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POTENTIAL OF ENDOFITING FUNGALE EXTRACT FROM THE LEAVES OF MANGO PARASITE (*Dendrophthoe pentandra* (L.) Miq) AS ANTIOXIDANTS

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ABSTRACT

The importance of the presence of bioactive compounds that have the potential as antioxidants from various medicinal plants has become a topic that is often discussed by several researchers today. One of the higher-classification plants that potentially has antioxidant activity is the mango parasite (*Dendrophthoe pentandra* (L.) Miq), classified as a parasite of the Loranthaceae family. The use of specific endophytic fungi microbes from mango parasites of leaf tissue is expected to produce several bioactive compounds that act as antioxidants needed for large-scale production in a short time without excessive exploitation of nature. This primary research aims to determine the potential antioxidant activity of endophytic fungi extracts from the parasite of mango leaves Integrated Microbiology Laboratory and Halal Center of the Islamic University of Malang. Antioxidant testing using DPPH (2,2-diphenyl-1-picrylhydrazyl) free-radical capture of activity method. The results showed that KEDBM 1 (*Aspergillus* spp.) isolates had no potential antioxidant activity. While KEDBM 2 (*Aspergillus* spp.) isolate has the highest antioxidant potential with an IC50 value of 13.71 µg/ml and KEDBM 3 (*Colletotrichum* spp.) has a high potential as an antioxidant with IC50 of 36.29 µg/ml.

Key words: antioxidant, mango balu, DPPH, endophyte

INTRODUCTION

The importance of the presence of bioactive compounds with antioxidant potential from various medicinal plants has been a frequent topic of discussion by some researchers nowadays. Several compounds such as phenolic compounds and diterpenes are known to be responsible for the antioxidant activity of many plant species (1).

Based on the exposure of (2), a number of studies have shown that higher classification of plants has been identified as containing polyphenols that can be used as antioxidants against various diseases. Antioxidants are essential compounds that can inhibit cell damage due to oxidative stress from free radical molecules. Free radicals are molecules with unpaired electrons in their outer orbit. As unstable molecules, free radicals can quickly react and attack nearby molecules and create a chain reaction that causes cell death (3).

One of the superior plants that potentially has antioxidant activity is the mango parasite. The parasitic plant itself is classified as a higher plant as well as a parasite of the Loranthaceae family (4). The mango parasite (*Dendrophthoe pentandra* (L.) Miq) is one of the parasite types that has potential as a traditional medicine such as herbal medicine (5), although it is often considered a parasitic plant because it lives on and takes food from its host plant (6). It has been proven to be used as an anti-inflammatory, antiviral, anticancer, and analgesic, lowers blood pressure,

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lowers free radicals, and increases immunity. Several studies both in vitro and in vivo have shown that mango parasite can be utilized as an antihypertensive (7-9), antidiabetic, anticancer (10), antidiuretic, and can treat various infections and inflammation.

The need for new bioactive compounds used in medicine, industry, and agriculture is increasing. While plants have been the main source of new compounds for drug discovery, the concern has recently turned to endophytes as these microorganisms show a great potential source for new bioactive compounds (11). Endophytic fungi are available in almost all plant species and have been reported to be a potential source of bioactive secondary metabolite compounds. Many researchers have proven that the microbial ability of endophytic fungi to produce secondary metabolite compounds by their host plants has been confirmed and has enormous potential (12) and can be used in modern medicine, agriculture, and industry, antiviral, anticancer, antidiabetic, and antimicrobial effects, but less is known about their antioxidant capacity (11).

This information indicates that endophytic fungi can be used as an alternative source in producing the required secondary metabolite compounds that can function as antioxidants. So that there is a way to streamline the production of secondary metabolites from host plants, using specific endophytic fungi from the leaf tissue of mango parasite which is expected to have potential as an antioxidant without having to extract it from the plant. One way to determine whether or not there is a potential antioxidant activity in the extract of endophytic fungi can be known using the DPPH free radical scavenging activity method which has been widely used due to its practicality. Therefore, this research was conducted in the hope of providing the latest information on the potential of endophytic fungi extracts from mango parasite leaves as antioxidants.

TOOLS AND METHOD

Time and Place

The research was conducted at the Integrated Microbiology Laboratory and Halal Center of the Islamic University of Malang, Malang, East Java from November 2022 to February 2023.

Purification of Endophytic Fungus Isolate

Endophytic fungi isolates of mango parasite leaves were obtained from previous research (13) which were purified several times on Potato Dextrose Agar (PDA) media. Purification is performed by taking a little of the original isolate as much as 1 x 1 cm2 using a round ose needle, then inoculating (14). Purification is carried out every two weeks by checking the condition of the isolate in between days.

Fermentation

Isolates were inoculated into the liquid medium of sterile Potato Dextrose Yeast Broth (PDYB) aseptically. The isolates were then fermented in a shaker incubator at the speed of 140-170 rpm at room temperature for 14-21 days.

Ekstraction

Endophytic fungus isolates were filtrated to separate between the media and mycelium, then extracted by maceration method using ethyl acetate solvent 1:1 for 24 hours. The endophytic

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fungus extract was then concentrated using a Rotary Vacuum Evaporator at 40 ^{O}C with a speed of 100 rpm.

Antioxidant Activity Test

Concentrated extract of the endophytic fungi of Mango Parasite leaves as much as 10 mg, dissolved in 10 ml of 96% ethanol as the parent solvent. Then a concentration series solvent was made with the size of 120 µg/ml, 140 µg/ml, 160 µg/ml, 180 µg/ml, and 200 µg/ml. Each 0.1 mL concentration solvent of the Mango parasite is endophytic of fungus extract was reacted with 3 mL of 0.1 mM DPPH solvent, then homogenized and incubated for 30 minutes. Ascorbic acid was used as a standard or comparison solvent with concentrations of 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml, and 100 µg/ml. Absorbance measurements using UV-Vis Spectrophotometer at a wavelength of 517 nm and calculated the percentage level of inhibition using formula (1).

$$Free\ radical\ inhibition\ DPPH\ (\%) = \frac{A_0 - A_1}{A_0} \times 100\% \text{.....(1)}$$

Keterangan: A0 = Absorbance Control
A1 = Absorbance Sample/ Standard

The control absorbance is the absorbance value of the DPPH free radical solvent added with 96% ethanol and the sample absorbance is the absorbance value of the sample/standard solvent added with DPPH free radicals (11).

Data Analysis

Antioxidant activity is depicted in a relationship graph and expressed in IC50 values obtained from the linear regression equation formula of the relationship curve between percent inhibition against sample concentration using Microsoft Excel. Data analysis was conducted descriptively by considering the absorbance value and obtaining the percent of inhibition.

RESULT AND DISCUSSION

Endophytic fungus isolates from mango parasite leaves are known to have the genus Aspergillus and Colletotrichum. The acquisition of this genus is based on the results of research conducted previously, stated in the research of (13) and (15). Endophytic mold isolates that were successfully purified have different morphological characteristics in each genus. The macroscopic observations were made visually by matching the purified isolates with the parent isolates based on the identification book Introduction to Common Tropical Funugs (16).

Aspergillus spp. in isolate KEDBM 1 has macroscopic characters such as velvety or sandy with a blackish-green color, has a growth zone, zonation, no radial lines, and colony exudate drops (13). While the KEDBM 2 isolate also has the genus *Aspergillus* spp. and almost the same characteristics as the KEDBM 1 isolate, which has a velvety or sandy texture, a blackish-green color, a growth zone, zonation, but has radial lines and exudate drops. The isolate KEDBM 3 or *Colletotrichum* spp. has characteristics such as cotton or blackish brown velvet, does not have radial stripes, zonation, and growth zones, and does not have exudate drops (15).

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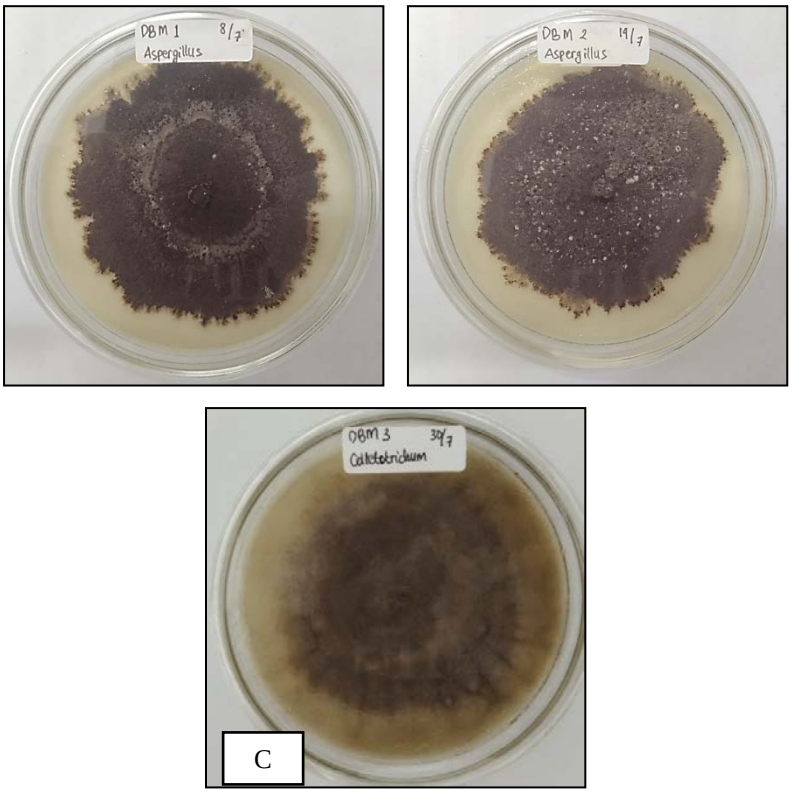

Figure 1. The Macroscopic photographs of KEDBM 1 or *Aspergillus* spp. (A), KEDBM 2 or *Aspergillus* spp. (B), and KEDBM 3 or *Colletotrichum* spp. (C) isolates.

The fermentation of fungi isolates that have been inoculated from PDA media to PDYB media aims to obtain the maximum results of secondary metabolite and can be processed easily before testing on endophytic fungus. The characteristics of the emergence of secondary metabolites from the results of this fermentation are the growth of endophytic fungi following the macroscopic morphological characteristics of the endophytic fungi and the changing color of the liquid media. This characteristic can be seen by the appearance of white fibers like cotton on the inoculated endophytic fungi of plate pieces. These white fibers are the mycelium of the endophytic fungus (17). The presence of the distribution of oxygen and temperature during the fermentation process caused the endophytic fungus to grow optimally on day 21.

Fermented isolates have mycelium characteristics such as white fine fibers for *Aspergillus* spp. and dense clumps with yellowish white pale for *Colletotrhichum* spp. This shows that each endophytic fungi isolate can produce different secondary metabolites, namely intracellular or extracellular. Therefore, the filtering of fermented fungus isolates was carried out to separate the mycelium from the medium.

Extraction is carried out by separating the mycelium and growth media of endophytic fungi and then doing a maceration. This maceration method aims to separate secondary metabolite compounds produced from the previous fermentation process. The solvent used is based on the polarity and effectiveness of a solvent in binding secondary metabolite compounds on endophytic fungi that will be macerated, which is ethyl acetate solvent. The maceration process

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is carried out for 24 hours to get the maximum binding of secondary metabolite compounds by the solvent.

Testing the antioxidant activity of the concentrated extract of endophytic fungi of mango parasite of leaves using the method of DPPH (2, 2-diphenyl-1- picrylhydrazyl) free-radical scavenging activity. The concentrated extract that has been obtained is then dissolved in 96% ethanol and made a concentrated solvent to be reacted with 0.1 mM DPPH solvent. The results of absorbance measurements on UV-Vis Spectrophotometer with a wavelength of 517 nm.

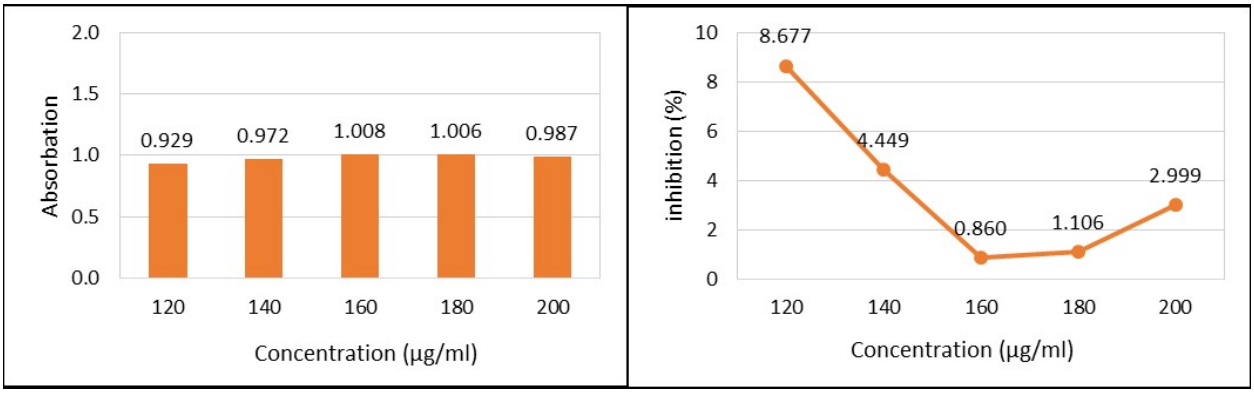


Figure 2. Graph of concentrated relationship, average absorbance, and inhibition of KEDBM isolate 1 (*Aspergillus* spp.) (A1: absorbance; A2: inhibition)

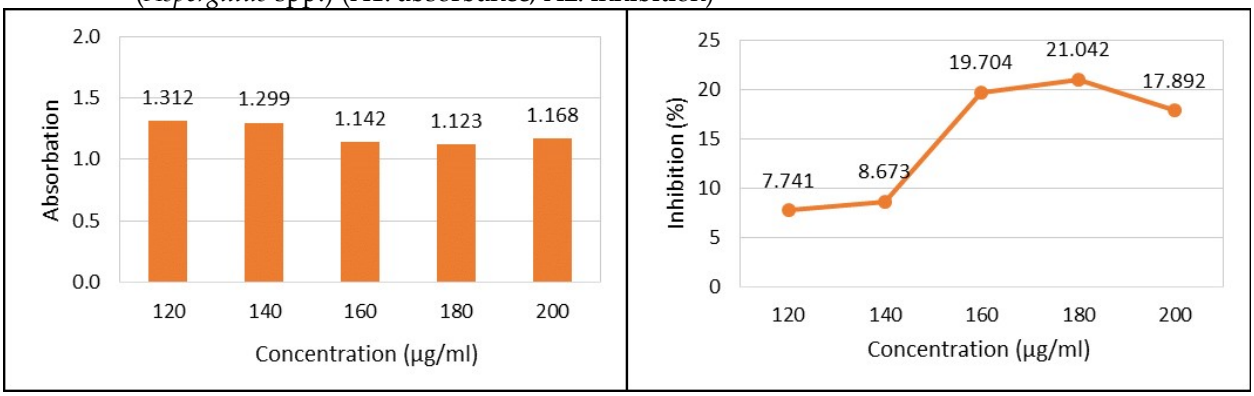
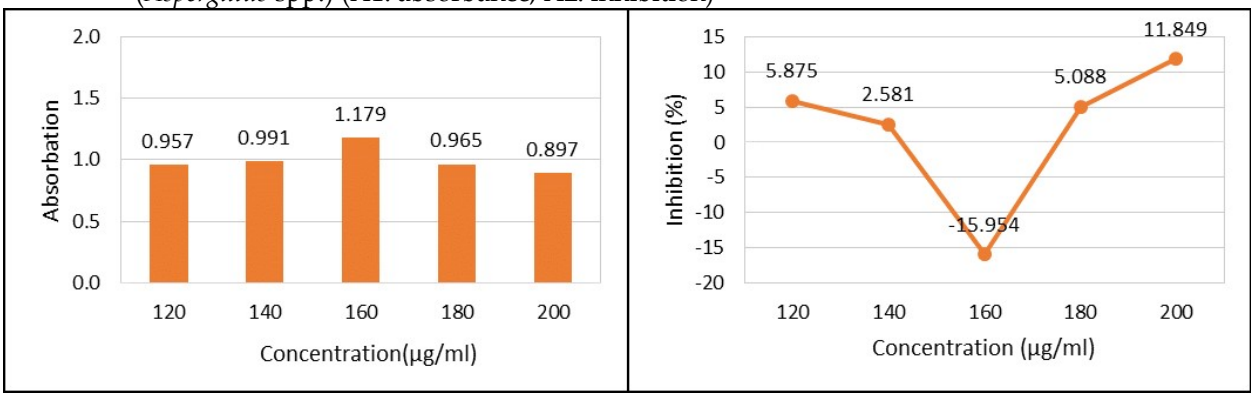


Figure 3. Graph of concentrated relationship, average absorbance, and inhibition of KEDBM 2 isolate (*Aspergillus* spp.) (A1: absorbance; A2: inhibition)



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Gambar 4. Graph of concentrated relationship, average absorbance, and inhibition of KEDBM 3 isolate (*Colletotrichum* spp.) (A1: absorbance; A2: inhibition)

The graphs (Figures 2, 3, 4) show that the number of concentrations used is not proportional to the obtained inhibition value. This explains that there are differences in the rate of inhibition of DPPH free radicals at each concentration (18). Meanwhile, to determine the effectiveness of the mango parasite of endophytic fungi extract as an antioxidant, the determination of free-radical inhibitory activity is expressed by the IC50 (Inhibitory Concentration) value. This value illustrates how much the concentration of test compounds in the sample could capture 50% of free radicals in DPPH (19), so that the IC50 of each isolate can be calculated. The ability to capture 50% of free radicals by an organic material can be classified as a very strong antioxidant if it has IC50 < 50µg/ml, classified as strong if 50µg/ml < IC50 < 100µg/ml, classified as moderate if 100µg/ml < IC50 < 150µg/ml, classified as weak if the value of 150µg/ml < IC50 < 200µg/ml, and very weak if the IC50 value is above 200µg/ml (20). The results of the calculation of percent free-radical inhibition by DPPH can be seen in Table 1.

Table 1. Linear regression equation and IC50 value of endophytic fungus extract of mango parasite (*Dendrophthoe pentandra* (L.) Miq)

Sample	Genus	Linier Regression Equation	Score IC_{50} (µg/ml)	Antioxidant Category
KEDBM 1	*Aspergillus*	y = -1,47x + 8,0285	-28,55	None
KEDBM 2	*Aspergillus*	y = 3,2671x + 5,2094	13,71	Very Strong
KEDBM 3	*Colletotrichum*	y = 1,4454x - 2,4484	36,29	Strong
AA	Ascorbic Acid	y = 4,4227x + 1,6407	10,93	Very Strong

Description: Ascorbic Acid (AA) is a positive control as a comparator

KEDBM 1 has an IC50 value below zero or negative, which is -28.55µg/ml. This negative value can also mean that no amount of concentration can inhibit free radicals in the concentration range used. So it can be stated that the isolate KEDBM 1 (*Aspergillus* spp.) has no potential as an antioxidant. While KEDBM 2, which has the same genus as KEDBM 1, is known to have an IC50 value of 13.71µg/ml, so it can inhibit free radicals very strongly. The isolate KEDBM 3 (*Colletotrichum* spp.) has an IC50 value of 36.29µg/ml, so it is categorized as a strong antioxidant in counteracting free-radical compounds.

The absence of antioxidant activity in the endophytic fungi isolates of KEDBM 1 (*Aspergillus* spp.) is different from the research by (21) which stated that the ethyl acetate extract of Aspergillus sp. from red algae Eucheuma sp. which is classified as very strong with an IC50 of 38.64µg/ml. While another Aspergillus genus, KEDBM 2, has very strong potential. This may occur due to differences in growing habitat so that the endophytic fungi of the Aspergillus genus that grow in the leaf tissue of mango parasite are found to have less antioxidant potential than those that grow outside the tissue. This also applies to isolating KEDBM 3 with the genus Colletotrichum which showed different results from previous studies, such as in the research of

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(22) which stated that the extract of endophytic fungi *Colletotrichum* sp. from Kina (Cinchona calisaya Wedd.) has antioxidant activity that is classified as inactive because it has a very high-value IC50, which is 837.143µg/ml to 1900.46µg/ml.

Ascorbic acid itself is a water-soluble form of vitamin C and is absorbed in the form of ascorbic acid or dehydroascorbic acid in the small intestine. Vitamin C circulates in the body in an unbound form and is available as a reducing substance or antioxidant in the blood and interstitial fluid (23). However, antioxidant activity describes the kinetics (speed) of any reaction formed between the antioxidant and the target radical reflecting the ability of the antioxidant to block the propagation stage in the oxidative chain (24).

CONCLUSIONS AND SUGGESTIONS

Endophytic fungi extract from Mango parasite leaves are obtained from isolates with the genus Aspergillus and Colletotrichum. Potential test of antioxidant activity using the DPPH method of (2, 2-diphenyl-1-picrylhydrazyl) free-radical scavenging activity. The results showed that KEDBM 1 (*Aspergillus* spp.) had no antioxidant potential, while KEDBM 2 (*Aspergillus* spp.) and KEDBM 3 (*Colletotricum* spp.) had very strong antioxidant potential with IC50 values of 13.71 µg/ml and 36.29 µg/ml. This indicates that endophytic fungi from parasite mango leaves have high antioxidant potential.

As for the development of future research, it is necessary to optimize the concentration range of the endophytic fungi extract used, as well as add variables by testing the growth medium of the endophytic fungi to obtain more optimum antioxidant activity in the endophytic fungi of mango parasite leaves.

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