Evaluation of Efficacy of Green Chiretta Leaf Extracts Against Anthracnose Disease

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Abstract. Plant secondary metabolites are synthesized from primary metabolites and play a role in plant defense against pathogens. Several secondary metabolite compounds isolated from several types of plants have biological activities, such as being cytotoxic against fungi and bacteria. Bitter plants— green chiretta [*Andrographis paniculata* (Burm. f.) Wall. ex Nees.] are plants that contain flavonoid compounds. Flavonoid compounds can damage the fungal cell wall, which causes cell denaturation in the fungus. Anthracnose is caused by the fungus *Colletotrichum gloesporioides* (Penz.) Penz. & Sacc is a post-harvest disease that is often found in various tropical and subtropical fruits, which can reduce production and can even lead to crop failure. The study was conducted to determine secondary metabolite compounds of green chiretta leaves to suppress the phytopathogen *C. gloesporioides*, which causes anthracnose disease. The research used a completely randomized design and was repeated four times with the treatment extracts of green chiretta leaf with concentrations of 5 %, 10 %, 15 %, 20 %, 25 %, and 30 %. The results showed that the extract from green chiretta leaves could suppress *C. gloesporioides* by more than 70 % at 8 d after inoculation with a concentration of 5 % to 30 %.

Keywords: *Andrographis paniculata*, *Colletotrichum gloesporioides*, environmentally friendly, natural fungicide, phytochemicals.

1 Introduction

Plant secondary metabolites are synthesized from primary metabolites and play a role in plant defense against pathogens. Secondary metabolites contain bioactive compounds, including: flavonoids, steroids, phenol hydroquinone, and tannins which can be useful as

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anti-microbial ingredients [1–4]. Several secondary metabolites alkaloids, terpenoids, flavonoids, steroids isolated from several plants have biological activities such as being cytotoxic against cancer cells, inhibiting the release of histamine, anti-inflammatory, anti-fungal and anti-bacterial [5–10]. Green chiretta [*Andrographis paniculata* (Burm. f.) Wall. ex Nees.] was a seasonal plant that has high adaptability to its growing environment. This plant contains anti-fungal compounds including flavonoid compounds [9–16]. The results showed that the total flavonoid content of the ethanol soluble extract from bitter herb—green chiretta was 4.64 % ± 0.05 % [4].

Secondary metabolite compounds produced by plants, namely: carotinoids, phytosterins, saponins, phenolic compounds, alkaloids, glycosinate and terpenes have great potential as a source of fungicides to control post-harvest pathogens [2, 4]. *Colletotrichum* ssp. be a facultative parasitic fungus as the main pathogen causing anthracnose [17, 18]. Furthermore, it was argued that *Colletotrichum* ssp. not only attacks the fruit but also attacks the leaves, flowers, twigs, and seedlings. *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. can develop and spread well in a temperature range of 23 °C to 25 °C [19, 20]. The research objective was to determine the content of green chiretta leaves extracted so that it was able to suppress anthracnose phytopathogens.

2 Material and methods

The research was carried out in the agrotechnology laboratory of the Faculty of Agricultuture and Animal Science and pharmacy laboratory, University of Muhammadiyah Malang from February to May 2020. The concentration test of the secondary metabolite extract from green chiretta leaves used a completely randomized design, and was repeated four times. Follow by testing the phytochemical content of the leaf extract. The treatment concentrations were: 5 %, 10 %, 15 %, 20 %, 25 %, and 30 %. The extraction method uses a combination of methods [21, 22].

2.1 Sample preparation

Samples were taken from fresh bitter leaf, washed, drained, dried, and cut into small pieces, blended until smooth and filtered using a 65 meshs sieve. Extraction using the maceration method, namely 100 g of crushed bitter leaf samples soaked in 500 mL ethanol 96 % for 2 × 24 h then filtered. The residue was soaked again with 250 mL 96 % ethanols, shaken and stored for 2 × 24 h then filtered. The filtrate from the first and second maceration is mixed and evaporated, then ovens at a temperature of 40 °C to 50 °C until a thick extract is obtained. The thick extract was used for toxicity tests of the fungal pathogen *C. gloesporioides* and for analysis of secondary metabolites.

2.2 Toxicity test

Preparation of stock solution, namely extract from the sample of green chiretta leaves fresh as much as 200 mg dissolved to 100 mL with water, then diluted according to the concentration treatment, namely: 5 %, 10 %, 15 %, 20 %, 25 %, and 30 %.

2.3 Green chiretta leaf phytochemichal screening

2.3.1 Tannin test

Amount of 1 mL of bitter leaf extracts plus a few drop of 10 % Fe_3Cl solution. If there is a color change in the solution, namely dark blue or green black, it indicates that there is a tannin content [23, 6, 7].

2.3.2 Alkaloid test

Amount of 2 mL of extract plus 10 mL of 0.05 M ammonia chloroform, stirred and filtered. The filtrate is collected in a test tube plus 0.5 mL 10^{-1} drops of 2 N sulfuric acid, shaken and allowed to form two layers. Take a layer of sulfuric acid and put it in a test tube, adding one drop of Mayer reagent [7].

2.3.3 Flavonoid test

Amount of 2 mL of the extract was boiled with 25 mL of ethanol for 25 min and filtered. Amount of 5 mL of filtrate plus 0.05 mg of magnesium powder and 1 mL of concentrated HCl, shaken then left to stand until two layers of chloroform (bottom) and water (top) are formed. Amount of 2 mL of the extract was boiled with 25 mL of ethanol for 25 min and filtered. Amount of 5 mL of filtrate plus 0.05 mg of magnesium powder and 1 mL of concentrated HCl, shaken then left to stand until two layers of chloroform (bottom) and water (top) are formed [6, 7].

2.3.4 Saponin test

Amount of 5 g of bitter leaves extract sample was boiled in 100 mL waters for 5 min then filtered. Amount of 10 mL of the solution was shaken vertically in a test tube for 10 s. The presence of saponin content is indicated by the presence of foam that is stable between 1 cm to 10 cm and does not disappear by administering one drop of 2 N HCl [23].

3 Results and discussion

3.1 Result

3.1.1 Phytochemical tests for green chiretta leaves extract

The results of the qualitative phytochemical test of green chiretta leaves extract from several compounds, such as: tannin compounds, saponin compounds, flavonoid compounds and alkaloid compounds, the results are presented in Table 1.

Table 1. Qualitative test results from tannin compounds, saponin compounds, flavonoid compounds, and alkaloid compounds.

Compound	Description	The result
Tannins	There is no sediment	Negative
Saponins	Produces a blue green, red purple, or light-yellow color	Positive
Flavonoids	Produces a bright red color	Positive
Alkaloids	Produce sediment	Positive

In Table 1 it appears that the results of the phytochemical test of green chiretta leaves extract to contain saponin compounds, flavonoid compounds, and alkaloid compounds, but do not contain tannin compounds. This shows that the secondary metabolites contained in the extract from bitter leaves are saponins, flavonoids and alkaloids.

The presence of saponin compounds in the phytochemical test is shown at the end of the reaction to produce a red purple color. The results of the phytochemical test of flavonoid compounds produce a red color where according to Roeswitawati *et al.* [1], Ikhwan *et al.* [3], Baud *et al.* [6], Lanisthi *et al.* [7] that the addition of magnesium powder and hydrochloric acid to the flavonoid test will reduce the existing flavonoid compounds, causing a red reaction which is a characteristic of flavonoids.

3.1.2 *Toxicity tests for secondary metabolites of green chiretta leaves suppressed the pathogen* C. gloesporioides *(%)*

The results of the analysis of variance showed that there was a significant difference between the treatment of bitter leaves extract from suppressing the growth of the pathogen *C. gloesporioides* in vitro compared to without treatment of bitter leaves extract. Treatment of the concentration of bitter leaves extracts tested for the pathogen *C. gloesporioides* did not show a significant difference.

The test results from the ability of secondary metabolites of bitter leaves to inhibit the development of the pathogenic fungus *C. gloesporioides* in vitro is presented in Figure 1.

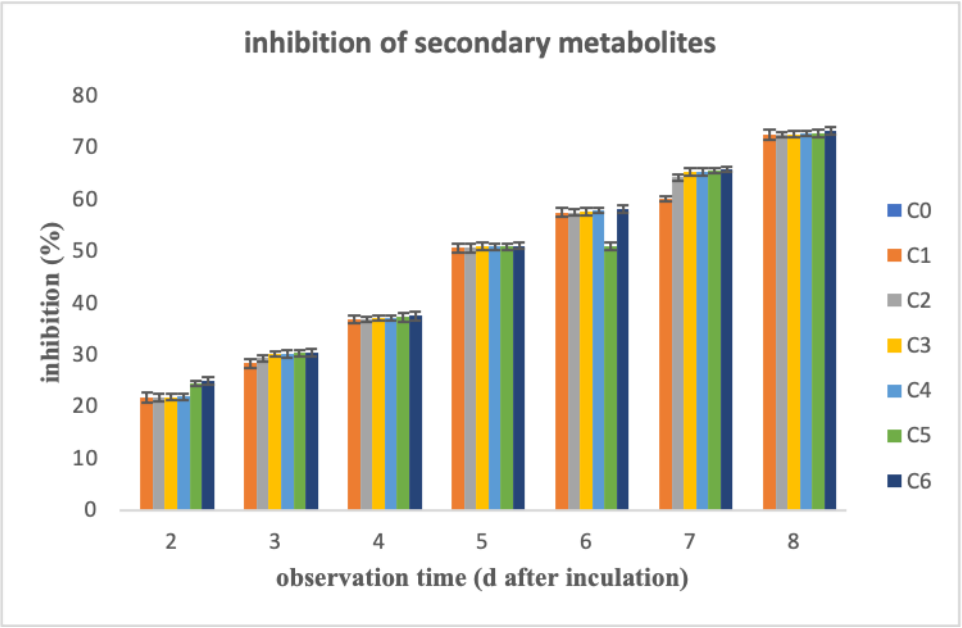

Fig. 1. The ability of secondary metabolites of bitter leaves to inhibit the growth of the pathogenic fungus *C. gloesporioides* in vitro (%).

Note: C_0: concentration 5 %; C_1: concentration 5 %; C_2: concentration 10 %; C_3: concentration 15 %; C_4: concentration 20 %; C_5: concentration 25 %; C_6: concentration 30 %.

In Figure 1, it appears that secondary metabolites of green chiretta leaves are able to suppress the growth of the pathogenic fungus *C. gloesporioides*. The tested concentration treatment did not show any significant difference, such as: C_1: concentration 5 %; C_2: concentration 10 %; C_3: concentration 15 %; C_4: concentration 20 %; C_5: concentration 25 %; C_6: concentration 30 %. While the C_0 treatment was unable to suppress the development of *C. gloesporioides* fungi, which was shown by an inhibition value of 0 %.

The toxicity of the secondary metabolites of green chiretta leaves can suppress the pathogen *C. gloesporioides* up to 72 % at 8 d after inoculation with a concentration on 5 % to 30 %.

3.2 Discussion

The qualitative test results of green chiretta leaves extract showed that the secondary metabolites contained in the leaf extract were saponins, alkaloid compounds and flavonoid compounds. The results of the alkaloid phytochemical test showed positive results, namely by the formation of sediment. The deposition of alkaloid compounds is caused by Dragendorff and Mayer reagents in which the nitrogen atom has a lone pair with Mayer's reagent, then a white solution is obtained [1, 3, 5–7]. If there are white deposits, it indicates alkaloid content [24–26]. Alkaloids when tested using Dragendorff's reagent will produce an orange sediment, but when used Mayer reagent it will produce a yellowish white precipitate [27, 6, 21]. The results of the phytochemical test of flavonoid compounds produce a red color where according to Roeswitawati *et al.* [1], Ikhwan *et al.* [3], Baud *et al.* [6], Lanisthi *et al.* [7] that the addition of magnesium powder and hydrochloric acid to the flavonoid test will reduce the existing flavonoid compounds, causing a red reaction which is a characteristic of flavonoids. The content of saponin compounds where saponin compounds in the phytochemical test produce a red purple color. According to the opinion Ikhwan *et al.* [3], Baud *et al.* [6], Iqrar *et al.* [26] that the secondary metabolite extracted after being given the reagent produces a blue, purple, or light-yellow green color.

The results of the analysis of variance showed that the presence of these compounds was able to suppress the pathogenic fungus *C. gloesporioides* compared to the treatment of bitter leaves extract. As with the results of the in vitro toxicity test of the laboratory, the extract from bitter leaves is able to suppress the growth of the pathogenic fungus *C. gloesporioides* by up to 72 % at a concentration of 5 % to 30 %. The inhibition power is due to the presence of saponin compounds, alkaloids and flavonoids contained in secondary metabolites of bitter leaves. Saponins form complex compounds with sterols, which are enzymes that make up the cell walls of fungi, so that saponin activity causes a loss of permeability of the cell wall of the pathogenic fungus *C. gloesporioides*. Likewise, flavonoid compounds can damage the fungal cell walls and cause denaturation of these walls. Some secondary metabolites, particularly alkaloids, are toxic to prokaryotic and eukaryotic cells. For example, berberine, is a benzylisoquinoline alkaloid produced in some plant species that inhibits DNA and protein biosynthesis in bacteria and functions as a chemical protectant against bacteria. Secondary metabolite compounds are alkaloids, flavonoids, saponins, tannins, steroids and terpenoids [2, 6]. The results of research Baud *et al.* [6] show that from methanol and chloroform extracts, plant twigs (*Euphorbia firucalli* L.) can empirically be used as anti-bacterial *Bacillus subtilis* (Ehrenberg 1835), *Staphylococcus aureus* (Rosenbach 1884), *Echerichia coli* (Migula 1895) and *Pseudomonas aeruginosa* (Schröter 1872) and have the potential as a pesticide. The compounds contained in methanol extract are flavonoids, tannins and saponins [7, 23, 27]. Most of the phenolic components, such as alkaloids and flavonoids, have strong biological activity as a component of the defense function as herbivores as pathogens [28].

Plant secondary metabolites are often referred to as components that have a number of basic roles in the maintenance of plant life processes. But what is important to these plants is to interact with their environment, namely adaptation and defense. However, the meaning of plant secondary metabolites is crucial to having a role as plant growth and development [10, 22, 26, 28]. Plant secondary metabolites such as alkaloids, phenolics, terpenoids play an important role in antagonistic activity against pathogens due to the presence of toxins and provide protection against environmental stresses [29, 30]. On the other hand, plant secondary metabolites function as attractants, such as color and aroma pigments, as well as

repellants [31, 32]. Secondary metabolites also contribute to specific colors, odors, and tastes in plants. Secondary metabolites are also a source of food additives, pharmaceuticals, and unique flavors [33–41].

4 Conclusion

Green chirettar leaf extracted to contain secondary metabolites of saponins, alkaloids and flavonoids which can suppress the growth of the pathogenic fungus *C. gloesporioides* which causes anthracnose disease by 72 % in vitro for 8 d after inoculation.

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