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The Anticoccidial Property of Sambiloto (*Andrographis paniculata*) Leaf Extract

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Abstract

Andrographis paniculata, known as sambiloto, is a plant widely used in herbal medicine in the community. The most commonly used bitter plant is the leaves. Leaf-boiled water has a deep, dark color and bitter taste. As an herbal plant, sambiloto has various benefits, such as antimalarial, antidiarrheal, antipyretic, and anti-inflammatory properties. Andrographolide is a typical compound found in Sambiloto extract. In addition, other compounds can be found in the quote, namely flavonoids, alkaloids, tannins, saponins, and steroids. Several studies have discussed the potential of these five compounds to overcome coccidial infections in poultry. *Eimeria tenella* infection in poultry occurs quickly in the field, and its impact is detrimental. Chickens become stunted and unhealthy. Therefore, this study aimed to determine the potential of sambiloto extract with water and 80% ethanol against *Eimeria tenella* infection. In addition, this study also aimed to determine the phytochemical content of Sambiloto extract in water and 80% ethanol. Andrographolide was detected using UPLC. A UV-vis spectrophotometer was used to test for flavonoids, tannins, alkaloids, saponins, and phenols, and densitometry TLC was used to test the total steroids in the extracts. It concluded that both extract contain flavonoids, tannins, alkaloids, saponins, phenols, and andrographolide, with ethanolic extract contains higher andrographolide and has steroid compound.

Keywords: Andrographis paniculata; andrographolide; *Eimeria tenella*; extract

Introduction

Andrographis paniculata is a Latin name for sambiloto. According to taxonomy, Sambiloto is classified into the Angiospermae division and Dicotyledoneae class because it has seeds with double plates. This plant belongs to the sub-class Gamopetalae, order Personales, family Acanthaceae, sub-family Acanthoidae, genus Andrographis, and Andrographis paniculata Nees (Anonymous 2000). This plant grows in hot areas in tropical and subtropical climates, such as Asia, India, Sri Lanka, Java, Pakistan, Indonesia, and Malaysia (Yunita, 2021). Sambiloto is an herbal plant that has a bitter taste. The leaves are shaped like lancets and have a plant height of 40-90 cm (Nyeem et al., 2017). Sambiloto has flowers in small tubes, growing from the tips of stems with a purple-white color. The fruit is in the form of an oblong capsule, 1.5 cm long, 0.5 cm wide. Small flat brown seeds. Sambiloto can be propagated using seeds or stem cuttings (Yuniarti, 2008).

All parts of the sambiloto, including flowers and fruits, can be used as medicines. However, the parts most often used as ingredients in traditional medicine are leaves and stems. The leaves, stems, flowers, and roots of sambiloto taste very bitter when eaten directly or boiled to drink. The people

of Indonesia have empirically used Sambiloto as traditional medicine. Sambiloto has several properties, including antimalarial, antidiarrheal, antidiabetic, anti-inflammatory, analgesic, antimicrobial, antipyretic, antithrombotic, and antidote properties for snake bites (Geetha et al., 2017; Cahyawati, 2021).

There are several main components of the diterpene lactones in Sambiloto which were identified in the leaves, namely andrographolide, neoandrographolide, deoxyandrographolide (Kumoro and Hasan 2006), deoxyandrographolide-19-β-D-glucose and dehydroandrographolide (Patarapanich et al. 2007). In addition to these main components, there are other compounds, including saponins, flavonoids, alkaloids, and tannins. Other chemical constituents in the leaves and stems are lactones, paniculin, kalmegin, and yellow crystals, which taste bitter (Yusron and Januwati, 2004; Sudarmi, et al. 2018). Several methods can be used to qualitatively and quantitatively measure the content of andrographolide and other compounds in extracts, including thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), spectrophotometry, ultraviolet spectrophotometry, and volumetric and colorimetric techniques (Aromdee et al. 2005; Mishra et al. 2007; Royani et al. 2014). This study aimed to determine the phytochemical profile of sambiloto extract obtained from farmers in the Kediri area, East Java, and to determine its potential for infection with Eimeria sp.

Material and Methods

Time and location

This research was conducted at the Pharmacology Laboratory, Department of Pharmacology, FKH UGM, and UGM Integrated Research and Testing Laboratory (LPPT).

Extraction

Sambiloto leaves used in the manufacture of extracts in this study were obtained from the Kediri area of East Java. The one-year-old bitter leaf was harvested and made into Simplicia powder. The Sambiloto simplicia powder was then placed in a maceration container, distilled water was added as a solvent for the water extract, and 80% ethanol was added to the ethanol extract at a ratio of 1:10. The maceration was performed with stirring. Every day for 3 × 24 h, the solvent was replaced, and the filtrate was stored in a different place. The remaining dregs of filtration were added to the solvent again, and the filtration stage was repeated. The resulting filtrate was then filtered using 0.2 µm PTFE. The filtered samples were then evaporated using a rotary evaporator until the final fixed weight was obtained.

Phytochemical Test

Phytochemical tests on water-distilled sambiloto extract were carried out to test the content of total andrographolid, total flavonoids, tannins, alkaloids, saponins, phenols, and steroids. Testing for total andrographolid was performed using ultra-performance liquid chromatography (UPLC). The tests were conducted at the Integrated Research and Testing Laboratory (LPPT) of Gadjah Mada University. The method used in this test was a UV-vis spectrophotometer for flavonoids, tannins, alkaloids, saponins, phenols, and TLC densitometry for total steroid testing.

The andrographolid content in the extract was measured using ultra-performance liquid chromatography (UPLC). Andrographolid was detected using the mobile phase of 0.5 formic acid in water: methanol: acetonitrile with a ratio of 43:40:17. The wavelength was set at 254 nm.

Quercetin total flavonoid equivalent measurement using the UV-vis spectrophotometer method was carried out by dissolving the sample in 5% sodium nitrite, 10% aluminum nitrate, and 1 M sodium hydroxide with a delay of 5 min each. Then, the solution was diluted 10x and the absorbance was read at λ510 nm.

The total tannin equivalent Tannic Acid was measured using the UV-Vis Spectrophotometer method by extracting the sample with diethyl ether for 20 h and filtering. The remaining diethyl ether was evaporated. Aquadest was added to the sample until the volume reached 10 ml. One milliliter of sample solution was added to Folin-Ciocalteu reagent and vortexed, and 20% sodium carbonate was added and vortexed. Distilled water was added until the volume reached 10 ml, and the mixture was incubated for 30 min at room temperature before reading the absorbance at λ760 nm.

Quinine equivalent total alkaloids were measured using the UV-vis spectrophotometer by adding 2N HCL to the sample and homogenizing it. The solution was then washed with chloroform 3x with a separatory funnel, and the chloroform phase was discarded. The solution was neutralized using 0.1 N NaOH, and 5 ml of BCG solution and phosphate buffer were added. Subsequently, the solution was extracted with chloroform, homogenized using a magnetic stirrer, repeated twice 2x, and evaporated with chloroform (5 ml of added) and the absorbance at λ470 nm.

Total saponins were measured using the UV-Vis spectrophotometer by adding 25% H2SO4 and autoclaving for 120 min at 110 °C. The plant was extracted with ether and dried. Distilled water was added, and the mixture was vortexed for 5 min. Then, they were anisaldehyde, shaken, and allowed to stand for 10 min. Then, 50% sulfuric acid was added, and the mixture was heated in a water bath at 60 °C for 10 min. Water was then added to a volume of 10 ml in a measuring flask and diluted by 5x. Absorbance was read at λ435 nm.

The total phenol equivalent of gallic acid was tested by adding Follin Ciocalteus phenol reagent H2SO4 and distilled water, followed by shaking and allowing it to stand for 10 min. Then, 20% sodium carbonate was added, and the mixture was shaken and allowed to stand for 10 min. Distilled water and dilute to 50x. Read the absorbance at λ760 nm.

Total steroids were tested using the densitometry thin-layer chromatography (TLC) method by adding ethanol to the sample that was placed in a microtube. The mixture was then vortexed for 30 s and sonicated for 2 min. The mixture was macerated for 24 hours at room temperature. The sample was then centrifuged, and the supernatant was collected on a 60 F254 silica gel plate as the stationary phase, including a comparator, namely β-sitosterol. Subsequently, it was placed in the chamber with a combined mobile phase with a Toluene: Ethyl Acetate ratio of 80:20. Expand to limit, remove, and dry. The mixture was heated at 110 °C for 2 min and densitometry TLC read the absorbance.

Result and Discussion

Phytochemical tests were carried out to detect the content of andrographolid, flavonoids, phenols, tannins, alkaloids, saponins, and steroids in the Sambiloto extract. Sambiloto leaves, used as raw material for the extract, were harvested from farmers in the Kediri area, East Java, with plant age of at least 1 year after harvest. The test results are presented in Table 1.

Royani et al. (2014) stated that the andrographolide content in Sambiloto plants in Java varies depending on the location where the plants are planted. His research found that the average andrographolid content in Java was 2.19% for the sambiloto extract with 80% ethanol-water dilution. Mishra (2007) stated that the andrographolid content for the water-distilled graboto extract was 0.29%. The ethanol extract of Sambiloto from the results of this study showed much higher results compared to the literature, whereas the extract with water as the solvent showed equivalent results. Research has found that andrographolide can kill filarial worms effectively in dogs, preventing obstruction of the lymphatic system. The ethanol extract of Sambiloto showed antibacterial and antifungal

Tabel 1. Phytochemical result of aqueous and 80% ethanolic sambiloto extract

No.	Testing Parameter	result	
		Aqueous extract	Ethanolic extract
1.	Andrographolide total	0,3% b/b	5,72% b/b
2.	Flavonoid total	1,17% b/b	0,82% b/b
3.	Phenol total equivalent Gallat Acid	7,21% b/b	7,02% b/b
4.	Tannin total equivalent tannic acid	8,57% b/b	8,55% b/b
5.	Alkaloid total equivalent Quinine	0,17% b/b	0,4% b/b
6.	Saponin total from Quillaja bark	1,53% b/b	1,34% b/b
7.	Steroid equivalent Beta Sitosterol	undetected*	0,28% b/b

*detection limit is 0,026 ppm

activities compared to standard antibiotics (Singha, et al. 2003). Andrographolide is also one of the three diterpenoid compounds in bitter plant extracts, inhibiting platelet aggregation in vitro (Thisoda, et al. 2003). Intragastric administration of sambiloto extract with 80% ethanol extract can stimulate antibody production and inhibit allergic reactions in sheep red blood cells (Puri, 1993; Nyeem, et al. 2017). The andrograpolid contained in Sambiloto can work directly through lymphocyte cells to produce interferon to increase the phagocytic activity response by macrophage cells, thus inhibiting the multiplication process of *E. tenella* (Sheeja & Kutan, 2007). Sambiloto plants also contain compounds capable of producing interferon-gamma cytokines and can induce macrophage cells, thereby increasing their ability to kill coccidia (Wang et al. 2010).

Das, et al. (2009) stated that phytochemical analysis showed that Sambiloto extracted using water showed higher levels of flavonoids than ethanol extract. Sambiloto extract also contained flavonoids. Flavonoids can induce oxidative stress in cells. Oxidative stress is known to cause an imbalance between oxidants and antioxidant compounds in the host, and is frequently observed in various microbial and parasitic infections, including coccidia (Allen, 1997; Muthamilselvan, et al. 2016; Zhang, et al. 2012). Oxidative stress triggers cellular damage, which is the basis of several physiological and pathophysiological events. It plays a role in various processes such as inflammation, aging, carcinogenesis, defense against protozoa, drug action, and toxicity (Sies, 2000). Table 1. shows that the levels of flavonoids in the water-extracted sambiloto extract were lower than those in the sambiloto with ethanol extraction. Flavonoids observed in phytochemical tests on sambiloto extract indicate the potential of flavonoids to interfere with the life cycle of *Eimeria sp*. by the mechanism of oxidative stress.

The other compound found in this test was total phenols. Phenol levels in water extracts of Sambiloto are known to be lower than those in ethanol extracts (Das et al., 2009). Phenol was the second dominant ingredient in the Sambiloto extract, with either water or 80% ethanol extraction. Several studies have documented the potential for inhibition due to phenolic compounds in plant extracts. Natural phenolic components obtained from medicinal plants have been reported to inhibit the invasion of sporozoites E. tenella in vitro (Arlette et al., 2019). Flavonoids and phenols exhibit anti-inflammatory and antioxidant activities (Gadelhaq et al., 2018).

The sambiloto extract had the highest tannin content. Sambiloto extract with water and 80% ethanol extract had a higher tannin content than the other phytochemical compounds. Tannins have been shown to inhibit the sporulation *of E. tenella, E. maxima*, and *E. acervulinna* oocysts. Tannins can actively penetrate the oocyst cell wall, damage the cytoplasm, and inactivate endogenous enzymes that play a role in the sporulation process (Molan, et al. 2009; Muthamilselvan, et al. 2016). Tannins can also increase humoral immune activity to fight coccidia infection in chickens (Muthamilselvan et al. 2016).

Saponins can bind sterol molecules on the surface of the oocyst cell membrane, allowing them to lyse the oocyst (Hassan et al. 2008). Felici, et al (2018) stated that saponins interact with cholesterol on the sporozoite cell membrane so that they can interfere with the life cycle of Eimeria tenella. The detection of saponin levels in the extract indicated the potential of Sambiloto extracts to disrupt the life cycle of *Eimeria tenella*.

Alkaloids can also be found in the bitter extract. Alkaloids are commonly found in herbal plants and are widely known to have a significant role as antioxidants in the body. Together with phenols, alkaloids can help increase body weight and improve feed conversion ratio (FCR) efficiency. In addition, alkaloids and phenols could reduce the cecum lesion score and the amount of oocyst excretion in chickens with coccidiosis (Dakpogan et al. 2019). This study shows that sambiloto extract has the potential to be effective against coccidia infection. Various compounds detected in sambiloto extract can have a remedial effect on the cecum of chickens with coccidiosis.

Conclusion

Sambiloto extract has potential as an anticoccidial because it contains andrographolide, flavonoids, phenols, tannins, alkaloids, saponins in Sambiloto extract with water extraction, and additional steroids that are detected in Sambiloto

extract with 80% ethanol extraction. The ethanol extract contains higher andrographolide than water extract.

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