity.

Lingzhi mushrooms are rich in secondary metabolites with potent antioxidant properties. These include phenolic acids like 1-Caffeoylquinic acid, Caffeoyl-2-hydroxyethane-1,1,2-tricarboxylic acids, and Yunnaneic acid F, as well as flavonoids such as Isorhamnetin 3-O-rutinoside, Quercetin derivatives, B-type procyanidin tetramer, and Apigenin, all of which have hydroxy groups attached directly to the benzene ring. These compounds can neutralize free radicals by donating protons and stabilizing the resulting radicals. Additionally, lingzhi mushrooms contain triterpenoids such as ganoderic acid and ganoderic acid B, which also exhibit high antioxidant activity (Kolniak-Ostek et al., 2022).

Strong antioxidants extract from natural products are considered safer than synthetic alternatives. The antiradical action of antioxidant substances may be explained by a hydrogen atom transfer (HAT) mechanism. Studies have indicated that phenolics, triterpenoids, and flavonoids, which are plentiful in lingzhi extract, exhibit high antioxidant activity.

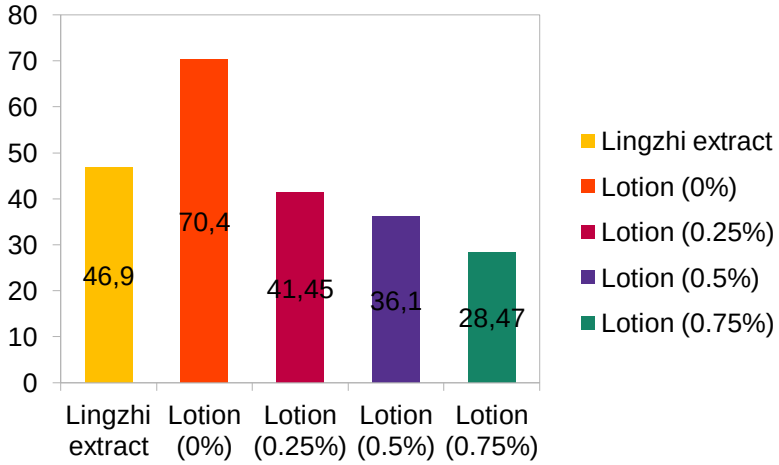

Figure 2. Present an Antioxidant Test IC_{50} Value Against DPPH from a Variety of Test Samples

Based on **Figure 2**, present an antioxidant test IC_{50} value against DPPH from a variety of test samples. Variation of lotion 0% or without addition of lingzhi mushroom extract obtained an IC_{50} of 70.4 ppm. The IC_{50} values of the lotion with the addition of lingzhi fungus extract of 0.25%;

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0.5% and 0.75% (b/v) in succession are 41.45, 36.10 and 28.47 ppm. Based on the data, it can be concluded that increasing the addition of lingzhi mushroom extract in the preparation of lotions can increase the potential to inhibit free radical attacks.

CONCLUSION

Lingzhi mushrooms offer significant natural benefits. The extract of Lingzhi mushrooms exhibits potent antioxidant activity, with an IC_{50} value of 46.9 ppm. Incorporating Lingzhi extract into hand and body lotion formulations influences the antioxidant activity level. Increasing the amount of Lingzhi extract can enhance the antioxidant capacity of these lotions.

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