Phytochemical Screening and KLT Analysis of Endophytic Fungi Secondary Metabolite of *Vetiveria zizanioides* L.

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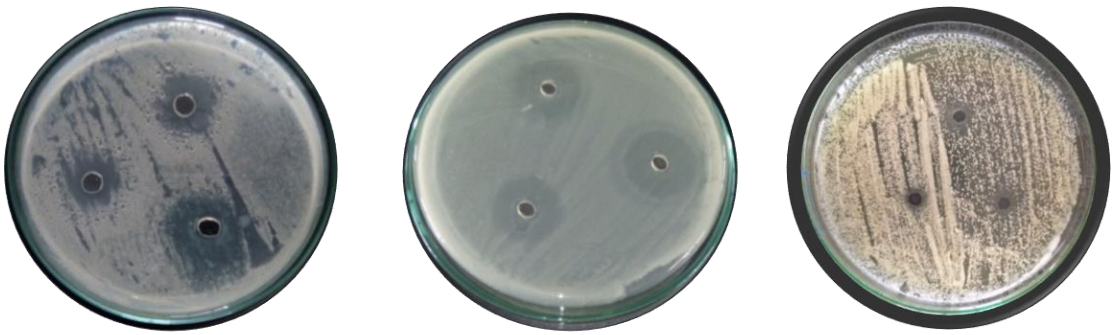
Abstract. Endophytic fungi are microorganisms that live in the healthy tissue of the host plant without causing disease. Endophytic fungi grow in every plant, including vetiver roots of Vetiveria zizanioides L. both intracellular and intercellular. This study aims to do phytochemical screening and Thin Layer Chromatography(KLT) analysis of secondary metabolites produced by endophytic fungi from vetiver roots. The results showed that secondary metabolites produced by endophytic fungi contain one or more compounds, namely in the form of terpenoids, steroids, alkaloids, phenolics, flavonoids, and saponins. All fungal isolates produce secondary metabolites in the form of alkaloids except IH2 and IH11 isolates. The results ofKLT strengthened the phytochemical screening results on secondary metabolites produced by endophytic fungi with Rf values of 0.6-0.8.

1. Introduction

Endophytic fungi are fungi that live in the tissues of host plants without causing symptoms of disease [1,2]. Endophytic fungi play a role in protecting the host against insect pests [3], as a growth regulator [4] producing hydrolytic enzymes such as amylase, cellulase, xylanase, ligninase [5] and chitinase [6]. In addition, endophytic fungi are able to produce the same secondary metabolites as the host [7]. Secondary metabolite products can function as antimicrobials [8], antifungal and cytotoxic [9], antiviral, anticancer, antidiabetic, antimalarial, antioxidant and antiimmunosuppressive [1]. Endophytic fungi can be found in various types of plants, especially medicinal plants. Endophytic fungi isolated from medicinal plants can produce secondary metabolites that are higher than the original plants [10]. One type of aromatic plants that are widely used in treating various diseases are vetiver roots (Vetiveria zizanioides L.) Based on phytochemical analysis of vetiver extract, it is known to contain alkaloids, amino acids, flavonoids, saponins and tannins [11] which are useful as antifungal agents, antioxidant and antibacterial [12]. Harahap & Elsie (2017) have successfully isolated 34 endophytic fungus isolates from vetiver root (V. zizaniodes L.). Of the 34 isolates that were isolated, 22 isolates were able to produce bioactive compounds that could inhibit the growth of Staphylococcus aureus, Escherichia coli and Candida albicans (Figure 1) in the strong category.



(a) (b) (c)

Figure 1. Antimicrobial activity test of endophytic fungi isolates from vetiver roots (V. zizaniodes L.) in inhibiting the growth of (a) Staphylococcus aureus; (b) Escherichia coli, (c) Candida albicans

Endophytic fungi are useful in the pharmaceutical industry because of their ability to produce secondary metabolites such as alkaloids, flavonoids, steroids, terpenes, quinones, phenols, which have great potential as bioactive compounds [13]. The magnitude of the potential for endophytic fungi isolates in producing bioactive compounds is the potential that must be developed. However, the bioactive compounds produced by the fungus are unknown. Therefore, phytochemical screening and KLT analysis are needed from the crude extract of endophytic fungus fermentation media of vetiver root to determine the secondary metabolite content produced and expected to be the same as the host plant.

2.Material and Methods

2.1 Preparation of Endophytic Fungus Isolates

A total of 22 from 34 isolates of endophytic fungi from vetiver root that have high ability in inhibiting microbial growth were selected and then grown on MEA media (Malt Extract Agar) at room temperature for 7 days.

2.2 Endophytic Fungus Fermentation

After fungus isolates had been grown for ± 7 days in MEA media, each isolate was taken using 3 sterile straws. Isolates were fermented to get secondary metabolites in the media of PDB as much as 20 ml in erlenmeyer. Medium containing endophytic fungi isolates was in static condition and placed at room temperature. This fermentation process lasted for ± 21 days.

2.3 Screening of Endophytic Fungi Phytochemical Compounds

Coarse extracts of secondary metabolites of endophytic fungi which have antimicrobial activity in previous studies were then carried out by phytochemical screening to determine the class of compounds contained therein, including terpenoids, steroids, alkaloids, flavonoids, phenolics, and saponins. Positive extracts of secondary metabolites were then monitored using thin layer chromatography (KLT).

3.Results and Discussion

3.1. Phytochemical Screening of Endophytic Fungus Secondary Metabolite

Phytochemical screening was carried out on endophytic fungi secondary metabolites which included examination of terpenoids, steroids, alkaloids, phenolics, flavonoids, and saponins. Phytochemical screening results revealed that the secondary metabolites produced by endophytic fungi from vetiver mostly contained alkaloids except IH 2 and IH isolates. Some isolates produced secondary metabolites containing terpenoids, steroids, alkaloids, phenolics, flavonoids, and saponins (Table 1.) This is in line with the phytochemical analysis of vetiver extract which is known to contain alkaloids, flavonoids, saponins, tannins and steroids [14]. The same type of secondary metabolite produced by endophytic fungi with host plants is thought to be the result of coevolution or genetic transfer (genetic recombination) from host plants into endophytic microbes [4]. Some endophytic fungi isolates produce one or more chemical compounds, IH2 isolates, IH3, IH4, IH11, IH 18, IH 22, IH 29 and IH30 contain terpenoids. Steroids are found in secondary metabolites produced by IH 33 isolates while saponins are found in secondary metabolites produced by IH 24 isolates. This is because the formation of secondary metabolites is coded by a number of genes found in chromosomal DNA or plasmid DNA. The gene will appear when given induction first. The induction process can be the addition of a precursor compound or the addition of a certain amount of isolate inoculum in the fermentation process. Some conditions that affect secondary metabolites are limited nutrients available in a microbial growing environment, addition of inducing compounds and decreased growth velocity [15].

Flavonoid compounds are synthesized by plants as a defense system and in response to infections by microorganisms, so these compounds are effective as antimicrobial compounds against a number of microorganisms [16]. Flavonoid compounds that act directly as antibacterial work by denaturing bacterial cell proteins and damaging cell membranes irreparably [17]. The content of the compounds produced by endophytic fungi has an antimicrobial role, and this can be seen in previous studies where the endophytic fungus is able to inhibit the growth of S. aureus, E.coli and C.albicans. Saponins and steroids / triterpenoids produced by endophytic fungi have the ability to be antimicrobial [18,19].

Table 1. Phytochemical Screening of Endophytic Fungus Secondary Metabolite from Vetiver Root

No	Compound	Endophytic Fungi Isolate																					Positive Reaction (Discoloration)
		IH 1	IH 2	IH 3	IH 4	IH 8	IH 9	IH 11	IH 13	IH 15	IH 18	IH 19	IH 21	IH 22	IH 23	IH 24	IH 26	IH 27	IH 29	IH 30	IH 31	IH 32	IH 33	
1	Terpenoid	-	+	+	+	-	-	+	-	-	+	-	-	+	-	-	-	-	+	+	-	-	-	Purplish red
2	Steroid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	Blue-green
3	Alkaloid																							
	a.Dragendorff	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Orange
	b.Mayer	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	White
4	Fenolik	+	+	+	+	-	+	+	+	-	+	+	+	+	-	-	+	-	-	+	+	+	-	Blackish green
5	Flavonoid	-	-	-	-	+	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	+	-	Orange-red
6	Saponin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	Stable foam ± 10 minutes

Description: (+) detected, (-) not detected

Analysis of Secondary Metabolites by Using KLT

The results of the analysis of secondary metabolites using Thin Layer Chromatography (KLT) and seen in UV light 254 nm (Table 2) were carried out with the aim to further strengthen the results obtained from the phytochemical test. The results of the analysis with KLT show that the Rf value is 0.6-0.8. These results indicate that the antimicrobial activity produced by secondary metabolites from endophytic fungi is due to containing flavonoid compounds. Analysis of thin layer chromatography (KLT) was carried out on vetiver extracts and obtained Rf values of 0.63-0.75. The Rf value shows that vetiver extract contains flavonoids which play a role in inhibiting bacterial growth [14].

Table 2. The Results of KLT Analysis of endophytic fungi isolate secondary metabolites of vetiver root (*V.zizanioides* L.)

No	Isolate Code	Motion Phase (N-hexane:ethyl acetate)
1.	IH 1	0,77
2.	IH 2	0,67
3.	IH 3	0,65
4.	IH 4	0,72
5.	IH 8	0,67
6.	IH 9	0,75
7.	IH 11	0,7
8.	IH 13	0,67
9.	IH 15	0,7
10.	IH 18	0,72
11.	IH 19	0,8
12.	IH 21	0,75
13.	IH 22	0,7
14.	IH 23	0,7
15.	IH 24	0,72
16.	IH 26	0,77
17.	IH 27	0,75
18.	IH 29	0,67
19.	IH 30	0,65
20.	IH 31	0,77
21.	IH 32	0,75
22.	IH 33	0,65

4. Conclusions

Based on phytochemical screening, secondary metabolites produced by all endophytic fungi isolates from vetiver contain at least one or more compounds namely terpenoids, alkaloids, steroids, saponins, phenolics and flavonoids. This phytochemical screening is strengthened by the KLT test. The KLT test results showed that the Rf value for all endophytic fungi ranged from 0.6-0.8.

5. Reference

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