Screening of Some Plant Extracts from Toba Regions-North Sumatra for controlling Wood-Rotting fungi

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Abstract. The wood-rot fungus is one of the most destructive organisms for wood, wood products and building structures besides termites and beetles. Until today, wood-root fungi are still difficult to control, so it is necessary to search for new natural ingredients to be developed as wood preservatives. The purpose of this study was to screen some plant extracts from the area surrounding Lake Toba as anti-wood-rot fungi. This study evaluated antifungal properties of 6 different plant extracts dissolved in methanol against 9 species of wood-rot fungi. The activity was determined using the method of Mohareb [1] by growing the fungi on PDA supplemented with the plant extracts. Parameters observed included diameter of fungal growth and inhibition percentage of fungal growth by plant extracts. Extracts of *Tithonia diversifolia*, *Chromolaena odorata*, *Saurauia bracteosa* dan *Azadirachta indica* displayed extraordinary activity in inhibiting the growth of wood rot, up to 100%, therefore these plant extracts have the potential to be further investigated as wood rot control agent.

1. Introduction

Wood is a renewable natural resource that plays an important role in the world economy, specifically in the construction and furniture sector. However, wood degradation caused by fungi, bacteria, termites and beetles has been an important problem during storage, transportation, manufacturing and others. Wood rot fungal infection is one of the biological factors of wood damage that must be controlled. White and brown rot fungi are groups of wood decomposers that have known to cause much losses to industrial forest and building construction.

Naturally, there are three types of wood rot fungi, namely white rot, brown rot, and soft rot fungi. These funguses are able to degrade lignocellulosic component of wood. White rot fungi break down lignin, while brown rot fungus break down cellulose and leave the lignin behind. Soft rot fungi secrete enzymes to degrade polysaccharides and then creating microscopic cavities resulted in softening of inhabited wood [2, 3].

Although the use of synthetic preservatives is effective to prevent wood decay, the chemicals are non-biodegradable thus have the potential to pollute the environment because they generally contain chemicals derived from minerals, crude oil or metals [4]. In addition, preservatives synthesized chemically from
organic compounds are poisonous to the human body [5, 6]. Therefore, the search for wood preservatives that are safe for the environment is needed, one of them by utilizing natural ingredients derived from plants.

Plant extracts used in this study have been reported to have several potentials including as a pesticide, antifungal, antibacterial and other pharmacological activities due to bioactive compounds contained in the plant organs such as leaves, stems, flowers or roots. The six plant extracts used in this study were *Tithonia diversifolia*, *Chromolaena odorata*, *Saurauia bracteosa*, *Turpinia sphaerocarpa*, *Toona sinensis* and *Azadirachta indica*. Based on the results of phytochemical tests conducted by Meisyara et al. [7], these plant extract contained flavonoids, steroids, triterpenoids, triterpenoids, saponins and tannins. Matejic et al. [8] reported that metabolites produced by *T. diversifolia* have various pharmacological activities including antimalarial, antibacterial, anti-viral, anti-fungal and anti-diabetic. This plant also contains the active compounds Tagitinins C (1), F (2), and A (3) which are reported to reduce lipopolysaccharide-6 induced interleukin-6, interleukin-8 and tumor necrosis [9]. The *C. odorata* leaves extracted with various solvents such as ethanol, methanol and dichloromethane also reported to have activities as anti-malaria, anti-inflammatory, anti-bacterial, anti-fungal, anti-oxidant and anticancer [10]. Meanwhile, according to Runtuwene et al. [11], Tonsawang ethnic, Tombatu, North Sulawesi have used *S. bracteosa* leaves for the treatment of degenerative diseases such as tumors and cancer. The leaves of this plant are also known to contain antioxidants because they contain secondary metabolite compounds such as phenolic, flavonoids, steroids and saponins [12]. The *T. sinensis* leaves have been used for thousands of years as an herbal medicine because they are believed to have pharmacological activity. In Chinese traditional medicine *T. sinensis* is described as a drug that has good anti-inflammatory, detoxifying and hemostatic effects, and this plant is commonly used to treat enteritis, dysentery, urinary tract infections, leukorheal diseases, and itching on the skin [13]. In addition, *T. sinensis* also contains anti-bacterial activity, especially against *Escherichia coli*, *Bacillus subtilis* and *Colletotrichum gloeosporioides*. *A. indica* seed kernel extract was reported to significantly reduce the growth of four types of pathogenic fungi causing postharvest disease in plums (*Prunus salicina*) or pears (*Pyrus bretschneideri*) [6]. According to Grosvenor et al. [14], *T. sphaerocarpa* has the potential as an anti-infectious agent in human digestive system and also anti-bacterial activity especially against *Staphylococcus aureus* and *E. coli*. The six extracts used in this study also showed toxicity activity to pest insect, especially for controlling termites and *Spodoptera litura* [7].

However, information on the potential of plant extracts as controlling agent of wood rot fungus is still very little to be found. This research aim to provide scientific information about the utilization of several plant extracts originating from the Toba region, northern Sumatra as a natural fungicide to control wood rot fungus.

2. Materials and method

2.1. Sample preparation

The samples used in this study were plant extracts collected from the Toba and surrounding areas. Leaves and bark (Table 1) were dried at 32 °C ± 5 °C until the moisture content of the material reached ± 12%. Materials were crushed by a disc mill and sifted using 40-80 mesh. Sifted material was extracted in three stages; in each stage, plant materials were extracted in methanol (Merck, Darmstadt, Germany) in the ratio of 1:7 following agitation maceration method (72 hours, 150 rpm, 30 °C). The extracted solvent was then concentrated to obtain crude extractives using a rotary evaporator [7].

| Sample code | Local name          | Plant part | Species                        |
|-------------|---------------------|------------|--------------------------------|
| SP          | Simar pahit-pahit    | Leaf       | *Tithonia diversifolia* (Hemsl.) A.Gray |
| SH          | Simar huting-huting  | Leaf       | *Chromolaena odorata* (L.) R.M King & H.Rob. |
| P           | Pirdot              | Leaf       | *Saurauia bracteosa* DC.       |
| KKS         | Sikkam              | Bark       | *Turpinia sphaerocarpa* Hassk.  |
| I           | Ingul               | Leaf       | *Toona sinensis*               |
| DH          | Haurese             | Leaf       | *Azadirachta indica* A. Juss.  |
2.2. Preparation of wood-rot fungi isolates

This study used 9 species of white rot and brown rot fungi that were isolated from the Batam Botanical Gardens [15] and collection of Microbiology Laboratory, Research Center for Biomaterials-LIPI, Cibinong- Bogor (Table 1). The wood-rot fungi were inoculated and grown on PDA (Potato Dextrose Agar) media for 7 days.

Table 2. List of wood-rot fungi isolates [16].

| Species                  | Isolate code | Origin of                                      |
|--------------------------|--------------|-----------------------------------------------|
| White rot                |              |                                               |
| *Trametes versicolor*    | COR          | Collection of RC of Biomaterials, LIPI        |
| *Schizophyllum commune*  | SC           | Collection of RC of Biomaterials, LIPI        |
| *Trametes sp.*           | Gano         | Collection of RC of Biomaterials, LIPI        |
| *Pycnoporus sanguineus*  | M3           | Botanical Garden of Batam                    |
| *Trametes ijubarskii*    | M4           | Botanical Garden of Batam                    |
| *Flavodon flavus*        | H2A          | Botanical Garden of Batam                    |
| *Phanerochaete chrysosporium* | PC   | Collection of RC of Biomaterials, LIPI        |
| Brown rot                |              |                                               |
| *Fomitopsis palustris*   | TP           | Collection of RC of Biomaterials, LIPI        |
| *Antrodia wangii*        | M7           | Botanical Garden of Batam                    |

2.3. Determination of antifungal activities

The plants extracts were tested for their antifungal activity against wood-rot fungi by growing wood rot fungi on PDA media containing plant extract, respectively. The wood rot fungus was grown on PDA media that have been amended with leaf extract with a ratio of 2 ml extract per 20 ml of media (v/v). The plant extract were tested at 10,000 ppm concentrations. Each of plant extracts was mixed separately into molten autoclaved PDA medium (121 °C for 15 minutes) and thoroughly shaken to ensure the mixture uniformity. Next, the mixed medium was poured into petri dish and allowed to solidified. A mycelium disc (6 mm diameter) of tested wood-rot fungi was inoculated at the center, respectively [1]. Three replicates were maintained for each wood–rot fungus. The petri dish were incubated for 10 days and observation on diameter of fungal growth were recorded every 2 days (Figure 1). The diameter of colony growth and antifungal activity were determined using the following formula:

\[
\text{Diameter of colony} = \frac{R_1 + R_2}{2}
\]  

Figure 1. Determination of diameter of wood-rot fungi colony [16].

R1 R2
% (G) = \frac{A - B}{A} \times 100\% \quad (2)

A = diameter of growing fungal colony of control petri dish
B = diameter of growing fungal colony of treatment petri dish
G = percentage of inhibitory power of fungal growth (%)

3. Result and Discussion

The plant extracts used in this study, namely *T. diversifolia*, *C. odorata*, *S. bracteosa*, *T. sphaerocarpa*, *T. sinensis* and *A. indica* demonstrated the potential to inhibit growth of white rot and brown rot fungi. The toxicity of the plant extracts against 7 types of white-rot fungi including *T. versicolor* (COR), *S. commune* (SC), *Ganoderma* sp. (Gano), *P. sanguineus* (M3), *T. ijubarkii* (M4), *F. flavus* (H2A), *P. chrysosporium* (PC) and 2 types of brown-rot fungi namely *F. palustris* (TP), *A. wangi* (M7) were observed from the diameter of fungus growth for 10 days. Antifungal activity was determined by comparing the growth of wood-rotting fungus between control and treated petri dish amended with plant extract.

Based on Figure 2 and Figure 3, the diameter of colony growth of all wood-rotting fungi tested in control treatment reached 9-10 cm on the 10th day of incubation. Meanwhile, wood-rotting fungal colony added with plant extracts showed smaller diameter, between 0 - 5.14 cm in 10 days of testing. The leaf extract of *T. diversifolia*, *C. odorata*, *S. bracteosa* and *A. indica* were completely inhibited the growth of SC, Gano, M3, M4, M7, H2A, and PC, but these extracts were less effective against COR and TP, where the fungi still showed diameter growth of 1.8 - 3.74 cm. Meanwhile the extract of *T sinensis* leaves showed 100% growth inhibitory activity only on M7, H2A and PC, while the growth media of other 6 wood-rotting fungi amended with *T sinensis* extract still showed colony growth ranged from 3.14 to 5.14 cm.

![Figure 2](image-url)  
*Figure 2.* Wood-rotting fungal diameter growth in petri dish amended with various plant extracts for 10 days

4
|          | Control | I   | KKS | SP  | SH  | P   | DH |
|----------|---------|-----|-----|-----|-----|-----|----|
| *T. versicolor* (COR) | ![Image](image1.png) | ![Image](image2.png) | ![Image](image3.png) | ![Image](image4.png) | ![Image](image5.png) | ![Image](image6.png) | ![Image](image7.png) |
| *S. commune* (SC) | ![Image](image8.png) | ![Image](image9.png) | ![Image](image10.png) | ![Image](image11.png) | ![Image](image12.png) | ![Image](image13.png) | ![Image](image14.png) |
| *Trametes sp.* (Gano) | ![Image](image15.png) | ![Image](image16.png) | ![Image](image17.png) | ![Image](image18.png) | ![Image](image19.png) | ![Image](image20.png) | ![Image](image21.png) |
| *P. sanguineus* (M3) | ![Image](image22.png) | ![Image](image23.png) | ![Image](image24.png) | ![Image](image25.png) | ![Image](image26.png) | ![Image](image27.png) | ![Image](image28.png) |
| *T. ijuborskii* (M4) | ![Image](image29.png) | ![Image](image30.png) | ![Image](image31.png) | ![Image](image32.png) | ![Image](image33.png) | ![Image](image34.png) | ![Image](image35.png) |
| *F. flavus* (H2A) | ![Image](image36.png) | ![Image](image37.png) | ![Image](image38.png) | ![Image](image39.png) | ![Image](image40.png) | ![Image](image41.png) | ![Image](image42.png) |
| *P. chrysosporium* (PC) | ![Image](image43.png) | ![Image](image44.png) | ![Image](image45.png) | ![Image](image46.png) | ![Image](image47.png) | ![Image](image48.png) | ![Image](image49.png) |
| *F. palustris* (TP) | ![Image](image50.png) | ![Image](image51.png) | ![Image](image52.png) | ![Image](image53.png) | ![Image](image54.png) | ![Image](image55.png) | ![Image](image56.png) |
| *A. Wangii* (M7) | ![Image](image57.png) | ![Image](image58.png) | ![Image](image59.png) | ![Image](image60.png) | ![Image](image61.png) | ![Image](image62.png) | ![Image](image63.png) |

**Figure 3.** Appearance fungal growth after 10 days on media treated with treatment and control.

Based on the antifungal activity against selected wood-rotting fungi, the leaves extract of *T. diversifolia*, *C. odorata*, *S. bracteosa* and *A. indica* had the highest inhibitory effect on the growth of 9 species of wood-rotting fungi up to 100%. The highest antifungal activity, ranged from 78-100%, was found in *A. indica* leaf extract compared to other plant extracts. The strong antifungal activity may due to antifungal properties contained in the active compounds of the extract. Previous study by Meisyara et al. [7] had conducted phytochemical analysis of leaf plant extracts used in this study and found that *A. indica* leaf extract contained the most abundance phytochemical compounds compared to other extracts collected from the
Toba region including flavonoids, steroids, saponins, and tannins. According to Alzhoairy [17], these active compounds are suggested to have the potential as an anti-cancer, anti-inflammatory, antibacterial, antifungal, antioxidant and also as an insecticide. The smallest percentage of growth inhibition of wood-rotting fungi was shown by T. sinensis leaf extract. The extract can only inhibited 100% growth of three wood-rotting fungi namely S. commune, A. wangii and F. flavus while inhibitory activity to other wood-rooting fungi were ranged from 42.96% to 66.67% (Table 2).

Table 2. Antifungal activity of plant extracts against wood rot fungi

| Fungi                  | Growth inhibition (%) |
|------------------------|-----------------------|
|                        | I         | KKS       | SP        | SH        | P         | DH        |
| **White rot fungi**    |           |           |           |           |           |           |
| T. versicolor (COR)    | 61.57 ± 1.43 | 71.97 ± 1.91 | 78.42 ± 1.99 | 92.79 ± 0.27 | 92.79 ± 0.27 | 92.79 ± 0.27 |
| S. commune (SC)        | 63.7 ± 5.13 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 |
| Trametes sp. (Gano)    | 58.52 ± 2.57 | 73.33 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 |
| P. sanguineus (M3)     | 42.96 ± 1.28 | 65.93 ± 1.28 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 |
| T. iijubarskii (M4)    | 65.19 ± 1.28 | 77.78 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 |
| F. flavus (H2A)        | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 |
| P. chrysosporium (PC)  | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 |
| **Brown rot fungi**    |           |           |           |           |           |           |
| F. palustris (TP)      | 66.67 ± 3.06 | 65.33 ± 4.16 | 74.00 ± 0.0 | 62.67 ± 2.31 | 65.33 ± 2.31 | 78.00 ± 3.06 |
| A. wangii (M7)         | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 |

Note: I= T. sinensis; KKS= T. sphaerocarpa; SP= T. diversifolia; SH= C. odorata; P= S. bracteosa; DH= A. indica

White rot and brown rot fungal growth inhibition was caused by active compounds contained in plant extracts added to the growth media were toxic to the growth of fungi. Ngegba et al. [18] reported that A. indica, T. diversifolia dan C. odorata demonstrated antifungal activity while another study found that in general, all T. diversifolia organs such as leaves, stems, flowers and roots contain active ingredients (nutritional or non-nutritional) that can be used for pharmacological purposes [19]. The leaves, stems and roots of T. diversifolia contained alkaloids, flavonoids, phenols, saponins, tannins and terpenoids with the highest phytochemical content was found in leaves [20]. Terpenoids are the most common metabolites contained in T. diversifolia, especially sesquiterpenes, which are known to have antibacterial, antifungal, antiviral, antidiabetic and anti-cancer activities [8]. In addition, according to Linthoingambi and Singh [21], T. diversifolia has antifungal activity against Alternaria alternata, Aspergillus flavus, Aspergillus niger, Curvularia lunata, Fusarium oxysporum, Penicillium expansum, Penicillium italicum and Trichoderma viridae. Some of these fungi are known as causal agent of blue stain on wood [3].

The C. odorata leaf are rich in flavonoids including quercetin, sinensetin, sakuranetin, padmatin, kaempfenol and salvagenin [22, 23]. It also contains saponins, triterpenoids, tannins, organic acids etc [24]. Ethanol extract of C. odorata inhibited the growth of Phytophthora megakarya, the cause of cocoa pod infection, resulted in an inhibiting clear zone diameter of 15mm - 32 mm [25].

The A. indica seed oil is known as a bio preservative which effectively inhibits the growth of wood-rotting fungi [26]. In addition, 3% of ethanol extract of A. indica leaves showed inhibitory effect to the growth of wood-rotting fungi due to a high phenolic content and few flavonoids. According to Dhyani et al. [27], phenolic compounds are known to have anti-fungal activity up to 98-99%.
The *T. diversifolia*, *C. odorata*, *S. bracteosa* and *A. indica* extracts had better antifungal activity against *T. versicolor* which was 78.42-92.97% and inhibitory effect to the growth of *F. palustris* ranged from 62.67 to 78%. These results were also found on *Cinchona* sp. and *Homalanthus populneus* leaves which showed antifungal activity to the growth of the nine wood-rotting fungi by 84.68-100% [16]. These results are better than that reported by Celimene et al. [28] on antifungal activity of Pynosilvins compound extracted from pine seeds (pine cone) against white and brown-rotting fungi which showed the compound inhibited the growth of *T. versicolor* by 11.1% and *Postia placenta* by 11.5%. This study results are also better than antifungal activity of deoxylapachol compound extracted from teak wood which showed 64-75% inhibition to the growth of *T. versicolor* and *F. palustris* [3, 29]. In addition, *T. diversifolia*, *C. odorata*, *S. bracteosa* and *A. indica* extracts in this study also demonstrated a higher activity to inhibit the growth of wood rot fungus compared to mangrove leaf extract (*Rhizophora* sp.) which can only inhibit 59% growth of *T. versicolor* and 21.6% growth of *F. palustris* [30].

4. Conclusion
Methanol extracts of *T. diversifolia*, *C. odorata*, *S. bracteosa* dan *A. indica* leaf displayed remarkable fungal growth inhibition activity, up to 100%, against selected wood-rotting fungi, therefore are potentially be studied further as anti-wood-rotting fungal agent.

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