

Bioactivities of Catechin from Gambir (*Uncaria gambir* Roxb.) Against Wood-decaying Fungi

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Gambir is one of the most economically important natural products of Indonesia. Indonesia accounts for 80% of the global exports of this product. The product contains catechin, a phenolic compound of the flavonoid group, which has demonstrated bioactivity against horticulture-destroying fungi. However, its bioactivity in controlling wood-decaying fungi has not yet been reported. A laboratory study was conducted to examine the characteristics of the catechin of gambir and its bioactivity against the wood-decaying fungi *Schizophyllum commune* Fr. Extraction of catechin from gambir was conducted *via* a gradual maceration process using hot water (70 °C, 3 h) followed by ethyl acetate (1:10 w/v, 4 h). The chemical components of catechin were analyzed by gas chromatography mass spectrometry (GCMS), while its bioactivity against *S. commune* was examined according to EN 113 (1986). The results showed that there were five chemical components in catechins, *i.e.*, 1,2-benzenediol, catechol, 1,3,5-benzenetriol, dimethyl terephthalate, and terephthalic acid. These compounds demonstrated the ability to remarkably inhibit the growth of *S. commune*.

Keywords: Catechin; Ethyl acetate; Gambir; Gas chromatography mass spectrometry; Wood-decaying fungi

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INTRODUCTION

Indonesia is well known for its diversity of herbs and medicinal plants that are especially prized by some ethnic groups. This includes gambir (*Uncaria gambir* Roxb.), which is found in Sumatera, Java, and Bali. In these areas, the plant has been cultivated for decades by farmers, especially in West Sumatera. The leaves and young twigs of gambir are the part of the plant that is processed into gambir blocks or gambir powder. Gambir block or gambir powder has a specific aroma and induces a fresh bitter taste on the tongue, which makes it unique. This product has been used by various ethnicities for centuries as a complement to betel. The product is also one of the Indonesian export commodities. It contributes to approximately 80% of the gambir block trading in the world (Gumbira-Said 2009). It contains several chemical components, one of which is catechin. Catechin is a bioactive compound that can be found abundantly in gambir blocks (Taniguchi *et al.* 2007; Apea-Bah *et al.* 2009; Anggraini *et al.* 2011) and is known to be a complex flavonoid compound from the polyphenol group (Taniguchi *et al.* 2017).

In the last few decades, research on the use of catechin from gambir has mostly focused on the development of pharmaceutical products. Studies have revealed that catechin has some antioxidant and antibacterial properties (Taniguchi *et al.* 2007) as well as pharmacological effects (Desmarchelier *et al.* 1997). The presence of catechin in green tea and fermented tea is associated with health-protective and cancer-preventative effects in animals due to its antioxidant activity (Sang *et al.* 2002). Rosada *et al.* (2017) reported that 24 mg of gambir extract is effective in decreasing bacterial colonies in male Wistar rats. In addition, Merta *et al.* (2013) reported that ethyl acetate gambir extracts inhibit the growth of *Staphylococcus aureus*. Pambayun *et al.* (2007) stated that a 22.26% gambir extract has antibacterial properties against the Gram-positive bacteria *Streptococcus mutans*, *Bacillus subtilis*, and *Staphylococcus aureus*. Katu *et al.* (2016) showed that a 1% concentration of gambir extract with a contact time of 24 h effectively inhibits the growth of *Enterococcus faecalis*.

The antimicrobial activity of catechin is due to its ability to damage the cell membrane and bind to adenosine triphosphate sites on the DNA gyrase b subunit (Gopal *et al.* 2016). Magdalena and Kusnadi (2015) reported that gambir is more effective in inhibiting Gram-positive bacteria than Gram-negative bacteria. Catechin derived from gambir penetrates easily to peptidoglycan, disrupts cell wall structures and functionality, and leads to cell lysis (Bai *et al.* 2016). In addition, catechins are also known to exhibit bioactivity against fungi that destroy horticultural products (Farkas and Kiraly 1962). However, there is a lack of scientific information regarding the bioactivity of catechin against wood-decaying fungi, even though the occurrence of wood decay, such as on housing constructions, in tropical countries like Indonesia is responsible for huge economic losses.

It has been deemed necessary to conduct research to determine the bioactive compounds of catechin extracted from gambir and its potential as an active wood preservative (anti-wood-decaying fungi) ingredient. This is related to the fact that today the wood preservatives that are used are almost entirely synthetic organic compounds that are harmful to human health and are potential environmental pollutants. In addition, all synthetic wood preservatives that are marketed in tropical countries are imported products that are quite expensive.

Therefore, it is crucial to explore and develop organic active ingredients for the development of environmentally friendly wood preservatives. Up to now there has been no scientific information regarding the potential of catechin from gambir as anti-wood destroying fungi. Moreover, gambir is known as one of Indonesian indigenous products. Thus, by utilizing this commodity into natural wood preservative can give additional economic value to the final products.

EXPERIMENTAL

Materials

The materials used in this study was gambir (*Uncaria gambir* Roxb.) cylindrical blocks obtained from Talang Maua Village, Mungka District, Lima Puluh Kota Regency, West Sumatra Province (Fig. 1).

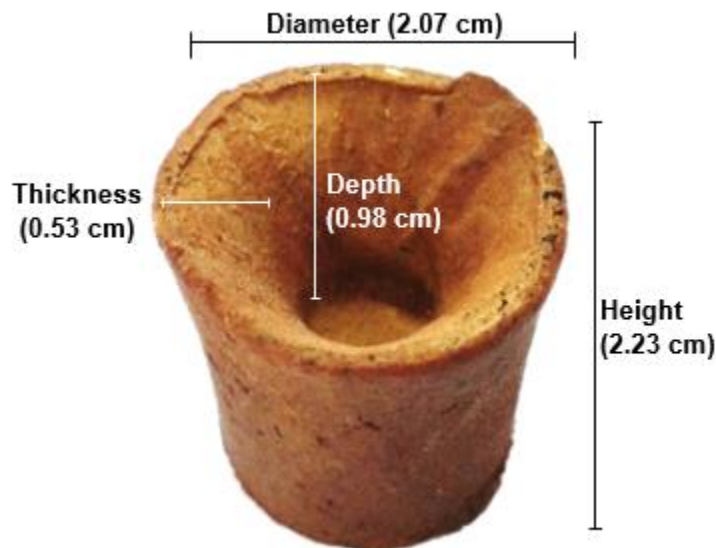


Fig. 1. Gambir block obtained from Indonesian market used in this research

Methods

Catechin extraction process

Gambir blocks were milled and then screened on 100 mesh screeners. After that, the catechin contents was extracted with 1:5 (w/v) hot water (70 °C) for 3 h according to TAPPI T207 cm-99 (1999). The extraction with hot water was performed to separate the water-soluble compounds that caused impurities in the extract. According to Sousa *et al.* (2007), hot water extraction proved to be a better method to extract phenolic antioxidant compound than methanol at room or boiling temperature. The extraction result was precipitated for 24 h, and repeated sedimentation was completed using cold water (20 °C) to separate the extracts from the tannins. The residual results of repeated washing were then dried using a freeze dryer for 24 h. The dried filtrate was macerated for 4 h using ethyl acetate 50%, and then filtered with Whatman 42 (GE Healthcare Companies, Buckinghamshire, UK) filter paper. The filtrate obtained was dried with a spray dryer at an inlet temperature of 175 ± 5 °C and an outlet temperature of 60 ± 5 °C.

Chemical component analysis of catechin

The extracted catechins from the powder were then analyzed for their chemical components using gas chromatography mass spectrometry (GCMS) with an Agilent column type 19091S-433 (Agilent Technologies, Santa Clara, CA, USA). Samples (catechin solution, 1:10 w/v) were injected into with a temperature of 300 °C, then passed into the front inlet (GC) mode split with an initial temperature of 300 °C, a pressure of 13.21 psi, and a flow rate of 33.7 mL / minute for two minutes. The Gas Chromatography (GC) system was connected to a Mass Spectrometer (MS) equipped with a fused silica capillary column having dimensions 30 mm x 0.25 mm x 0.25 μ m. Components are separated using helium as a carrier gas at a constant flow of 1 mL/minute and flow to the detector. The difference in substance mass and conductivity is then defined as mass spectrum. Interpretation of the GCMS mass spectrum was compared to the spectrum of components in the W10N14.L database. The chemical structures of catechin and its derivative were generated using SIGMA (Sigma-Aldrich Inc., Darmstadt, Germany).

Evaluation of bioactivity performance on wood

The bioactivity of catechin against wood-decaying fungi was completed according to the EN 113 (1986) standard. The wood samples used were rubber wood (*Hevea brasiliensis* Muell. Arg.) from 20-year-old trees grown at Bogor, West Java, Indonesia with a moisture content of $15 \pm 3\%$. The samples were then cut into the dimension of 2.5 cm x 1.5 cm x 0.5 cm and were impregnated with a catechin solution of concentrations 6%, 12%, and 18% (w/v) using five replications each. These solutions were made by diluting catechin powder (40-mesh) into ethyl acetate 90% as a solvent. The impregnation was conducted in a vacuum-pressure chamber as a closed system (Fig. 2). This process began with a vacuum of 50 mbar for 1 h, followed by the process of pressing 2.5 psi for 2 h. The samples were conditioned for 24 h. After that, catechin-impregnated wood samples were exposed to white rot *Schizophyllum commune* Fr. in the laboratory for 16 weeks inside a potato dextrose agar media. The weight percentage gain (WPG) of catechin in the wood samples and the weight loss of the wood sample after exposure to *S. commune* fungi were evaluated. *S. commune* is one of the most widely distributed wood decaying fungi and is also recognized to be associated with buildings (Schmidt and Kebernik 1988). This fungus is intermediate between white-rot and brown-rot species, as well as deviate from the classical model of white rot in that they lack of ligninolytic class II peroxidases (corresponding to brown-rot fungi) but possess diverse arrays of enzymes acting in crystalline cellulose like white rot fungi (Riley *et al.* 2014). Djarwanto *et al.* (2018) reported that *S. commune* was one of the most virulent fungi capable of attacking almost all wood species.

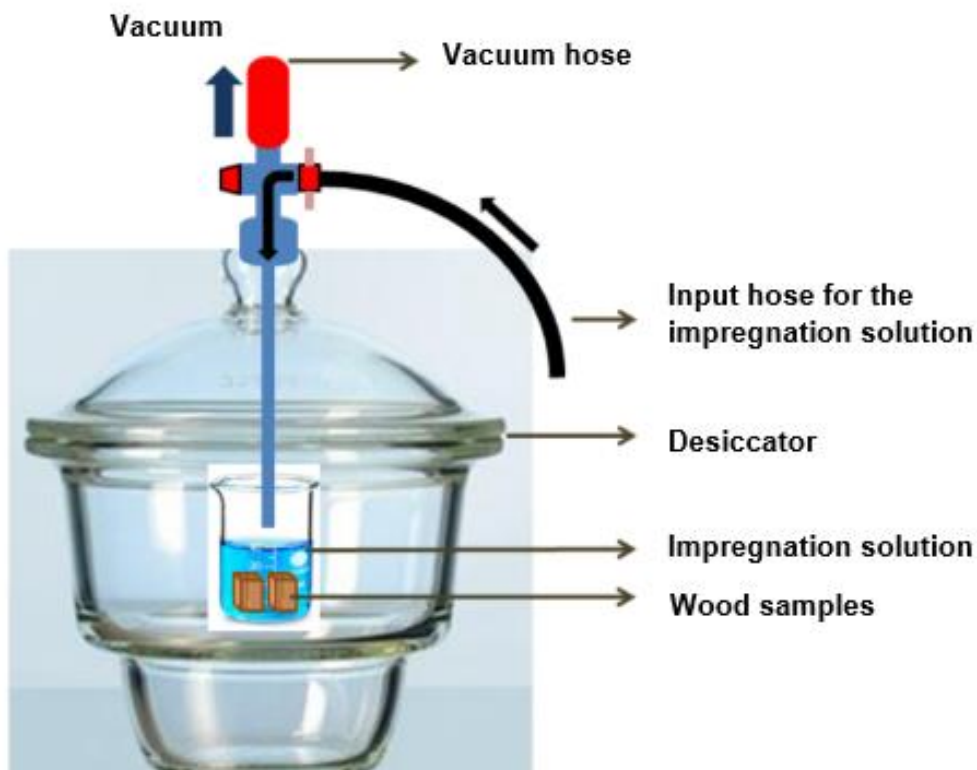


Fig. 2. Close system vacuum pressure chamber

Data Analysis

The SPSS software (SPSS 19, 2010) was utilized as a statistical tool. Single factor analyses of variation (ANOVA) tests of catechin concentration levels and its interactions on mass losses were evaluated.

RESULTS AND DISCUSSION

Physical Characteristics of Catechins

The results showed that the multilevel extraction method using hot water (70 °C) and ethyl acetate (1:10 w/v) was able to extract catechins from gambir with a yield of 33.5%. The extracted catechins were in the form of a fine yellowish-white powder and were odorless, with a water content of 8.8% (Fig. 3). Rismana *et al.* (2017) also tried to extract catechin from gambir block using 50% and 96% ethanol, resulting in yields of 66.8% and 76.4%, respectively. The difference in the yields could have been due to the temperature and time of extraction, size of the gambir blocks, as well as difference in the solvent used. Nevertheless, several publications (Yeni *et al.* 2014; Rahman *et al.* 2018; Failisnur *et al.* 2018) stated that based on the considerations of time and cost effectivity, the use of ethyl acetate on gambir extraction is considered to be the best decision.



Fig. 3. Catechin powder extracted from gambir

At the initial stage of extraction, the gambir powder underwent a weight loss of 51.0%. This reflected the occurrence of tannin separation from catechins. The maceration in hot water separated the catechins from tannins due to their different polarity properties. Tannins are phenol compounds that contain many OH groups that render them soluble in water or alcohol (Pambayun *et al.* 2007). Meanwhile, catechins tend to have semi-polar properties, therefore the dissolution of this compound in cold water or polar solvents was taking a long time. Hence, the utilization of high temperatures (70 °C) in the extraction process caused higher interparticle activity that was dissolving more substance.

Catechin Chemical Characteristics

The analysis of the chemical components of catechins using GCMS showed that the catechins contained five chemical components with high equality values (≥ 90), namely 1,2-benzenediol, catechol, 1,3,5-benzenetriol, dimethyl terephthalate, and terephthalic acid (Fig. 4). Two of the five chemical compounds, 1,2-benzenediol and catechol, were derivatives of catechins with a total relative concentration of 62.4%. Catechol had the structures of a simple catechin that had been reduced ($C_6H_6O_2$), as presented at Fig. 5.

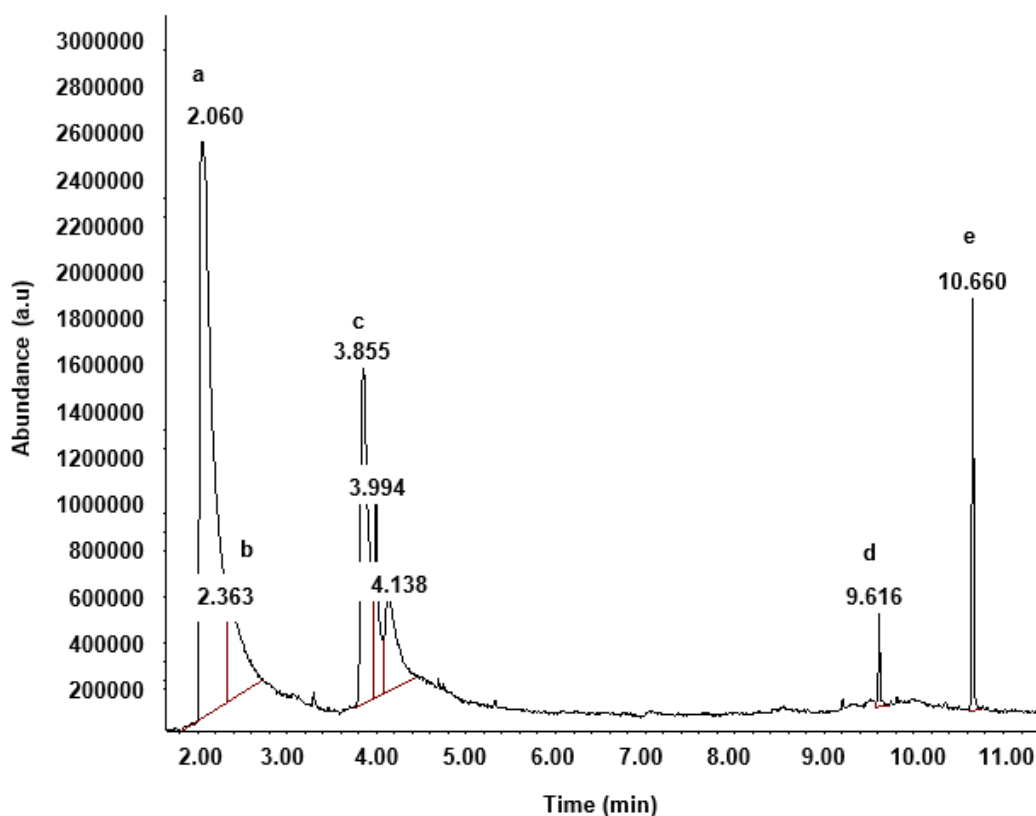


Fig. 4. The spectra of catechin chemical components as revealed by GCMS: 1,2-benzenediol (a); catechol (b); 1,3,5-benzenetriol (c); dimethyl terephthalate (d); and terephthalic acid (e)

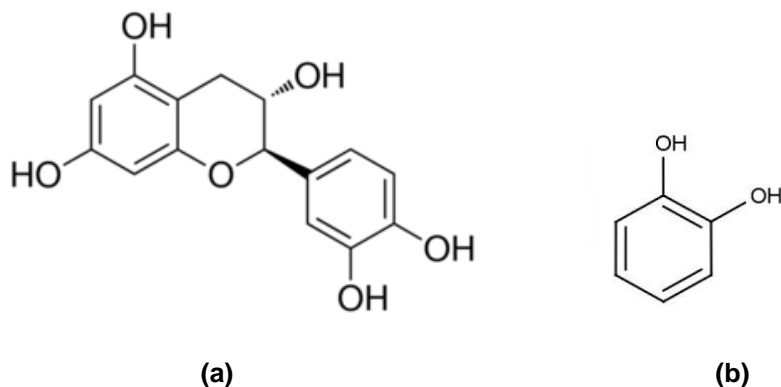


Fig. 5. Chemical structure of catechin (a) and catechol (b)

WPG Catechin in Samples

The results showed that the highest catechin WPG (21.45%) was found in the samples that were impregnated with a 12% concentration of catechin solution, followed by an impregnated sample of 18% catechin solution and 6% impregnated catechin solution (Fig. 6). Gabrielli and Kamke (2010) stated that weight percent gain (WPG) provides information regarding the degree of impregnant substance to penetrate the wood's cellular structure, thus higher WPGs indicate greater penetration. The high WPG

value indicated that many catechins had been deposited in the wood samples. In other words, catechins were relatively easily impregnated into wood with the help of a vacuum followed by pressure process in the closed system. The decrease in the WPG of the 18% catechins solution was attributed to the high molecular weight of the compound. There were several factors affecting the process of wood treatment, such as the solubility of impregnant in water, the molecular size or molecular weight, and the viscosity of impregnant solution (Pittman *et al.* 1994).

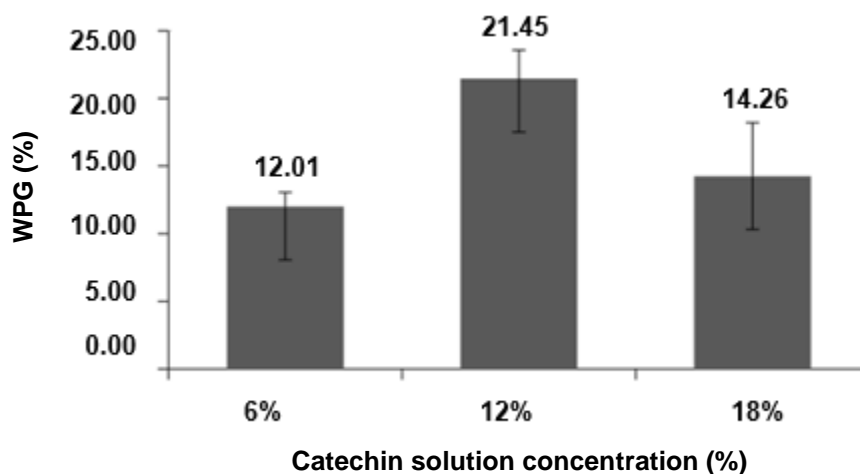


Fig. 6. The average WPG of catechins in the test samples in each level of the concentration of catechin solution

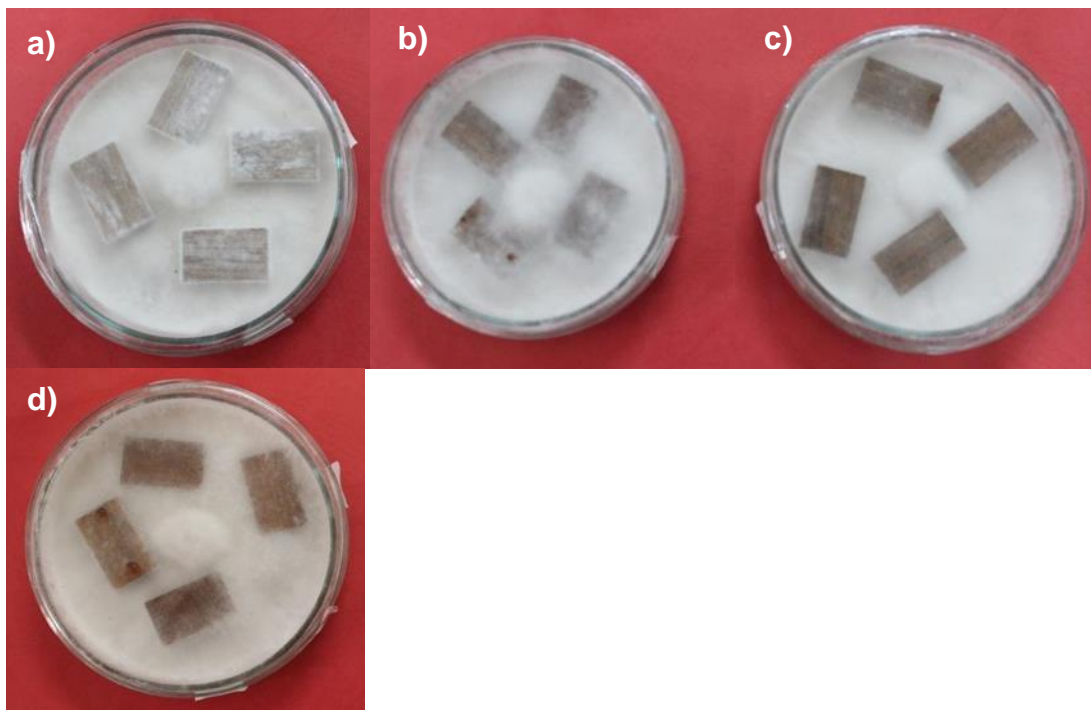


Fig. 7. Surface conditions of the untreated wood samples (a), 6% catechin impregnated wood samples (b), 12% catechin impregnated wood samples (c), and 18% catechin impregnated wood samples (d) after 16 weeks exposure to *S. commune*

Catechin Bioactivities Against Wood-decaying Fungi *S. commune*

The bio-activity of catechin against *S. commune* fungi was shown by the relatively low average weight loss of the wood samples impregnated by the catechin solution at concentrations of 12% and 18%, which were only 1.82% and 1.33%, respectively. In contrast, the average weight loss of the untreated wood samples reached 14.0%. Meanwhile the average weight loss of the impregnated wood sample of the 6% catechin solution was 3.75% (Fig. 8). Thus, the catechin solution exhibited remarkable bio-activity against *S. commune* fungi. In addition, these results were also supported by the absence of the fungal mycelia cover (0%) in the test sample impregnated with the catechin solution with a concentration of 12% and 18% (Figs. 7c through 7d). The intensity of covering of the mycelia on the test sample impregnated with a catechin solution with a concentration of 6% reached 50% (Fig. 7b). In contrast, almost all of the surfaces of the untreated sample were covered by the fungal mycelia of *S. commune* (Fig. 7a). A higher concentration of the catechin solution resulted in a lower weight loss of the wood sample. This due to the active component in catechin acted as anti-fungi; therefore, higher concentration of catechin will give better protection to the wood samples.

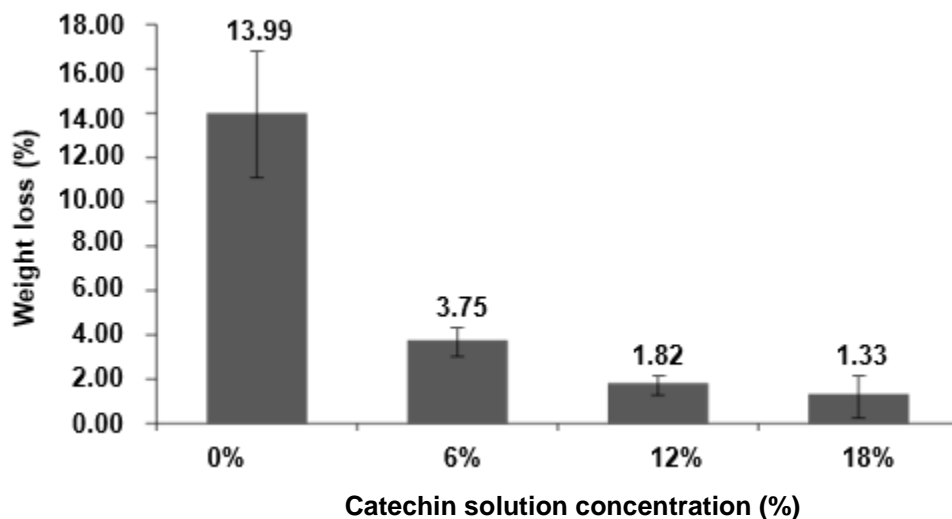


Fig. 8. Average weight loss of wood samples after 16 weeks exposed to *S. commune* fungi

CONCLUSIONS

1. The extracted catechin from gambir contained five main compounds, namely 1,2-benzenediol, catechol, 1,3,5-benzenetriol, dimethyl terephthalate, and terephthalic acid. The two of the five compounds that had the highest content were 1,2-benzenediol and catechol, which are monomers of the catechin.
2. At a concentration of 12% or more, catechins showed remarkable bio-activity in inhibiting the growth of wood-decaying fungi *S. commune*.

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