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Original Article

ANTIMICROBIAL AND CYTOTOXIC SCREENING OF ENDOPHYTIC FUNGI ISOLATED FROM *GRAPTOPHYLLUM PICTUM* L. GRIFF

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ABSTRACT

Objective: Endophytic fungus secondary metabolites often demonstrate potential pharmacological activity against various diseases. This study aims to isolate fungi from *Graptophyllum pictum* and test for its antibacterial and cytotoxic activity.

Methods: Potato Dextrose Agar (PDA), a common fungus culture medium, was utilised. The streak plate method was used to isolate fungi. Each pure fungal isolate was grown on rice media for 4-8 w at room temperature before being extracted with ethyl acetate. Ethyl acetate extract of all isolated fungi was tested for antimicrobial activity against pathogenic microbes *Staphylococcus aureus* (SA) ATCC 2592 and *Escherichia coli* (EC) ATCC 25922 using the disk diffusion method. Each extract was further screened for cytotoxic activity using the BSLT method using shrimp larvae of *Artemia salina*.

Results: Six pure fungal isolates were successfully obtained from *G. pictum*. Only two isolated fungi showed antimicrobial activity. WD1 and WD2 showed antimicrobial activity against SA with a diameter of inhibition zone 13.90 mm±0.56; 13.92 mm±0.83 and EC, 11.97 mm±0.22; 11.86±0.18, respectively. Based on the screening cytotoxic result, LC50 values from each extract WD1, WD2 and WA2 were toxic against Artemisia salina l with an LC50 value of less than 200 ppm. Microscopic identification showed that WD1 was *Fusarium sp*, and WD2 and WA2 was *Aspergillus sp*.

Results: These findings suggest that two fungi isolated from *G. pictum* have substantial antibacterial and cytotoxic properties.

Keywords: Endophytic fungi, *Graptophyllum pictum*, Antimicrobial activity, Cytotoxic activity

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INTRODUCTION

The potential of endophytic fungi, which are microorganisms that reside within the tissues of plants, producing a wide variety of bioactive metabolites, has attracted interest in recent years. This rich source of natural substances has promising pharmacological properties [1–3]. On the other hand, exploring endophytic fungi that inhabit medicinal plants provides numerous opportunities to find new metabolite compounds with potential bioactivity [4–7]. Furthermore, research on endophytes from tropical plants is very sparse, particularly when considering the therapeutic potential of these isolates [8, 9].

The use of crude extracts from endophytic fungi may be a promising alternative drug discovery because these microorganisms are an endless source of new metabolites and because their bioactive compounds can be produced on an industrial scale, which helps to lower the cost of the end product and preserve biodiversity [10]. In this regard, endophytes from plants that flourish in unique ecological niches, such as the Indonesian rainforest, may be capable of producing a wide range of secondary metabolites. The bioactive compounds produced by these microbes' secondary metabolism directly contribute to species' adaptation and survival [11, 12]. The compounds produced by endophytic fungi include flavonoids, alkaloids, steroids, terpenoids, iso-coumarins, and phenols. These compounds exhibit a wide range of biological activities, including hormonal, antitumor, cytotoxic, antiviral, immunosuppressive, antiparasitic, antimicrobial, and antioxidant activities [3, 13].

In Indonesia, *Graptophyllum pictum* (L.) Griff., a member of the Acanthaceae family, is known as "Daun Ungu"[12]. Several tropical locations, including the Pacific and Western and Central Africa, support the growth of this plant [14]. Numerous traditional medical uses for *G. pictum* include the treatment of tonsillitis, abscesses, rheumatism, haemorrhoids, swellings, constipation, and urinary infections. Several studies have revealed *G. pictum* pharmacology activities. The determination of analgesic and anti-inflammatory capabilities [15], the analysis of phagocytosis behaviour and immunoglobulin formation and the activity on the classical pathway of complement and chemoattractant activity [12, 16, 17].

The study of endophytic fungi's isolation from various plant sources is still in its infancy despite their interesting promise. No research has been done on the isolation of endophytic fungi from *G. pictum*. Exploring the endophytic fungi found in *G. pictum* may result in the discovery of novel bioactive substances with high antibacterial properties that could help develop treatments for infectious diseases and the fight against bacterial resistance and also for cytotoxic effect in many cell cancer lines. Therefore, the present study seeks to isolate the endophytic fungi from *G. pictum*, investigate their antibacterial and cytotoxic activity, and identify the constituents from the extract of the fungal isolate.

MATERIALS AND METHODS

Plant collection and identification

The material used was ±100 g of leaves, bark, and roots of *G. pictum* harvested at Nanggalo, Padang, West Sumatera, Indonesia. The voucher number 195/K-ID/ANDA/XI/2022 served as the sample's identification and authentication at the Andalas University Herbarium.

Isolation of the endophytic fungi

Part of the plant surface (leaves, bark, and roots) were sterilized, then rinse under running water for 10 min, followed by a 1 min rinse under ethanol 70%. The samples were then rinsed one more for 30 sec with ethanol 70% after being immersed in bleach solvent (NaOCl 5.3%) for 5 min. Under laminar airflow, the isolation process was carried out. Part of the plant were cut into small pieces. Each component was injected into a Sabouraud Dextrose Agar (SDA) medium and cultured for 3-5 d at 25 °C. The fungus colonies that developed on SDA media were gradually purified. Colonies with unique forms and colours are classified as distinct isolates. Endophytic fungus pure isolates were grown in 100 g rice medium

and incubated at 25 °C for 30 d. The cultivation procedure was carried out using Kjer *et al.* (2010) as a guide [18].

Extraction of the endophytic fungi

Handayani *et al.*, 2018 carried out the secondary metabolite extraction procedure. Following that, ethyl acetate was used to extract the substrate at each incubation time macerate. The extracts were then dried by evaporation [10, 19].

Antimicrobial activity

Escherichia coli ATCC 25922 and Staphylococcus aureus ATCC 25923 were purchased from the Microbiology Laboratory at the M. Djamil General Hospital in Padang, West Sumatra, Indonesia, and were utilized as test microorganisms. The agar diffusion method was used at a 5 mg/ml dose in dimethyl sulfoxide (DMSO). The 6 mm sterile paper discs were saturated with ten microliters of 5% extract were pipetted in order to conduct antibacterial and antifungal tests, the saturated paper discs were placed on nutrient agar (NA) and SDA, respectively. As a negative control, dimethyl sulfoxide (DMSO) was employed. Positive controls contained the antibiotic ciprofloxacin in a concentration of 30 µg/ml. After 24 h of incubation at room temperature, the diameter of the inhibitory zone was measured [20].

Cytotoxic activity-BSLT method

The nauplii of brine prawns (Artemia salina L.: Artemiidae) were utilised to assess cytotoxic activities. The eggs were incubated in 500 ml of filtered saltwater with continual aeration for 48 h at 27 °C±2 °C. Following hatching, active nauplii free of eggshells were collected and employed in the test. The extracts utilised in this experiment had final concentrations of 1,000, 100, and 10 µg/ml in triplicate. Negative controls include filtered saltwater and DMSO. The final concentrations of the extracts used in this assay were 1,000, 100, and 10 µg/ml in triplicate. The LC50 value was calculated utilising the curve technique and probit analysis to measure cytotoxic activity. Data were analyzed with IBM SPSS statistical analysis software version 23.0 [19].

Phytochemical screening

Harborne's (1973) standard method carried out the phytochemical screening. Alkaloids: The G60 F254 silica plate was spotted with ethyl acetate extract and eluted with n-hexane: ethyl acetate eluent (1:4). The spots were treated with Dragendorff's reagent to make it visible. If the extract became orange, it contained alkaloids. Steroids and terpenoids: The G60 F254 silica plate were spotted with ethyl acetate extract and eluted with n-hexane: ethyl acetate eluent (1:4). Lieberman Burdcharts were placed on cotton buds and exchanged evenly and thinly. Heating was required if the reaction was not spontaneous. If positive extracts produced pink, they contained terpenoids, and positive steroids formed blue and green colours. Phenolic compound. The G60 F254 silica plate was spotted with ethyl acetate extract and eluted with n-hexane: ethyl acetate eluent (1:4). Using a cotton bud, swap the FeCl3 reagent evenly and thinly on the plate. It includes phenolic if the extracts form purple, crimson, or pink. Flavonoid. The G60 F254 silica plate was spotted with ethyl acetate extract and eluted with n-hexane: ethyl acetate eluent (1:4). Using a cotton bud, the Citroborat reagent was applied precisely and thinly on the plate. If the extracts formed a green colour, they contained flavonoids [21].

Fungal identification

The morphological characteristics of the fungi were studied macroscopically by analysing colony features such as colour, shape, size, and hyphens. A small piece of fungal mycelium was put on a glass slide, coloured with lactophenol cotton blue, and examined microscopically with an optical microscope.

RESULTS

Antimicrobial activity of isolated endophytic fungi

The antibacterial activity of nie endophytic fungi isolated from *G. pictum* (WD1, WD2, WD3, WKB1, WKB2, WKB3, WA1, WA2 and WA3) was tested. Two extracts tested demonstrated activity against S. *aureus* and E. *coli* (table 1). The fungus extract WD1 and WD2 isolated from *G. pictum* leaves, were the most promising in producing antibacterial metabolites. Gram-negative E. *coli* and Gram-positive S. *aureus* were both inhibited by this fungus.

Cytotoxic activity of fungal extracts

The Brine Shrimp Lethality Test (BSLT) method was used as a preliminary test for cytotoxic activity screening. Meyer *et al.* categorized the toxicity of the extracts based on LC50 value. The LC50 value below 1,000 ppm is classified as toxic; if greater than 1,000 ppm, it is classified as non-toxic. The results showed that four isolates of the fungus (77.78% of total fungal isolates) have cytotoxic activity.

The preliminary screening for cytotoxic activity was performed using the BSLT method [18]. The cytotoxic isolates were WD1, WD2, WKB1, WAKB2, WKB3, WA2 and WA3. The isolate which showed the highest cytotoxic activity was WD1, WD2 and WA2 (fig. 1).

Table 1: Antimicrobial activity of endophytic fungi isolated from (*Graptophyllum pictum* L. *Griff*). (n=3)

No	Isolate code	Inhibition zone (mm)±SD	
		E. coli	***S. aureus***
1.	WD1	13.90±0.56	11.97±0.22
2.	WD2	13.92±0.83	11.86±0.18
3.	WD3	9.55±0.84	9.08±0.82
4.	WKB1	8.48±0.54	9.48±0.28
5.	WKB2	7.52±0.42	8.78±0.66
6.	WKB3	5.37±0.52	7.08±0.62
7.	WA1	6.87±0.26	7.82±0.28
8.	WA2	7.26±0.57	8.02±0.93
9.	WA3	4.20±0.56	6.78±0.44
10.	Positive Control	33.57±0.86	35.08±0.31
11.	Negative Control	-	-

Table 2: Phytochemical screening test of active fungi isolates from *G. pictum*

Code of extract	Alkaloid	Flavonoid	Terpenoid	Steroid	Phenolic	Saponin
WD1	+	+	-	-	+	+
WD2	-	+	-	-	+	+
WA2	+	-	+	-	+	+

Phytochemical profile of promising fungal extracts

The extracts of the fungi WD1, WD2 and WA2 were analyzed using standard method by Harborne (1973), in order to identify the chemical group present in the active extracts. WD1, WA2 and WA2 extract indicates the presence of alkaloid, phenolic and

saponin, while in WA2 extract the presence of flavonoid is abstained (table 2).

Identification of active endophytic fungi

The fungi that produce active extracts were identified. Fig. 2 and 3 displays the macro and micro morphological characteristics of an active isolate. The endophytic fungus identification results of WD1 are Aspergillus sp (fig. 2). This fungus appears to have conidia that are round in shape and blackish-white in color. The head of the conodia is a structure located at the terminal part of the conidiophore, round (globose) or semi-spherical (sub-globose) and composed of vesicles, metula (if any), fialids and conidia. Most of the species Aspergillus sp. has unbranched conidiophores that each produce a single conidia head [22].

The endophytic fungus WD2 and WA2 isolated from the leaves of *G. pictum* are suspected to be Fusarium sp (fig. 3 and 4). With its microscopic characteristics: hyaline microconids, ovoid (egg-shaped with one end narrowed) or oblong with a slightly bent end. Hyaline macroconids, shaped like a sickle or canoe with a slightly bent tip it should reveal the findings of works.

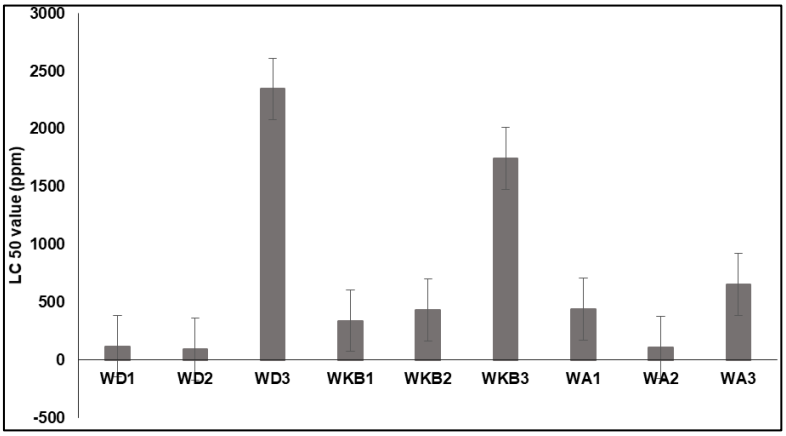
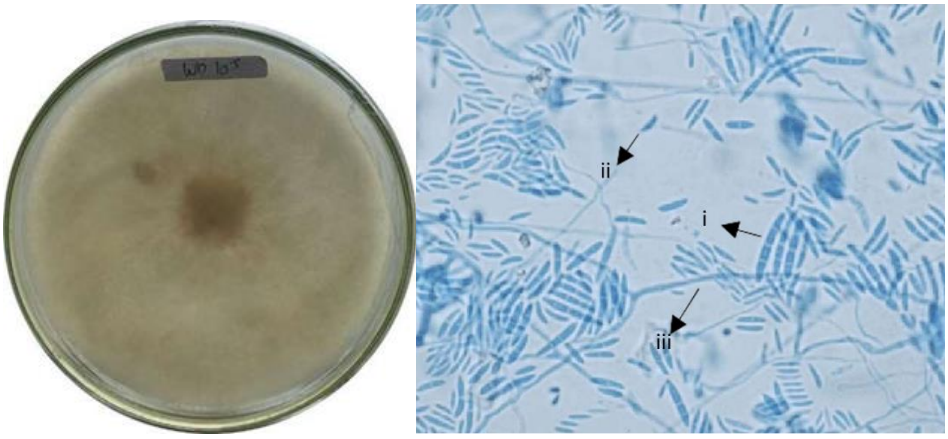
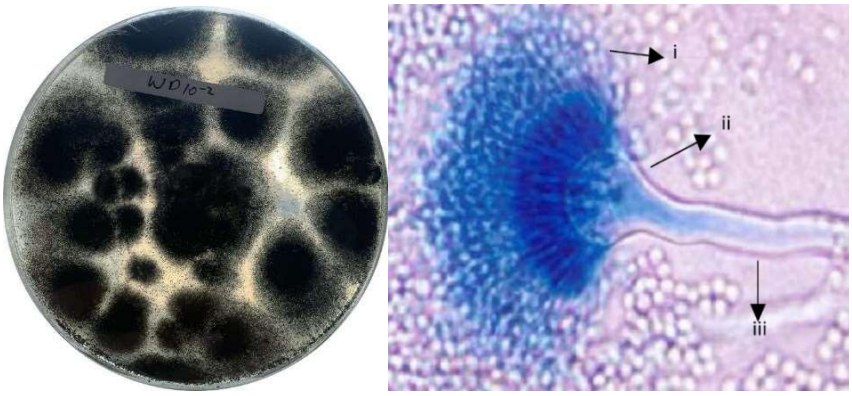


Fig. 1: The cytotoxic activity of ethyl acetate extract of endophytic fungi from *G. pictum.* Error bars indicate the SD values



A **B**

Fig. 2: Macroscopic (A) and microscopic (B) observation of fungal strain WD1 at 40x magnification. Description: i. macroconidia, ii. microconidia, iii. Hyphae



A **B**

Fig. 3: Macroscopic (A) and microscopic (B) observation of fungal strain WD2 at 40x magnification. Description: i. conidia, ii. conidiofor, iii. vesicle

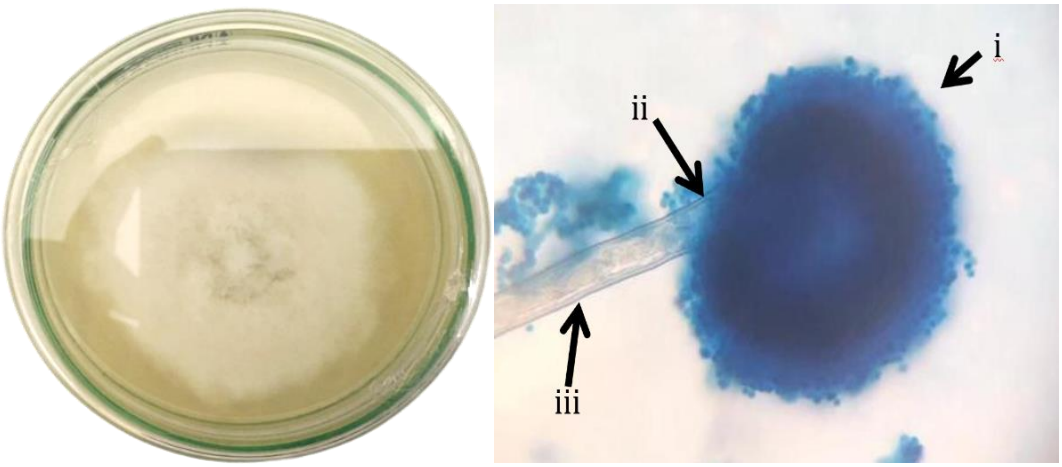


Fig. 4: Macroscopic (A) and microscopic (B) observation of fungal strain WA2 at 40x magnification. Description: i. conidia, ii. conidiofor, iii. Vesicle

DISCUSSION

Endophytic fungus plays a biological/biochemical role in plants and interacts with the host and other endophytes and creatures related to the plant species [1, 23]. However, the microbiological diversity of different plant species and the diversity of the metabolites produced by endophytic fungi provide an opportunity to discover new bioactive compounds [13]. Furthermore, a number of studies have established the importance of endophytic microbes in host survival because endophytes directly regulate plant metabolism, allowing plants to tolerate severe temperatures, dehydration, and the presence of plant pathogens [23]. As a result, the plant's traditional function and the region where it lives are crucial characteristics to consider when isolating endophytes [24].

G. pictum is widely grown in Southeast Asia, and its leaves have traditionally been used as astringents, treatment of inflammations, skin diseases and wound healing, intestinal colic, dysentery, leukorrhea, and anaemia [25], as well as antimicrobial and antioxidant activity [26]. Given the demand for novel compounds with antimicrobial and antioxidant action, we investigated the endophytic fungus of *G. pictum* for antimicrobial and antioxidant potential. Extracts of endophytic fungi obtained from *G. pictum* were tested for antibacterial activity against types of bacteria known to be harmful to humans. Compared to endophytes isolated from other parts of the plant, fungi isolated from the leaves of *G. pictum* were revealed to be more promising in antibacterial activity. Both fungi from the leaves produced antimicrobial compounds [27].

Secondary metabolites are responsible for the antimicrobial activity of endophytic fungi identified from *G. pictum*. As a result, the phytochemical test was carried out to determine whether these fungi's secondary metabolites have the characteristics mentioned earlier. WD1 and WD2 demonstrated the most potent antibacterial activity compared to other strains. The phytochemical test results revealed that strain WD1 included flavonoid, saponin, and phenolic components. Strain WD2 produced positive results due to the presence of saponin and phenolic groups.

It's important to highlight that Bhore *et al.*, research in 2010 divided bacterial growth inhibitory activity into four categories: very strong (20 mm), strong (10-20 mm), moderate (5-10 mm), and weak (5 mm) [28]. According to this classification, the extracts from *G. pictum* strains WD1 and WD2 are strong. Furthermore, the extract from strains WD1 and WD2 demonstrated strong antibacterial activity, indicating that they are a promising target for further study into natural antibacterial medicines. In comparison to this study, research on endophytic fungi from marine sponge-derived fungus *Aspergillus nomius.* Showed potential antibacterial activity that can inhibit ten pathogenic bacteria with an inhibition zone more than 10 mm [19].

Microbial sources have a high content of flavonoid and phenolic compounds, and these metabolites may be synthesised under regulated circumstances and at more rapid rates than when acquired from plants, which reduces costs on production [29]. The optimisation of *G. pictum* endophytic fungal growth in order to boost the synthesis of bioactive phenolic compounds might produce a higher level of bioactive molecules and, hence, should be pursued in future research.

It should be with the interpretation of the results and their comparison with those of other studies. There is no need to repeat the results, review literature and textbook knowledge, or cite references that do not have a close relationship with the present result.

CONCLUSION

Three fungus have been successfully isolated from the leaves, stem bark, and roots of *G. pictum* due to their potential antibacterial and cytotoxic effects. The fungus were identified as Aspergillus sp. WD1, Fusarium sp WD2 and WA2, respectively. More research into their bioactive components is required to fully understand their potential as prospective antibacterial and cytotoxic agents in the pharmaceutical industry. The extracts from strains WD1 and WD2 demonstrated promising antibacterial activity, particularly against S. aureus and E. coli, with significant inhibitory activity.

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Nil

AUTHORS CONTRIBUTIONS

All authors contributed to the manuscript and approved the submitted version. AE: Conceptualization, Methodology, Supervision; RA: Data curation, Writing-Original draft preparation, Writing-Revised and Editing, Project administration; FR: Validation, Visualization, Investigation; CPR: Investigation, Resources; AF: Formal analysis, Software, Validation.

CONFLICT OF INTERESTS

All authors declare that there is no conflict of interest regarding the publication of this article.

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