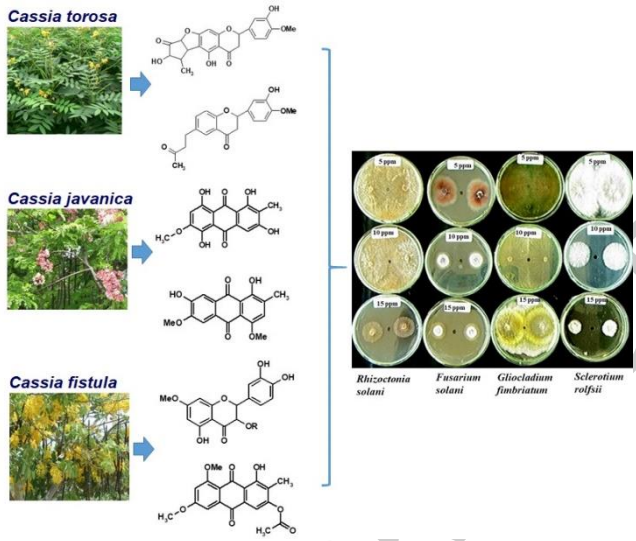
Global NEST Journal, Vol 27, No X, pp 1-12

Antifungal activity of flavonoids and anthraquinones derivatives from *Cassia* sp. against plant pathogenic fungus

Muhaimin Muhaimin[1]*, Harizon Harizon[2] and Anis Yohana Chaerunisaa[1]
[1]Department of Pharmaceutical Biology, Faculty of Pharmacy, Padjadjaran University, Sumedang 45363, Indonesia
[2]Department of Chemistry Education, Faculty of Education, University of Jambi, Jambi, 36361, Indonesia
[3]Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, Padjadjaran University, Sumedang 45363, Indonesia
Received: 06/04/2025, Accepted: 21/07/2025, Available online: 22/07/2025
*to whom all correspondence should be addressed: e-mail: muhaimin@unpad.ac.id
https://doi.org/10.30955/gnj.07529

Graphical abstract



Abstract

The Cassia plant comprises several species distributed throughout Indonesia, including *Cassia torosa*, *Cassia javanica*, and *Cassia fistula* L. Traditionally, these plants have been utilized by local communities for medicinal purposes and as biofungicides. This study explores the potential of *Cassia* species to yield bioactive compounds with strong antifungal activity. Specifically, the research aims to analyze the efficacy of flavonoid and anthraquinone compounds extracted from *Cassia* sp. against pathogenic fungi. The methodology involved isolating secondary metabolites, namely flavonoids and anthraquinones from *Cassia* sp., preparing fungal growth media, culturing pathogenic fungi, and evaluating the antifungal activity of the isolated compounds through bioassays. Identification of active compounds was carried out using spectroscopic techniques. In this study, three flavonoid compounds consist of Torosflavone C and Torosflavone D (from *Cassia torosa*) and Rhametin-3-O-Gentiobioside (from *Cassia fistula*), as well as three anthraquinone compounds consist of 1,3,5,8-tetrahydroxy-6-methoxy-2-methylanthraquinone and 1,7-dihydroxy-4,6-dimethoxy-2-methylanthraquinone (from *Cassia javanica*), and 1-hydroxy-3-ethanoate-6,8-dimethoxy-2-methylanthraquinone (from *Cassia fistula*) were successfully isolated. Antifungal assays revealed that Torosflavone D at 15 ppm exhibited the highest inhibition rate (48.75%) against *Fusarium oxysporum* f.sp. lycopersici. Meanwhile, 1-hydroxy-3-ethanoate-6,8-dimethoxy-2-methylanthraquinone at the same concentration inhibited *Rhizoctonia solani* growth by 40.20%. These findings suggest that Cassia sp. is a promising source of natural antifungal agents, which can be utilized to mitigate plant diseases caused by pathogenic fungi and support sustainable agricultural practices.

Keywords: Cassia torosa, Cassia Javanica, Cassia Fistula. L, flavonoids, anthraquinone

1. Introduction

Fungi are the leading cause of crop diseases and the resulting crop failures. Several hundred different fungal diseases of cultivated plants are known. These fungi destroy between 20 and 40 percent of the global annual harvest. The amount of food lost to fungal infections could feed between 600 million and four billion people each year (Blanchette *et al.* 1991; Duraipandiyan *et al.* 2007; Graine *et al.* 1998; Muhaimin *et al.* 2016; Pegg *et al.* 1987).

Most plant diseases caused by pathogenic fungi are responsible for major economic losses in the agricultural industry worldwide. Various fungal pathogens can infect plants and the appropriate amount of inoculum for infection is accompanied by variations in environmental conditions such as temperature, humidity, soil, water, air, and host susceptibility (Blanchette *et al.* 1991; Duraipandiyan *et al.* 2007; Aye *et al.* 2019; Belinda *et al.* 2022; Chaerunisaaa *et al.* 2020; Fiorito *et al.* 2018; Seawringht *et al.* 1985). The susceptible plant species or crop varieties may exhibit visible morphological symptoms in or on the tissues where the infection is initiated. If the

fungal pathogen can find favorable conditions for further development, systemic symptoms are induced in tissues or organs far away from the infection sites (Oka *et al.* 1993; Priyono *et al.* 2004; Verma *et al.* 2009; Lin *et al.* 2018; Muhaimin *et al.* 2020; Muhaimin *et al.* 2025). When the symptom of the infection is not expressed externally, it is termed latent infection. Some fungal pathogens infecting unripe fruits do not express any visible symptoms, as they remain dormant. Detection of fungal pathogens refers to the process of establishing the consistent presence of a particular target organism within the plant or in environments, irrespective of the development of visible symptoms in the plant suspected to be infected by the fungal pathogens in question (Oka *et al.* 1993; Priyono *et al.* 2004; Verma *et al.* 2009; Dewi *et al.* 2024; Ghosh *et al.* 1982; Indradi *et al.* 2023).

Continuous use of synthetic fungicides in addition to killing fungi has also had a negative impact on humans as users. In addition, fungicide residues cause pollution to the environment. In this regard, it has encouraged researchers to look for alternatives and development for the control of plant pathogenic fungi not from synthetic but from natural ingredients. The results of previous studies have known three species of plants from the clan of Cassia which have very interesting potential contents to study, *namely Cassia torosa, Cassia javanica* and *Cassia fistula* L (Gupta *et al.* 1989; Ismail *et al.* 2016; Kainsa *et al.* 2012; Leteane *et al.* 2012; Muhaimin *et al.* 2019; Muhaimin *et al.* 2024; Vaishnav *et al.* 1996). These three species have traditionally been used by people in rural areas as medicines, especially fungicidal pesticides. The search for the chemical content of the three species has been successfully isolated and identified as the main chemical content (Ismail *et al.* 2016; Kainsa *et al.* 2012; Leteane *et al.* 2012; Muhaimin *et al.* 2018; Morimoto *et al.* 1988; Heyne, 1987; Vaishnav *et al.* 1996).

Research on the antifungal activity of flavonoids and anthraquinones against plant pathogens has revealed promising results in various studies. Flavonoids, such as kaempferol and luteolin, exhibit significant antifungal effects by inhibiting mycelial growth and spore germination of plant pathogens such as *Botrytis cinerea* and *Fusarium* spp. Their mode of action involves disrupting the fungal cell membrane and interfering with enzymatic processes.

Anthraquinones, especially compounds such as chrysophanol and rhein, also exhibit potent antifungal properties. Studies have demonstrated their effectiveness against pathogens such as *Alternaria* spp. and *Phytophthora* spp., primarily through mechanisms that disrupt cell wall integrity and inhibit fungal growth (Ismail *et al.* 2016; Kainsa *et al.* 2012; Leteane *et al.* 2012; Muhaimin *et al.* 2019; Morimoto *et al.* 1988; Heyne, 1987; Vaishnav *et al.* 1996).

Both classes of compounds have been identified as environmentally friendly alternatives to synthetic fungicides, contributing to sustainable agriculture. Collectively, the existing literature underlines the potential of flavonoids and anthraquinones as valuable resources in developing natural antifungal agents for managing plant diseases. Further research is essential to elucidate their complete mechanisms and optimize their applications (Kainsa *et al.* 2012; Lestari *et al.* 2023; Leteane *et al.* 2012; Muhaimin *et al.* 2024; Morimoto *et al.* 1988; Heyne, 1987; Vaishnav *et al.* 1996).

The selection of *Cassia torosa*, *Cassia javanica*, and *Cassia fistula* for the study of antifungal properties against plant pathogens was based on several key factors. First, these species have demonstrated antifungal activity in previous studies, indicating their potential to combat fungal diseases in plants. Their rich chemical profiles, containing bioactive compounds such as flavonoids and phenolic acids, serve as a basis for exploring new antifungal agents. In addition, the agricultural significance of these species cannot be overlooked. Fungal pathogens pose *et al.* major threat to food security, and finding effective and sustainable solutions is essential. Utilizing these Cassia species offers opportunities for organic and sustainable agricultural practices, reducing reliance on synthetic fungicides that can damage the environment (Kainsa *et al.* 2012; Rostinawati *et al.* 2023; Leteane *et al.* 2012; Nurhasanah *et al.* 2024; Morimoto *et al.* 1988; Heyne, 1987; Vaishnav *et al.* 1996).

Furthermore, their cultural and economic relevance enhanced their selection, as these species are valued in traditional medicine and local economies. These dual benefits in promoting agricultural health and community livelihoods make them highly valuable for research. Overall, *Cassia torosa*, *Cassia javanica*, and *Cassia fistula* offer a promising focus for developing environmentally friendly antifungal treatments, contributing to the advancement of sustainable agricultural practices.

Based on its traditional use, the main chemical content and initial bioactivity test as anti fungus and literature studies on the relationship between structure and activity class of a compound can be predicted that the flavonoids and anthraquinone compounds of the Cassia genus have activeness against plant phatogenic fungi. For the development of compounds that have been found from Cassia, it is necessary to test the antifungal activity of flavonoids and anthraquinones on the following plant pathogenic fungi: *Fusarium oxysporum fsp. lycopersici* (Causes of wilting on tomatoes), *Colletotrichum capsici* (anthracnose cause in chili), *Rhizoctonia solani* (cause of disease in rice), and *Sclerotium rolfsii* (cause of disease in peanuts). Tests carried out in vitro and greenhouse scale. After knowing the activity of each of these compounds on the test fungus, it is hoped that it can be continued by making a new fungicide formula which contains the active ingredient of flavonoids or anthraquinones which are natural and do not have a large impact on the environment (Ismail *et al.* 2016; Kainsa *et al.* 2012; Leteane *et al.* 2012; Muhaimin *et al.* 2019; Morimoto *et al.* 1988; Heyne, 1987; Vaishnav *et al.* 1996).

2. Materials and methods

2.1. Materials

Plant material used were part of the stem bark *Cassia torosa, Cassia tavanica* and *Cassia fistula* L collected from

the Senami Batang Hari forest area, Jambi. The fungi used in this study were: *Fusarium oxysporum* fsp. lycopersici, *Colletotrichum capsici*, *Rhizoctonia solani*, and *Sclerotium rolfsii*.

The solvents used for extraction and chromatography were technical solvents which have been distilled, namely; n-hexane, benzene, diethyl ether, methylene chloride, acetone, ethyl acetate and methanol. The saturated solution $Ce(SO_4)_2$ 1.5% in H_2SO_4 2N and the Dragendorf reagent were used as stain appearances. Vacuum liquid chromatography with Merck 60 GF_{254} silica gel, gravity chromatography with Merck 60 silica gel (230 - 400 mesh), and compound purity analysis by thin layer chromatography on a silica gel coated plate Merck 60 GF_{254}, 0.25 mm will be carried out according to the procedure standard.

The tools used in this study were glassware commonly used in organic chemistry laboratories and microbiology laboratories, supported by other equipment such as tools for extraction, vacuum gasping sits, vacuum column chromatography and gravity column chromatography. Determination of melting point will be carried out with the Fisher John melting point apparatus, for the determination of the chemical structure from the prospective anti fungal compound requires UV-Vis and IR spectroscopy.

2.2. Methods

2.2.1. Sterilize tools and materials

The tools used in the experiment are sterilized according to the appropriate method for each tool, namely:

1. Glassware and other heating-resistant devices are sterilized by autoclaving at a temperature of 121 ^oC for 15 minutes. When finished drying it in the drying cabinet.

2. Mushroom breeding media (PDA, Czapek-Dox for Malt Extract, Komada and V-8 Juice agar), distilled water, and 1% NaOCl solution are sterilized by autoclaving at 121 ^oC for 15 minutes.

3. Aseptic work is carried out in a laminar air flow cabinet, which was previously cleaned with NaOCl solution and sterilized with the UV lamp turned on 2 hours before the cupboard is used.

2.2.2. Isolation of Flavonoid and Anthraquinone Compounds from Cassia torosa, Cassia javanica and Cassia fistula. L

A total of 10 kg of dried powder stem skin *Cassia torosa, Cassia javanica* and *Cassia fistula* L were macerated with n-hexane solvent and the pulp were macerated with 15 L of methanol for 3 x 24 hours. Then the initial methanol extract was separated for alkaloid compounds using 3% citric acid and followed by extraction using ethyl acetate. The residual part is partitioned with benzene solvents, methylene chloride, and ethyl acetate. Isolation of compounds was started from each extract through chromatographic techniques, namely liquid vacuum chromatography, gravity chromatography, chromatotron and press chromatography (Chaerunisaa *et al.* 2016; Muhaimin *et al.* 2019 and 2024).

2.3. UV-VIS Spectrum analysis

The isolate was centrifuged at 3000 rpm for 10 min and filtered through filter paper. The sample was diluted to 1:10 with the same solvent. The isolate was scanned from 190 to 1100 nm wavelength using UV-Visible Spectrophotometer (Shimadzu UV-1800) and the characteristic peaks were detected and recorded (Chaerunisaa *et al.* 2016; Muhaimin *et al.* 2016 and 2020).

2.4. Fourier Transform Infrared Spectrophotometer (FTIR) Analysis

Fourier Transform Infrared Spectrophotometer (FTIR) is a tool for identifying the types of functional groups present in compounds. The wavelength of light absorbed is characteristic of the chemical bond seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined. Dried powder of different isolate was used for FTIR analysis. 10 mg of the dried isolate powder was encapsulated in 100 mg of potassium bromide (KBr) pellet, to prepare sample discs. The thin pressed pellet was loaded in FTIR spectroscope (Agilent) and infrared spectra and peak values were recorded between 4,000–600 cm^{-1}. For each sample, 200 scans were averaged with a spectral resolution of 4 cm^{-1}. It took three minutes for a recording process. Then for a given material, a final average spectrum was calculated (Muhaimin *et al.* 2016 and 2020).

2.5. Preparation of test solution

The test solution (flavonoid and anthraquinone compounds) that would be used first was concentrated by evaporating on a water bath with temperature of no more than 50 ^oC.

2.6. Preparation of cultures and fungus inoculum

Each fungus was cultured on PDA media in petri dishes and incubated at 37 ^oC for 24 hours. Cultures of fungi that are 7 x 24 hours old are stored by culturing the appropriate media in a test tube with the media tilted. The media used for each pathogen / weathering fungus is sought in such a way as not to decrease its virulence / weathering ability.

2.7. Preparation of culture medium

Potato Dextrose Agar (PDA) Media Composition: Potatoes 200 g, Dextrose 20 g, Agarose 15 g and distilled water 1000 ml. The media was prepared and loaded into 250 ml Erlenmeyer and sterilized by autoclave at 121 ^0C (15 psi) for 15 minutes.

2.8. In vitro bioassay with perforation (well) method

Flavonoid and anthraquinone compounds, and azoxystrobin were tested for their antifungal activity on four types of plant pathogenic fungi, namely: *Fusarium oxysporum* fsp. lycopersici (Causes of wilting on tomatoes), *Colletotrichum capsici* (anthracnose cause in chili), *Rhizoctonia solani* (cause of disease in rice), and *Sclerotium rolfsii* (cause of disease in peanuts).

The sample was pure isolates which will be tested with concentrations of 1, 2.5, 5, 10 and 15 ppm. Then the

culture media in petri were made by pouring 10 ml of sterile PDA media that had been thawed (temperature 45 ^{o}C) on a sterile petri dish. Pure isolates were tested for anti-fungal activity against plant pathogenic fungi by including 20 µL into wells that had been made on culture media in sterile petri dishes, using five types of variations in concentrations of 1 ppm, 2.5 ppm, 5 ppm, 10 ppm and 15 ppm determined through orientation testing. Every test is repeated five times. Obstacles to fungus growth are seen as empty areas or zones around the well. The obstacle zone formed is measured by the calipers.

The variables observed were the diameter of the resistance zone formed around the fungus colonies, and expressed in 3 categories, namely: (1) The total obstacle zone, which is if the zone of resistance formed around the well looks clear and wide; (2) Partial obstacle zones, that is if in the zone of resistance formed still shows the existence of thin fungus colonies; (3) Zero obstacle zone, that is if there is no obstacle zone formed around the well. Observations are made every day until day 5.

To test the effect of solvents, a blank test was carried out, namely the solvent activity test entered in the well that had been made on culture media in sterile petri dishes. Then do the test in the same way as the test activity of isolates against fungus.

2.9. The perforation (well) method

The perforation (well) method for measuring antifungal activity involves making a well in the fungal growth medium, placing the isolate or compound in the well, and then measuring the zone of inhibition (a clear area where there is no fungal growth) around the well. Measurement times typically involve incubating the plate for 3-5 days at 30-35°C for fungi, followed by measuring the diameter of the zone of inhibition in millimeters.

2.10. Statistical analysis

The data obtained were statistically tested for analysis of variance in the treatment of each candidate fungicide, to determine the effect of each compound on the diameter of the fungus inhibitor. Data analysis used 2 factor factorial design in complete randomization. Further analysis with the Duncan Rare Test is intended to determine the compounds and concentrations that provide the best activity.

3. Results and discussion

3.1. Isolation, Separation and Purification of Flavonoid and Anthraquinones Compounds from Cassia sp

3.1.1. Isolation of two flavonoid compounds from Cassia torosa

As much as 10 kg of fresh ingredients of the *Cassia torosa* macerated with methanol. The results of maceration obtained concentrated methanol extract. Furthermore, the methanol extract was fractionated with solvents n-hexane, chloroform and ethyl acetate. After going through several processes of separation and purification of ethyl acetate fraction (EA fraction) two flavonoid compounds were obtained, namely Torosflavone C (45 mg) and Torosflavone D (58 mg). Torosflavone C was isolated from EA.II.2 fraction, while Torosflavone D from EA.III.1 fraction (Gupta *et al.* 1989; Ismail *et al.* 2016; Kainsa *et al.* 2012; Leteane *et al.* 2012; Muhaimin *et al.* 2019; Muhaimin *et al.* 2024; Vaishnav *et al.* 1996). Complete data for the isolated flavonoids can be seen in **Table 1** below :

Table 1. Data of melting point , UV and IR spectrum for flavonoid compounds from *Cassia torosa*

Type of Compounds	Melting point (^{o}C)		Infrared spectrum (cm^{-1})		UV spectrum (nm)	
	Research	Literature	Research	Literature	Research	Literature
Flavonoids compound (torosflavon C)	236-238	236-237	3600; 2927; 1652; 1658; 1461; 1136; 711;	3550; 2990; 1660; 1640; 1450; 1140; 715	212; 232; 250; 340; 375	212; 240; 310; 350
Flavonoids Compound (torosflavon D)	213-214	213-214	3428; 3110; 2950; 1651; 1628; 1456; 1211; 853	3500; 3050; 2975; 1650; 1610; 1450; 1200; 850	212; 250; 280; 375	215; 240; 300; 375

Furthermore, for the crystal shape and molecular structure of the compound can be seen in **Figure 1** below.

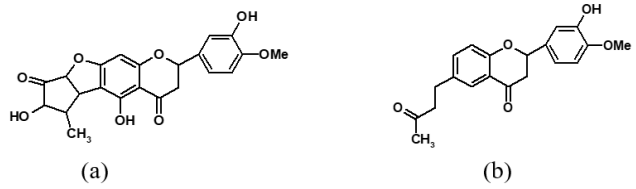


Figure 1. (a) Torosflavon C structure, and (b) Torosflavon D structure

3.1.2. Isolation of two anthraquinone compounds from Cassia javanica

As much as 10 kg of fresh ingredients of the plant *Cassia javanica* macerated with methanol. The results of maceration obtained concentrated methanol extract. Furthermore, the methanol extract was fractionated with n-hexane, dichloromethane and acetone solvents. After going through several processes of separation and purification of acetone fraction (as fraction) two anthraquinone compounds were obtained, namely 1,3,5,8-tetrahidroxy-6-methoxy-2-methylanthraquinone (36 mg) and 1,7-dihydroxy-4,6-dimethoxy-2-methylanthraquinone (67 mg). The 1,3,5,8-tetrahidroxy-6-methoxy-2-methyl anthraquinone was isolated from the As.I.2 fraction, while 1,7-dihydroxy-4,6-dimethoxy-2-methylanthraquinone from the As.II.1 fraction (Gupta *et al.* 1989; Ismail *et al.* 2016; Kainsa *et al.* 2012; Leteane *et al.* 2012; Muhaimin *et al.* 2016; Muhaimin *et al.* 2019; Vaishnav *et al.* 1996). Complete data for the isolated anthraquinone can be seen in **Table 2** below:

Table 2. Melting point, UV and IR spectrum for anthraquinone compounds from *Cassia javanica*

Type of Compounds	Melting point (°C)		Infrared spectrum (cm^{-1})		UV spectrum (nm)	
	Research	Literature	Research	Literature	Research	Literature
Anthraquinone compounds (1,3,5,8-tetrahidroxy-6-methoxy-2-methylantrakuinone)	167-169	167-168	34500; 2990; 2730; 1745; 1464; 1374; 1238; 1049; 745	3500; 3100; 2990; 1750; 1460; 1370; 1200; 1050; 750.	217; 242; 308	215; 245; 310
Anthraquinone compounds (1,7-dihidroxy-4,6-di methoxy-2-methyl anthraquinone)	201-203	201-202	3465; 2925; 2854; 1713; 1465; 1363; 1197; 1018; 723	3450; 2990; 2800; 1720; 1450; 1350; 1200; 1100; 780	212; 244; 338	215; 240; 340

Furthermore, for the crystal shape and molecular structure of the compound can be seen in **Figure 2** below.

(a) (b)

Figure 2. (a) Molecular Structure of 1,3,5,8-tetra hydroxy-6-methoxy-2-methyl anthraquinone, and (b) Molecular Structure of 1,7-dihidroxy-4,6-di methoxy-2-methyl anthraquinone

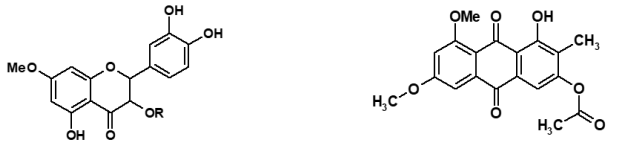
3.1.3. Isolation of flavonoids and anthraquinone compounds from Cassia fistula

As much as 10 kg of fresh ingredients of the plant *Cassia fistula* macerated with methanol. The results of maceration obtained concentrated methanol extract. Furthermore, the methanol extract was fractionated with n-hexane, acetone and ethyl acetate. After going through several processes of separation and purification of ethyl acetate fraction (EA fraction), flavonoid compounds were obtained, namely Rhametin-3-O-Gentiobiocide (46 mg). Rhametin-3-O-Gentiobioside was isolated from EA.II.1 fraction. Whereas the acetone fraction (As fraction) obtained anthraquinone compounds, namely 1-hydroxy-3-ethanoate-6,8-dimethoxy-2-methylanthraquinone (63 mg) isolated from the As.III.1 fraction (Gupta *et al.* 1989; Ismail *et al.* 2016; Kainsa *et al.* 2012; Leteane *et al.* 2012; Muhaimin *et al.* 2019 and 2020; Muhaimin *et al.* 2024; Vaishnav *et al.* 1996). Complete data for isolated flavonoids and anthraquinones can be seen in **Table 3** below :

Table 3. Melting point data, UV and IR spectrum for flavonoids and anthraquinone compounds from *Cassia fistula*

Type of Compounds Type of Compounds	Melting point (°C)		Infrared spectrum (cm^{-1})		UV spectrum (nm)	
	Melting point (°C)	Infrared spectrum (cm^{-1})	UV spectrum (nm)	Type of Compounds	Melting point (°C)	Infrared spectrum (cm^{-1})
Flavonoids compound (Rhametin-3-O-gentiobiocide)	227-229	227-228	3421; 3078; 2974; 1596; 1423; 1137; 821	3425; 3080; 2975; 1600; 1423; 1135; 820	242; 273	245; 275
Anthraquinone Compound (1-hydroxy-3-ethanoate-6,8-dimethoxy-2-methylanthraquinone)	191-192	191-192	3226; 3050; 2858; 1658; 1485; 1296; 1153; 890	3300; 3100; 2920; 1650; 1450; 1300; 1150; 870.	212; 242; 273	215; 245; 275

Furthermore, for the crystal shape and molecular structure of the compound can be seen in **Figure 3** below.



(a) (b)

Figure 3. (a) Rhametin-3-O-gentiobiocide molecular structure, and (b) 1-hydroxy-3-ethanoate-6,8-dimethoxy-2-methylanthraquinone molecular structure.

From the melting point data, UV and IR spectrum data showed that in this study had succeeded in isolating three flavonoid compounds and three pure anthraquinone compounds from *Cassia torosa*, *Cassia javanica* and *Cassia fistula*. This is reinforced by standard data, proven after the data from the above research are the melting and λmax points of the UV and IR spectrum compared to the literature data showing similarities.

3.2. In Vitro Bioassay for Plant Pathogen Fungus Activity

In this study, activity tests were carried out on four types of fungi, such as *Fusarium oxysporum fsp*. lycopersici (Causes of wilting on tomatoes), *Colletotrichum capsici* (anthracnose cause in chili), *Rhizoctonia solani* (cause of disease in rice), and *Sclerotium rolfsii* (cause of disease in peanuts). Before testing the activity of flavonoid and anthraquinone compounds on these fungi, orientation testing was first carried out to select the right method between the paper disc method and the well method, also to determine the concentration variation for testing. In orientation testing, the compounds tested are Torosflavone D and the concentrations used are 1, 2.5, 5,

10, and 15 ppm. The results show that the well method is better because the compounds tested for their activities will be more easily diffused in the media. While for good concentrations are 5, 10 and 15 ppm, because the concentrations that smaller than 5 ppm do not have the activity of inhibiting fungi, while concentrations greater than 15 ppm have endangered human health if these compounds are used in the field (Blanchette *et al.* 1991; Duraipandiyan *et al.* 2007; Aye *et al.* 2019; Belinda *et al.* 2022; Chaerunisaaa *et al.* 2020; Fiorito *et al.* 2018; Seawringht *et al.* 1985).

It should be noted that in this study the solvent used was chloroform, so before testing of flavonoid and anthraquinone compounds on plant pathogenic fungi, solvent activity testing was carried out first. It was done to find out whether the solvent has activity or not. It turned out that after testing, chloroform did not have plant anti-fungal pathogenic activity. The results of the activity test can be seen in **Figure 4** below:

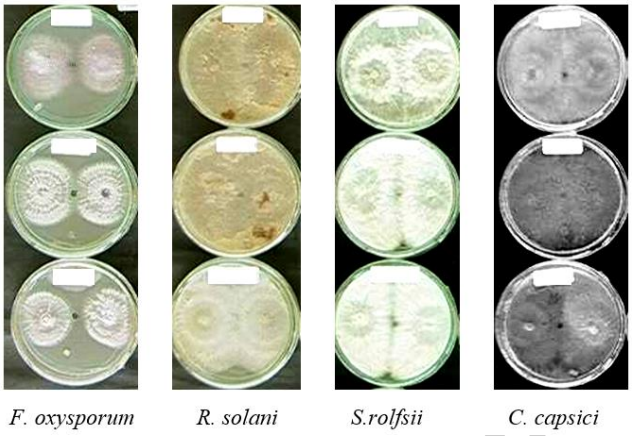


Figure 4. The testing results of anti-fungal activity, DMSO against several plant pathogenic fungi after five days of incubation

3.3. Antifungal Activity of Two Flavonoids Compounds from Cassia torosa Against Plant Pathogen Fungus

Testing the activity of Torosflavone C and Torosflavone D against *Fusarium oxysporum fsp. lycopersici*, *Colletotrichum capsici*, *Rhizoctonia solani*, and *Sclerotium rolfsii* performed by measuring the growth radius of the fungus colonies every day until the fifth day of incubation. Furthermore, the data obtained was converted into a percentage of inhibition of growth of fungus colonies.

Of the three kinds of variations in concentration (5, 10 and 15 ppm) tested on plant pathogenic fungi, it turns out Torosflavon D has the potential as a biological fungicide because it has a strong activity in inhibiting fungal growth. The results of the activity test for 5 days of incubation showed that 3 ppm Torosflavone D was able to inhibit the growth of *Fusarium oxysporum* fsp. lycopersici. As for other test fungi, Torosflavone D does not inhibit growth. Torosflavone D 5 ppm is the most effective percentage inhibition, because it can inhibit the growth of *Fusarium oxysporum* fsp. lycopersici (= 48.75%), the results of the activity test can be seen in **Figure 5** below:

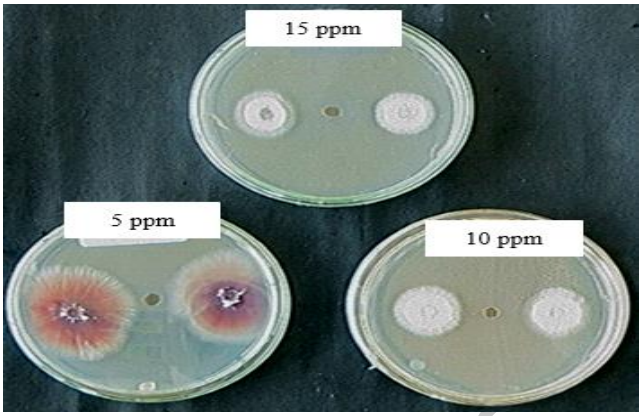


Figure 5. The results of the antifungal activity test of Torosflavone D against *Fusarium oxysporum* f.sp. lycopersici after five days of incubation

Of the three kinds of variations in concentration (5, 10 and 15 ppm) tested on plant pathogenic fungi, it turns out that Torosflavon C has no potential as a biological fungicide because it has no activity in inhibiting fungal growth. The results of the activity test for 5 days of incubation showed that Torosflavone C was not effective as a percentage of inhibition, because it could not inhibit the growth of the fungus colonies.

3.4. Antifungal Activity of Two Anthraquinone Compounds from Cassia javanica Against Plant Pathogen Fungus

Test the activity of 1,3,5,8-tetrahidroxy-6-methoxy-2-methylanthraquinone and 1,7-dihydroxy-4,6-dimethoxy-2-methylanthraquinone to *Fusarium oxysporum* fsp. *lycopersici*, *Colletotrichum capsici, Rhizoctonia solani*, and *Sclerotium rolfsii* performed by measuring the growth radius of the fungus colonies every day until the fifth day of incubation. Furthermore, the data obtained was converted into a percentage of inhibition of growth of fungus colonies. Of the three kinds of variations in concentration (5, 10 and 15 ppm) tested on plant pathogenic fungus, it was found that 1,3,5,8-tetrahidroxy-6-methoxy-2-methylanthraquinone and 1,7-dihydroxy-4,6-dimethoxy-2-methylanthraquinone has no potential as a biological fungicide because it has no activity in inhibiting fungal growth. The activity test results for 5 days of incubation showed 1,3,5,8-tetrahidroxy-6-methoxy-2-methylanthraquinone and 1,7-dihydroxy-4,6-dimethoxy-2-methylanthraquinone were very ineffective in inhibiting percentage, because it cannot inhibit the growth of fungal colonies at all (Blanchette *et al.* 1991; Duraipandiyan *et al.* 2007; Aye *et al.* 2019; Muhaimin *et al.* 2016; Chaerunisaaa *et al.* 2020; Fiorito *et al.* 2018; Seawringht *et al.* 1985).

3.5. Antifungal Activity of Flavonoids and Anthraquinone Compounds from Cassia fistula Against Plant Pathogen Fungus

Testing the activity of Rhametin-3-O-Gentiobiocide and 1-hydroxy-3-ethanoate-6,8-dimethoxy-2-methylanthraquinone to Fusarium oxysporum fsp. lycopersici, Colletotrichum capsici, Rhizoctonia solani, and Sclerotium rolfsii performed by measuring the growth radius of the fungus colonies every day until the fifth day of incubation. Furthermore, the data obtained was converted into a percentage of inhibition of growth of fungus colonies.

From three variations of concentration (5, 10 and 15 ppm) tested on plant pathogenic fungus, it turns out that 1-hydroxy-3-ethanoate-6,8-dimethoxy-2-methylanthraquin one has the potential as a biological fungicide because it has strong activity in inhibits the growth of *Rhizoctonia solani* fungi. The results of the activity test for 5 days of incubation showed that 1-hydroxy-3-ethanoate-6,8-dimethoxy-2-methylanthraquinone with a concentration of 5 ppm had been able to inhibit the growth of the *Rhizoctonia solani* colonies (Blanchette *et al.* 1991; Duraipandiyan *et al.* 2007; Aye *et al.* 2019; Muhaimin *et al.* 2016; Chaerunisaaa *et al.* 2020; Fiorito *et al.* 2018; Seawringht *et al.* 1985). Compounds of 1-hydroxy-3-ethanoate-6,8-dimethoxy-2-methylanthraquinone with a concentration of 15 ppm were the most effective inhibitory percentage, because they could inhibit the growth of radius of the fungus colonies of *Rhizoctonia solani* (= 40.20%). But the other fungi have less inhibition. While the Rhametin-3-O-Gentiobiocide compound after the activity tested did not have the power to inhibit fungal growth. So the Rhametin-3-O-Gentio compound biocide is not a fungicide candidate. The activity test results of Compounds 1-hydroxy-3-ethanoate-6,8-dimethoxy-2-met hylanthraquinone with a concentration of 15 ppm towards *Rhizoctonia solani* can be seen in **Figure 6** below:

Table 4. Percentage of growth inhibition of fungus colonies by flavonoids and anthraquinones from *Cassia* sp.

No.	Compounds	Average Percentage of Fungus Colony Growth Inhibition (r (%), n = 5)											
		R. solani			*F. oxysporum*			*C. capsici*			*S. rolfsii*		
		Concentration (ppm)			Concentration (ppm)			Concentration (ppm)			Concentration (ppm)		
		5	10	15	5	10	15	5	10	15	5	10	15
1.	Torosflavon C	0	0	1.5	0.6	2	8	0	0	0	1	2	7
		0	0	1.5	0.6	1.5	8.5	0	0	0	1	1.5	6
		0	0	1.5	0	2	7	0	0	0	1	2	6
		0	0	1	0	1.5	7	0	0	0	0.6	1.5	6.5
		0	0	1	0.6	2	7	0	0	0	0.6	2	7
	Average	0	0	1.3	0.36	1.8	7.5	0	0	0	0.84	1.8	6.5
2.	Torosflavon D	0.6	1.75	3	22	26.75	49.0	0	0	0	0	0	0
		0	1.5	4	22	27	49.0	0	0	0	0	0	0
		0	1	3.5	20	27.1	48.0	0	0	0	0	0	0
		0.6	1	3	21	26.75	48.5	0	0	0	0	0	0
		0	1.75	3	20	27	49.25	0	0	0	0	0	0
	Average	0.24	1.4	3.3	21	26.92	48.75	0	0	0	0	0	0
3.	1,3,5,8-tetrahidroxy-6-methoxy-2-methylantrakuinone	1	1	2	0	0	0	0.6	0.6	1.5	1	1	1
		1	1	1.5	0	0	0	1	1	1.5	1	1	1
		1	1	2	0	0	0	0.6	1	1	1	1	1
		1	1	1.5	0	0	0	0.6	0.6	1	1	1	1
		1	1	2	0	0	0	0.6	0.6	1.5	1	1	1
	Average	1	1	1.8	0	0	0	0.68	0.76	1.3	1	1	1
4.	1,7-dihydroxy-4,6-dimethoxy-2-methyl anthraquinone	0	0	0	0	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0	0	0	0	0
	Average	0	0	0	0	0	0	0	0	0	0	0	0
5.	Rhamnetin-3-O-Gentiobioside	0	0	0	0	0	0	1	1	1	1	1	1
		0	0	0	0	0	0	1	1	1	1	1	1
		0	0	0	0	0	0	1	1	1	1	1	1
		0	0	0	0	0	0	1	1	1	1	1	1
		0	0	0	0	0	0	1	1	1	1	1	1
	Average	0	0	0	0	0	0	1	1	1	1	1	1
6.	1-hydroxy-3-ethanoate-6,8-dimethoxy-2-methyl anthraquinone	22.5	23.5	40	0.6	1	5	0	0	0	0.6	1.75	6
		21.75	24.6	40.50	0.6	1.5	6	0	0	0	0.7	1	6.5
		22	24.25	40	0.6	1.5	5.75	0	0	0	0.6	1.5	6
		21.5	25	40	0	1	5.5	0	0	0	0.7	1	6.5
		22	25	40.50	0	1	5	0	0	0	0.6	1	6
	Average	21.95	24.47	40.2	0.36	1.2	5.45	0	0	0	0.64	1.25	6.2
7	Azoxystrobin	40.5	54.85	62.25	45.5	58.5	65.2	46.5	57.5	68	41.5	52.5	64
		40.75	55.5	62.5	44.75	57.6	65.50	45.75	58.6	67.50	41.75	51.6	63.50
		39.75	55.25	62.55	46	58.25	65.55	47	58.25	68	41	51.25	63.7
		40.5	56	64.85	45.5	59.15	65.35	46.5	59	68	42.5	50.85	64.9
		40	54.75	63.5	45	58.75	64.95	47	59	68.5	40	51	63.50
	Average	40.3	55.27	63.13	45.35	58.45	65.31	46.55	58.47	68	41.35	51.44	63.92

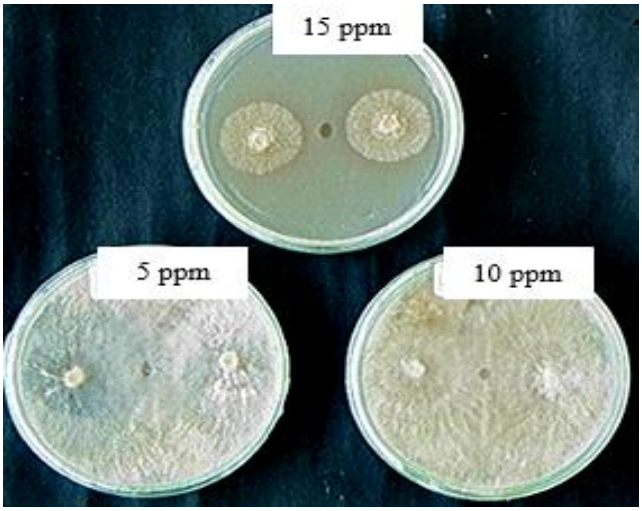


Figure 6. The results of the antifungal activity test of 1-hydroxy-3-ethanoate-6,8-dimethoxy-2-methylanthraquinone against *Rhizoctonia solani* after five days of incubation

3.6. Inhibition percentage of fungus colonies growth by flavonoid and anthraquinone compounds from Cassia sp. and its statistical analysis

Furthermore, complete data on the inhibition percentage of fungal colonies growth by flavonoids and anthraquinones from *Cassia* sp. for 5 times testing (n = 5) can be seen in **Table 4**.

From the table above, it can be seen that the 15 ppm D Torosflavone compound is the most effective inhibition percentage, because it can inhibit the growth of *Fusarium oxysporum fsp. lycopersici* (= 48.75%). Compounds of 1-hydroxy-3-ethanoate-6,8-dimethoxy-2-methylanthraquinone with a concentration of 15 ppm were also effective in inhibiting the percentage, because it could inhibit the growth the fungus colonies of *Rhizoctonia solani* (= 40.20%). While the other flavonoids and anthraquinone compounds are not effective, because they cannot inhibit the growth of the fungus colonies. According to **Table 4**, it shows and explains that azoxystrobin as a positive control (5, 10 and 15 ppm) is a highly effective fungicide known for its broad-spectrum activity against several plant pathogens. It excels in controlling major fungal threats such as *Rhizoctonia solani*, which causes various plant diseases such as root rot, damping off, and wire stem, *Fusarium oxysporum*, a common wilt pathogen, *Colletotrichum capsici*, which is responsible for late blight, and *Sclerotium rolfsii*, which cause collar rot, sclerotium wilt, stem rot, charcoal rot, seedling blight, damping-off, foot-rot, stem blight and root rot in many economically important agriculture and horticulture crops. By inhibiting mitochondrial respiration, azoxystrobin effectively reduces disease development and improves crop health and yield.

Data analysis from anti fungus activity testing was carried out using 2 factor factorial design in complete randomization. Further analysis with the Duncan Rare Test is intended to determine the compounds and concentrations that provide the best activity. Of all the results of the Anava test analysis the value of P <0.05 so that it can be concluded that the type of compound and concentration affect the inhibition percentage of growth of the fungus colonies. In other words, because Fcount (P = 0) was less than F table at the significance level α = 0.05, then the difference in compound and concentration affects the inhibition percentage of growth of the fungus colonies (At 95% confidence interval).

Further analysis with the Duncan Rare Test was intended to determine which compounds and concentrations give the best inhibitory activity of fungal colonies growth and which fungi are most sensitive to the test compounds. The conclusions obtained from the analysis are:

- Compound type factors

The activity of inhibition of growth of the best fungus colonies is given by the compound Torosflavon D.

- Concentration factor

The inhibition concentration of fungal colonies is best at 15 ppm.

- Fungus type factors

The most sensitive fungus was Fusarium oxysporum fsp. lycopersici.

- Factor interaction with the type of compound concentration

Interaction of Torosflavone D with a concentration of 15 ppm gave the best inhibitory growth activity of fungus colonies.

- Factor interaction type of compound with fungus

Torosflavone D provides the best inhibitory activity for the growth of fungus colonies in one type of fungus, *Fusarium oxysporum fsp. lycopersici*.

- Factors of fungus interaction with concentration of bioactive compounds

Fusarium oxysporum fsp*. lycopersici* was very sensitive to the use of a concentration of 15 ppm.

- Interaction of types of compounds, concentrations and types of fungi

Torosflavone D with a concentration of 15 ppm provides inhibition activity for the growth of the best fungus colonies against *Fusarium oxysporum fsp. lycopersici*.

Torosflavone D (Flavonoid) and 1-hydroxy-3-etanoate-6,8-dimethoxy-2-methylanthraquinone (Anthraquinones), two classes of compounds in plants, exhibit significant antifungal activity, which is largely due to their structural features and specific functional groups. Torosflavone D typically have a benzene ring structure with multiple hydroxyl (–OH) and carbonyl (C=O) groups. The presence of hydroxyl groups enhances their ability to chelate metal ions and interfere with cellular processes in fungi. For example, compounds such as quercetin and catechin exhibit antifungal properties by disrupting fungal cell membranes and inhibiting enzyme activity (Gupta *et al.* 1989; Ismail *et al.* 2016; Kainsa *et al.* 2012; Leteane *et al.* 2012; Muhaimin *et al.* 2019 and 2020; Muhaimin *et al.* 2024; Vaishnav *et al.* 1996). The degree of hydroxylation

can affect potency, with increased hydroxyl groups generally correlating with increased antifungal activity (Gupta *et al.* 1989; Ismail *et al.* 2016; Kainsa *et al.* 2012; Leteane *et al.* 2012; Muhaimin *et al.* 2019 and 2020; Muhaimin *et al.* 2024; Vaishnav *et al.* 1996). 1-hydroxy-3-etanoate-6,8-dimethoxy-2-methylanthraquinone, which are characterized by a three-ring structure with multiple hydroxyl and carbonyl groups, exhibit similar antifungal properties. The presence of hydroxyl groups facilitates hydrogen bonding and enhances cell entry, while carbonyl groups contribute to membrane disruption and oxidative stress in fungal cells (Gupta *et al.* 1989; Ismail *et al.* 2016; Kainsa *et al.* 2012; Leteane *et al.* 2012; Muhaimin *et al.* 2019 and 2020; Muhaimin *et al.* 2024; Vaishnav *et al.* 1996). Compounds such as emodin and chrysophanol have shown effectiveness against a variety of fungi by interfering with metabolic pathways. In short, the functional groups in Torosflavone D (Flavonoid) and 1-hydroxy-3-etanoate-6,8-dimethoxy-2-methylanthraquinone (Anthraquinones) are critical in determining their antifungal activity, affecting interactions with fungal pathogens at the cellular level.

Torosflavone D (Flavonoid) and 1-hydroxy-3-etanoate-6,8-dimethoxy-2-methylanthraquinone (Anthraquinones) exhibit antifungal activity through multiple mechanisms that target different fungal cellular processes. Torosflavone D (Flavonoid), a class of polyphenolic compounds, disrupt fungal cell membranes due to their amphiphilic nature, leading to increased permeability and cell lysis (Gupta *et al.* 1989; Ismail *et al.* 2016; Kainsa *et al.* 2012; Leteane *et al.* 2012; Muhaimin *et al.* 2019 and 2020; Muhaimin *et al.* 2024; Vaishnav *et al.* 1996). Torosflavone D (Flavonoid) also inhibit key enzymes involved in cell wall synthesis, such as chitin synthase, which is essential for maintaining cell integrity (Gupta *et al.* 1989; Ismail *et al.* 2016; Kainsa *et al.* 2012; Leteane *et al.* 2012; Muhaimin *et al.* 2019 and 2020; Muhaimin *et al.* 2024; Vaishnav *et al.* 1996). Furthermore, Torosflavone D (Flavonoid) can modulate signaling pathways in fungal cells, effectively disrupting growth and reproduction by interfering with cellular communication and hormonal regulation. 1-hydroxy-3-etanoate-6,8-dimethoxy-2-methylanthraquinone (Anthraquinones) is known to generate reactive oxygen species (ROS), which cause oxidative stress that damages cellular components, including lipids, proteins, and DNA, ultimately leading to cell death (Gupta *et al.* 1989; Ismail *et al.* 2016; Kainsa *et al.* 2012; Leteane *et al.* 2012; Muhaimin *et al.* 2019 and 2020; Muhaimin *et al.* 2024; Vaishnav *et al.* 1996). These compounds can also inhibit fungal enzymes, such as cytochrome P450 enzymes, which are essential for the biosynthesis of ergosterol, an essential component of the fungal cell membrane (Ismail *et al.* 2016; Kainsa *et al.* 2012; Leteane *et al.* 2012; Muhaimin *et al.* 2019 and 2020; Muhaimin *et al.* 2024; Vaishnav *et al.* 1996). This disruption of membrane integrity and function complements their oxidative effects, thereby enhancing their antifungal efficacy. Together, these mechanisms highlight the multifaceted approach of flavonoids and anthraquinones in combating fungal infections.

Comparative analysis of Torosflavone D (Flavonoid) and 1-hydroxy-3-etanoate-6,8-dimethoxy-2-methylanthraquinone (Anthraquinones) as antifungal agents against plant pathogens highlights important differences when compared to the commercial fungicide azoxystrobin. Azoxystrobin, a widely used strobilurin fungicide, acts by inhibiting mitochondrial respiration through interaction with the cytochrome bc1 complex, effectively disrupting energy production in fungi and exhibiting broad-spectrum efficacy against many phytopathogens, such as *Botrytis cinerea* and *Fusarium oxysporum* (Gupta *et al.* 1989; Ismail *et al.* 2016; Kainsa *et al.* 2012; Leteane *et al.* 2012; Muhaimin *et al.* 2019 and 2020; Muhaimin *et al.* 2024; Vaishnav *et al.* 1996). In contrast, flavonoids, such as quercetin and apigenin, exhibit antifungal properties through mechanisms such as disruption of cell membrane integrity and inhibition of key enzymes involved in fungal cell wall synthesis. Anthraquinones, especially emodin, exhibit antifungal activity by generating reactive oxygen species and disrupting cellular functions, thereby affecting a wide range of plant pathogens including *Alternaria* and *Sclerotinia* species (Gupta *et al.* 1989; Ismail *et al.* 2016; Kainsa *et al.* 2012; Leteane *et al.* 2012; Muhaimin *et al.* 2019; Muhaimin *et al.* 2020; Vaishnav *et al.* 1996). Although azoxystrobin is favored for its potent action against a wide range of fungi, the use of Torosflavone D (Flavonoid) and 1-hydroxy-3-etanoate-6,8-dimethoxy-2-methylanthraquinone (Anthraquinones) offers a promising alternative strategy, particularly in integrated pest management, to combat resistance and promote sustainable agriculture.

Practical applications of Torosflavone D (Flavonoid) and 1-hydroxy-3-etanoate-6,8-dimethoxy-2-methylanthraquinone (Anthraquinones) as antifungal agents against plant pathogens face several challenges, particularly related to stability and formulation development. Torosflavone D (Flavonoid), although effective, are often susceptible to photodegradation upon exposure to light, resulting in reduced antifungal efficacy (Zhang *et al.* 2021). Their water solubility is generally low, which poses additional challenges for effective application in agricultural environments. To address these issues, the development of microencapsulation or nano-emulsion techniques may be necessary to improve their stability and bioavailability (Gupta *et al.* 1989; Ismail *et al.* 2016; Kainsa *et al.* 2012; Leteane *et al.* 2012; Muhaimin *et al.* 2019 and 2020; Muhaimin *et al.* 2024; Vaishnav *et al.* 1996). 1-hydroxy-3-etanoate-6,8-dimethoxy-2-methylanthraquinone (Anthraquinones), present their own set of challenges. Although they show promising antifungal activity, their high molecular weight and hydrophobic nature may hamper formulation options. Stability under varying environmental conditions, including temperature and pH variations during storage, also present obstacles for practical applications. Furthermore, potential phytotoxic effects require careful assessment to ensure safe application in crops. Addressing these challenges through innovative formulation strategies will enhance the practical application of Torosflavone D (Flavonoid) and

1-hydroxy-3-etanoate-6,8-dimethoxy-2-methylanthraquinone (Anthraquinones), positioning them as viable alternatives or complements to traditional fungicides in crop management.

Based on the research data and explanation above, related to the antifungal activity of flavonoids and anthraquinones derivatives from *cassia* sp. against plant pathogenic fungus, there are several things and information that we can get further, such as:

3.7. Strengthening Sustainability Perspectives

The study provides valuable insights into natural antifungal agents. To enhance its relevance to sustainable agriculture, consider discussing the policy implications of replacing synthetic fungicides with plantderived alternatives. For instance, integrating environmental governance frameworks could contextualize the broader impact of this research. This study explores the potential of natural compounds—specifically flavonoids and anthraquinone derivatives extracted from *Cassia* species—to serve as environmentally friendly antifungal agents. The focus is on their effectiveness against plant disease-causing fungi, which could have significant impacts on agriculture and food security. The following are some of the benefits of bio-fungicides; Environmental benefits: Using plant-derived compounds reduces chemical residues in the environment and minimizes ecological disturbance. Using plant-derived compounds reduces chemical residues in the environment and minimizes ecological disturbance. Agricultural impact: Developing natural antifungal agents can help manage plant diseases more sustainably and potentially reduce crop losses. Scientific Contribution: Increasing the understanding of bioactive compounds from *Cassia* sp., opens the way for developing new environmentally friendly fungicides. This study highlights the promising antifungal properties of flavonoids and anthraquinone derivatives from *Cassia* species as sustainable options for controlling plant pathogenic fungi. These natural compounds can contribute to more environmentally responsible agricultural practices, supporting the broader goal of sustainable crop protection (Lei *et al.* 2024).

3.8. Cross Disciplinary Applications

The antifungal compounds' potential could be further contextualized by exploring technological integration in agricultural practices. For example, digital tools (e.g., precision agriculture) might optimize the deployment of plantderived fungicides. The study of antifungal compounds derived from natural sources such as *Cassia* sp. involves multiple disciplines. Recognizing how these compounds can be applied across multiple disciplines highlights the broad potential and innovative uses of such research, particularly in agriculture. Cross-disciplinary applications involve integrating knowledge, methods and insights from multiple scientific fields to solve complex problems or develop new technologies. In this context, this means applying findings from plant chemistry, microbiology, environmental science, plant pathology and agriculture. In the case of agriculture and plant pathology, the applications are developing natural fungicides to protect crops, reducing reliance on synthetic chemicals. Promoting sustainable agricultural practices through environmentally friendly disease control. The antifungal activity of flavonoids and anthraquinone derivatives from Cassia sp. demonstrates how natural compounds can serve multiple disciplines, particularly agriculture. Their application in agriculture can promote sustainable and innovative solutions to plant diseases (Jin *et al.* 2023).

3.9. Economic Viability

While the study emphasizes biological efficacy, a brief discussion on costbenefit analysis or market adoption challenges would strengthen its practical relevance. Linking to green financing mechanisms could provide actionable pathways. Economic feasibility assessment involves evaluating whether the development and application of these natural antifungal compounds is cost-effective, sustainable, and profitable for stakeholders such as farmers, producers, and industry (Guo *et al.* 2024). Key aspects of economic feasibility that need to be assessed are:

3.10. - Extraction and Production Costs:

Costs incurred to harvest *Cassia* sp., extract flavonoids and anthraquinone derivatives, and purify these compounds. Advances in extraction technology can reduce costs, making large-scale production possible.

3.11. - Efficacy and Dosage:

Determine the minimum effective concentration required to inhibit or kill plant pathogenic fungi. Lower effective doses mean less raw materials and lower costs.

3.12. - Comparison with Conventional Fungicides:

Evaluate whether these natural compounds are more affordable or offer better value compared to synthetic fungicides. Consider factors such as durability, shelf life, and ease of application.

3.13. - Market Demand and Acceptance:

Increasing consumer preference and regulations for organic and environmentally friendly products can increase market acceptance. Farmers' willingness to adopt new biopesticides influences economic viability.

3.14. Environmental and Regulatory Costs:

Natural compounds often face fewer regulatory hurdles and may incur lower costs associated with environmental cleanup or health safety compliance. Reduced environmental impacts can result in economic savings.

3.15. Long-Term Sustainability:

The renewable nature of *Cassia* sp. and the potential for sustainable cultivation support a sustainable supply. Avoid the development of resistant fungal strains, which can be costly, by using diverse and environmentally friendly antifungal agents.

4. Conclusions

This study highlights the significant antifungal activity of Torosflavone D (Flavonoid) and 1-hydroxy-3-etanoate-6,8-dimethoxy-2-methylanthraquinone (Anthraquinones)

derivatives derived from Cassia species against a variety of plant pathogenic fungi. Experiments show that these natural compounds effectively inhibit the growth of major phytopathogens, such as *Fusarium oxysporum* fsp. lycopersici and *Rhizoctonia solani*, making them promising candidates for sustainable agricultural practices. The underlying mechanism of this antifungal activity appears to involve disruption of fungal cell membrane integrity and interference with metabolic processes, thereby reducing pathogen viability. Furthermore, this study demonstrates the potential of these phytochemicals to serve as environmentally friendly alternatives to conventional fungicides, which often pose risks to human health and the environment. The utilization of plant-derived antifungal agents is in line with the increasing demand for sustainable and organic agricultural practices, which provide the dual benefits of enhancing crop protection while minimizing chemical residues in agricultural products. However, several challenges remain for the practical application of Torosflavone D (Flavonoid) and 1-hydroxy-3-etanoate-6,8-dimethoxy-2-methylanthraquinone (Anthraquinones) in agricultural settings. Issues such as stability, solubility, and formulation need to be addressed to enhance their effectiveness when used in the field. Future research should focus on developing innovative encapsulation or delivery systems that enhance the bioavailability and shelf life of these compounds. In addition, the safety and phytotoxicity assessment of these derivatives is essential for their integration into crop management strategies. The antifungal properties of Torosflavone D (Flavonoid) and 1-hydroxy-3-etanoate-6,8-dimethoxy-2-methylanthraquinone (Anthraquinones) from *Cassia* species provide valuable opportunities for developing new biopesticides that can contribute to more sustainable agricultural systems while effectively combating plant diseases. Antifungal compounds from *Cassia* sp. hold promise not only scientifically but also economically. For these natural antifungal agents to be a sustainable alternative in agriculture, their production must be cost-effective, marketable, and competitive with existing chemical fungicides. Careful economic evaluation helps determine whether their increased use is practical and beneficial to all stakeholders involved.

References

Abdelgaleil SAM and El-Aswad. (2005). Antifeedant and Growth inhibitory Effects of Tetranortriterpenoids Isolated from Three Meliaceous Species on the Cotton Leafworm, Spodoptera littoralis (Boisd). *Journal of Applied Science and Research* **1**(2): 234–241.

Aye M.M., Aung H.T., Sein M.M. and Armijos C. (2019). Review on the Phytochemistry, Medicinal Properties and Pharmacological Activities of 15 Selected Myanmar Medicinal Plants. *Molecules*, **24**(2): E293. DOI: 10.3390/molecules24020293.

Belinda A.M., Febriyanti R.M. and Muhaimin. (2022). Chemical and Pharmacological Properties of Selected Medical Plant Species from Genus Premna and Their Immunomodulatory Potentials. *International Journal of Applied Pharmaceutics*, **14**, Issue Special Issue 5, 24–31

Blanchette R.A. (1991). Delignification by Wood-decay Fungi. *Annual Review of Phytopathology*. **29**: 381–398.

Chaerunisaa A.Y. and Muhaimin. (2016). Comparative study on release of two drugs in fixed dose combination using zeroorder and first derivative spectrophotometry. *International Journal of PharmTech Research*, **9**(12): 581–590.

Chaerunisaaa A.Y., Susilawati Y., Muhaimin M., Milanda T., Hendriani R. and Subarnas A. (2020). Antibacterial activity and subchronic toxicity of Cassia fistula L. barks in rats. *Toxicology Reports*. **7**:649–657.

Dewi M.K., Muhaimin M., Joni I.M., Hermanto F. and Chaerunisaa A.Y. (2024). Fabrication of Phytosome with Enhanced Activity of Sonneratia alba: Formulation Modeling and in vivo Antimalarial Study. *International Journal of Nanomedicine*, **19**: 9411–9435.

Duraipandiyan V. and Ignacimuthu S. (2007). Antibacterial and antifungal activity of Cassia fistula L.: an ethnomedicinal plant. *Journal of Ethnopharmacology*. **112**(3): 590–4.

Fiorito S., Epifano F., Taddeo V.A., Genovese S. and Preziuso F. (2018). A Survey of the Anti-microbial Properties of Naturally Occurring Prenyloxyphenylpropanoids and Related Compounds. *Current Topics in Medicinal Chemistry*. **18**(24): 2097–2101. DOI: 10.2174/1568026618666181025091927.

Ghosh P., Thakur S., Iton T. and Matsumoto. (1982). Setrols from Flowers Cassia siamea Lam., Cassia sophera Linn and Cassia fistula Linn., *Indian Journal of Chemistry*, **21** B: 796–797.

Graine M. and Ahmad S. (1998). Handbook of Plant With Pest-control Properties, A Willey Interscience Publication, John Willey & Sons, New York.

Guo J, Lin O, Ye K, Guan J. and Lei X. (2024). Research on regulatory strategies of green finance and optimization of corporate green behavior under the framework of circular economy. *Global NEST Journal*. **26**(4), 05913.

Gupta V., Agrawl A., Singh and Tiwari H.P. (1989). Isolation and Characterization of Two Flavonol and a Xanthone Glycosides from the Stembark of Cassia sophera Linn, *Indian Journal of Chemistry*, **28** B: 282–284.

Heyne K. (1987). Tumbuhan Berguna Indonesia. Badan Litbang Kehutanan, Jakarta.

Ismail I.A. and Abdel-Rahman R.S. (2016). Economical evaluation of different treatments for Fig trees against long-horned Beetle, Hesperophanes griseus (Coleoptera: Cerambycidae). *International Journal of ChemTech Research*, **9**(4): 122–125.

Indradi R.B., Berliana M,I., Muhaimin M., Khatib A. (2023). Potential plant-based new antiplasmodial agent used in Papua island, Indonesia, Plants, 12(9).

Jin X, Lei X and Wu W. (2023). Can digital investment improve corporate environmental performance? -- Empirical evidence from China. *Journal of Cleaner Production*. **414**, (https://doi.org/10.1016/j.jclepro.2023.137669).

Kainsa S., Kumar P., Rani P. (2012). Pharmacological potentials of Cassia auriculata and Cassia fistula plants: A review. *Pakistan Journal of Biological Sciences*, **15**(9): 408–17.

Lei X and Zhao K. (2024). The impact of environmental protection tax policy on the financial performance of heavy pollution enterprises. *Global NEST Journal*. **26**(3), 05750.

Leteane M.M., Ngwenya B.N., Muzila M., Namushe A., Mwinga J., Musonda R., Moyo S., Mengestu Y.B., Abegaz B.M. and Andrae-Marobela K. (2012). Old plants newly discovered: Cassia sieberiana D.C. and Cassia abbreviata Oliv. Oliv. root extracts inhibit in vitro HIV-1c replication in peripheral blood

mononuclear cells (PBMCs) by different modes of action. *Journal of Ethnopharmacology*. **141**(1): 48–56. DOI: 10.1016/j.jep.2012.01.044.

Lestari U., Muhaimin M., Chaerunisaa A.Y., Sujarwo W. (2023). Anti-aging potential of plants of the Anak dalam tribe, Jambi, Indonesia. *Pharmaceuticals*, **16**(9):1300. https://doi.org/10.3390/ph16091300

Lin L., Liu Y.C., Huang J.L., Liu X.B., Qing Z.X., Zeng J.G., Liu Z.Y. (2018). Medicinal plants of the genus Macleaya (Macleaya cordata, Macleaya microcarpa): A review of their phytochemistry, pharmacology, and toxicology. *Phytotherapy Research* **32**(1): 19–48. DOI: 10.1002/ptr.5952.

Morimoto S., Nonaka G.I., Chen R.F. and Nishioka. (1988). Tannins and Related Compounds LXI. Isolation and Structures of Novel Bi and Triflanoids from the Leaves of Cassia fistula. L., *Chemical & Pharmaceutical Bulletin*, **36**(1): 39–47.

Muhaimin M., Yusnaidar Y., Syahri W., Latief M., Putri R.D., Utami A., Chaerunisaa A.Y., Aditama A.Y., Siregar J.E. (2019). Antiplasmodial Activity of Ethanolic Extract of Macaranga gigantea Leaf and Its Major Constituent, *Pharmacognosy Journal*, **11**(6): 1181–1188.

Muhaimin, Syamsurizal, Chaerunisaa A.Y., Sinaga M.S. (2016). Eusiderin I from Eusideroxylon zwagery as antifungal agent against plant pathogenic fungus. *International Journal of ChemTech Research*, **9**(5): 418–424.

Muhaimin, Yusnaidar, Syahri W., Latief M., Utami A., Bemis R., Amanda H., Heriyanti, Chaerunisaa A.Y. (2018). Screening and Potential Analysis of Methanolic Leaf Extract of Mangrove Plants at East Coast Sumatera as Repellent against Aedes aegypti. *Journal of Pharmaceutical Sciences and Research*, **10**(9): 2228–2231.

Muhaimin M., Syamsurizal S., Latief M., Iskandar R., Chaerunisaa A.Y., Mujahidin D. (2020). Synthesis of 7,3'-Epoxy-8,4'-Oxyneolignane-1'-Carboxylic Acid from Natural Eusiderin A and its Activity Against Trichophyton mentagrophytes. *Current Organocatalysis*, **7**(1): 44–54. https://doi.org/10.2174/2213337206666190730144041.

Muhaimin M., Latief M., Putri R.D., Chaerunisaa A.Y., Aditama A.Y., Pravitasari N.E., Siregar J.E. (2019). Antiplasmodial Activity of Methanolic Leaf Extract of Mangrove Plants against Plasmodium berghei, *Pharmacognosy Journal*, **11**(5): 929–935.

Muhaimin M., Hazrina A., Hirzan R., Iskandar Y., Khatib A. (2024). Antioxidant Activity of Premna serratifolia Linn. Leaf Extracts: A Comprehensive Analysis Using Various Testing Methods, *Indonesian Food Science and Technology Journal*, **8**(1).

Muhaimin M., Nor L., Chaerunisaa A.Y., Subarnas A., Susilawati Y. and Hirzan R. (2024) Antiplasmodial Activity of Ethanol Extract of Sonneratia alba Leaves. *Tropical Journal of Natural Product Research*. **8**(4): 6884-6890.

Muhaimin M., Lestari U., Hirzan R. and Chaerunisaa A.Y. (2025). The potential of medicinal plants in tuberculosis treatment: Indigenous plants used by the Anak Dalam tribe of Jambi, Indonesia. *South African Journal of Botany*, **180**(4): 688–709.

Nurhasanah S., Iskandar Y., Muhaimin M., Hazrina A., Hirzan R. and Syahri W. (2024). Polymer type effect on Premna serratifolia extract-loaded microparticles preparation by solvent evaporation method with single emulsion system. *International Journal of Applied Pharmaceutics*, **16**(4):250–256.

Oka I.N. (1993). Usage, Problems and Prospects of Vegetable Pesticides in Integrated Pest Management, *Proceeding: Research Result Seminar in the Use of Vegetable Pesticides*, 1–9.

Pegg G.F. (1987). Infection of Plants. Cambridge University Press, Cambridge.

Priyono D., Adnan A.M. (2004). Pengujian Pestisida Berbahan Aktif Majemuk (Bahan Pelatihan), Pusat Kajian Pengendalian Hama Terpadu. Departemen HPT IPB.

Rostinawati T., Muhaimin M., Chaerunisaa A.Y., Hazrina A. (2023). Development of casticin-loaded ethyl cellulose microparticles by solvent evaporation method with single emulsion system. *International Journal of Applied Pharmaceutics*, **15**(6): 235–240.

Seawringht A.A., Hegarty M.P., James L.F. and Keeler R.F. (1985). Plant Toxicology, *Dominion Press-Hedges & Bell*, Melbourne, 395–398.

Vaishnav M.M., Triphati A.K. and Gupta K.R. (1996). Contituents of Cassia sophera Linn roots, Fitoterapi, LXVII: 93–94.

Verma V.C., Kharwar R.N. and Strobel G.A. (2009). Chemical and functional diversity of natural products from plant associated endophytic fungi. *Natural Product Communications*. **4**(11): 1511–32.