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RESEARCH ARTICLE

Assessment of the phytochemicals and anthelminthic activities of *Adiantum raddianum* and *Kibatalia arborea* extracts

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Abstract

Background: A limited spectrum and inappropriate use of anthelmintics lead to anthelmintic resistance. Among Indonesian medicinal plants, *Adiantum raddianum* (Suplir) and *Kibatalia arborea* (Jelutong Pipit) have shown antihelmintic properties. **Objective:** To reveal the anthelmintic activity as preliminary screening of *A. raddianum* and *K. arborea* leaves. **Method:** This study used the antihelmintic microdilution test towards methanol extracts at 500 µg/mL. The mortality of *Caenorhabditis elegans* expresses the presence of anthelmintic activity. Phytochemical screening was determined using thin-layer chromatography. **Result:** The methanol extracts of both extracts had anthelmintic activity with mortality values of 14.941% and 15.134%, respectively. Both extracts contain polyphenols, flavonoids, terpenoids, and alkaloids found only in *K. arborea*. **Conclusion:** This study reveals that the methanol extract of *A. raddianum* and *K. arborea* leaves have anthelmintic activity, which could be related to the findings of phytochemical constituents.

Introduction

Worm infections are infectious diseases caused by parasitic worms that cause various diseases globally. Worm infections are associated with tropical climatic conditions, population density, personal hygiene, and environmental sanitation (Kurscheid *et al*., 2020). Anthelmintic agents are generally used to treat and control offing and controlling helminth infections. Most anthelmintics work with a limited spectrum, where inappropriate and excessive use can lead to resistance (Holden-Dye & Walker, 2007). One way to overcome the problem of synthetic drug resistance is to look for new anthelmintic agents from medicinal plants. In Indonesia, 90 medicinal plants have been used traditionally for worm therapy. Among these medicinal plants, the leaves of *Adiantum raddianum* and *Kibatalia arborea* can be potential sources for exploring their anthelmintic potential.

Suplir (*Adiantum raddianum* C. Presl.) is a fern from the family Pteridaceae that is reported to have the potential as antimicrobial, antinociceptive, anti-hyperplastic, antidiuretic, and antioxidant (Reinaldo *et al*., 2018; Souza *et al*., 2009). Previous study shows that the Soxhlet extract of Suplir leaves contained flavonoids and phenol (Thomas, 2014). Jelutong pipit (*Kibatalia arborea* (Blume) G. Don.) belongs to the family Apocynaceae and is used as an antihelmintic empirically (Eisai Indonesia, 1986). Previous studies found that the maceration extract of the leaves contained alkaloids, flavonoids, polyphenols, and terpenoids (Chotimah *et al.*, 2020).

No previous studies have reported the antihelmintic activity of the leaf extracts of either plant. Thus, in this study, preliminary phytochemical screening based on ultrasonic extraction and anthelmintic test on the leaves of *A. raddianum* and *K. arborea* will be carried out against *Caenorhabditis elegans* as a model organism.

Methods

Preparation of plant extract

Simplicia leaves of *Adiantum raddianum* C. Presl and *Kibatalia arborea* (Blume.) Don was obtained from the Technical Unit Laboratory Herbal Materia Medica Batu, Malang, Indonesia. Each leaf simplicia powder (1g) was soaked in methanol (10mL) and extracted using ultrasonication (ELMA) for 30 minutes. The mixture was filtered and dried at room temperature, then the w/w yield of each sample was calculated.

Preliminary phytochemical screening

Phytochemical screening was carried out qualitatively using Thin Layer Chromatography (TLC) (MERCK) described in Harborne (1973). The dried extract of each sample (1 mg) was dissolved in 200µL methanol and then applied to silica gel TLC plate F_{254} (5x3cm). The TLC plate was eluted in the mobile phase of ethyl acetate:methanol:water (4.5:1:1) (technical grade) to identify the phytochemical class of alkaloids, polyphenols, flavonoids, and terpenoids using specific reagents. Orange spots will appear on the alkaloids when sprayed by Dragendorff (MERCK). The sample may contain polyphenols when it showed black spots when sprayed with $FeCl_3$ (MERCK). Flavonoids show a yellow spot when exposed to ammonia vapour (MERCK). Terpenoids will be reddish purple when sprayed with Vanillin-H_2SO_4 (MERCK) and then heated at 105ºC.

Anthelmintic assay

The procedure for maintaining worms and preparing food sources is based on the standard protocol by Stiernagle (2006). Testing of anthelmintic activity refers to the procedure by Thomsen (2012) and Nugraha (2020) with some modifications.

Preparation of control and extract solution

Dimethyl sulfoxide (MERCK) was used as the negative control solution. DMSO was dissolved in M9 buffer to obtain a 16% solution. The M9 buffer was obtained referring to the protocol by Stiernagle (2006). The positive control was Ivermectin (SIGMA) 80 µg/mL, dissolved by 16% DMSO in M9 buffer. The extract solutions at 4000 µg/mL were prepared by dissolving 4mg of dried methanol extract in 1mL of 16% DMSO. The control and extract solutions must be ensured to be completely dissolved.

Preparation of worm suspension

Four days old *C. elegans* was rinsed with 2mL of M9 buffer at 20˚C. It was pipetted all the worms suspension into a 15mL centrifuge tube (BIOLOGIX) and centrifuged at 800 G/rcf for 1 minute. The supernatant was discarded and a 2mL M9 buffer was added and centrifuged again. After discarding the second supernatant, resuspended the pellet with 2mL of M9 buffer.

Antihelmintic microdilution test

The test was carried out using a 384-well clear round bottom polypropylene microplate (THERMO FISHER) with an incubation time of 30 minutes three times. Each test was replicated five times for each treatment. The solutions in each well included 15µL of M9 buffer, 20µL of worm suspension, and 5µL of control and fresh extract solutions. Pipetted the entire solution in each well and placed it on an object glass to be checked under the microscope. Dead worms are characterised by their body moving stiffly or not moving. Count the number of live and dead worms in each well by slowly sliding the object glass until all solution parts are observed under the microscope. Each well must be rinsed with an M9 buffer to ensure no worms are left behind.

Data analysis

Anthelmintic activity was represented as the percentage of worm mortality calculated using the formula: mortality percentage = (number of dead worms/total worms) x 100%, then the standard deviation (SD) and the coefficient of variation (CV) were calculated. Data were analysed using One Way Anova followed by the Least Significant Difference (LSD) test using SPSS software to determine which groups had a significant difference with a significance value of $p < 0.05$ and 95% confidence level.

Results

Extraction

Ultrasonic extraction showed that the yield of methanol extract of Suplir leaf was 11.65%, and of Jelutong Pipit leaf was 19.13%.

Preliminary phytochemical screening

The results of qualitative phytochemical screening can be seen in Table I. Identification of phytochemical classes showed the presence of polyphenols, flavonoids, and terpenoids in both extracts. At the same time, alkaloids were found in the methanol extract of *K. arborea* leaves.

Anthelmintic activity

The results of the anthelmintic activity for the methanol extract of Suplir and Jelutong Pipit can be seen in Table II. Both extracts showed anthelmintic activity against *C. elegans*. The percentage of mortality in the methanol extract of *K. arborea* was higher than that of *A. raddianum*. However, statistically, the mortality percentage of the two samples was not significantly different, indicated by the significance value of $p > 0.05$.

Table I: Identification of phytochemical class

Tests	*Adiantum raddianum* leaf	*Kibatalia arborea* leaf
Alkaloid	-	+
Polyphenols	+	+
Flavonoid	+	+
Terpenoids	+	+

* (+) =Present, (-) =Absent

Table II: The percentage of worm's mortality

Variable	Worms mortality rate (%) ± SD (n=3)	CV (%)
DMSO 2%	0 ± 0^{a}	0
Ivermectin 10 µg/mL	100 ± 0^{b}	0
Methanol extract of *A. raddianum* leaf (500 µg/mL)	14.941 ± 2.059^{c}	13.781
Methanol extract of *K. arborea* leaf (500 µg/mL)	15.134 ± 1.993^{c}	13.166

* (a, b, c) = The same letter notation indicates insignificant differences ($p > 0.05$)

Discussion

In this study, the yield percentage obtained from the ultrasonication extraction gave a higher yield than Suplir leaf with Soxhlet extraction, as reported in previous studies, which had 5.1% (Thomas, 2014), and Jelutong Pipit macerated methanol extract yielded 6.402% (Chotimah *et al*., 2020). These results indicate that extraction using methanol solvent with ultrasonication method on *A. raddianum* and *K. arborea* leaves was more efficient than previous methods. The intensity of ultrasonic waves disrupts intramolecular forces. It forms cavitation, which plays a role in the mass transfer rate from plant cells into the solvent, making the extraction process more efficient (Gupta *et al*., 2012).

The leaves of *A. raddianum* and *K. arborea* were documented to have been used in traditional medicine to treat worm infections. There are no recent studies on anthelmintic activity, but several plants from the same family have been reported previously. Plants from the same family tend to contain the same metabolites and are also likely to have anthelmintic activity (Jayanti & Ersam, 2018).

The results of this study indicated that the two extracts had anthelmintic activity against the worm model *Caenorhabditis elegans* at 500 µg/mL concentration (Table II). This activity is based on worm mortality, represented as the mortality percentage. The single concentration of the samples in this study is intended only as an initial screening for the presence of anthelmintic activity that the extract can provide to the worm model. The antihelmintic activity of both extracts was not significantly different based on a one-way ANOVA statistical test with *p*-values more than 0.05 ($p > 0.05$).

Based on the previous study of the Pteridaceae family by Rajesh and colleagues (Rajesh *et al*., 2015), an in vitro test on the ethanol extract of *Pityrogramma calomelanos* against *Haemonchus contortus* at 100 mg/mL caused mortality. These plants contain terpenoids, tannins, phenols, and steroids. In another study on the Apocynaceae family, the methanol extract of *Tabernaemontana divaricata* leaves against *Pheretima posthuma* gave mortality within 146 minutes at 20 mg/mL in the presence of alkaloids, phenols, flavonoids, tannins and terpenoids which are thought to provide antihelmintic activity (Radhika & Vilasini, 2016).

Referring to previous studies on plants of the same family, the anthelmintic activity of the methanol extract of Suplir and Jelutong pipit leaves in this study was presumably due to the presence of compounds detected on phytochemical screening (Table I). The

phytochemical constituents obtained from this study have also been reported to provide anthelmintic activity. Several studies have revealed the role and mechanism of these phytochemical constituents in anthelmintic activity (Rajesh *et al.*, 2015; Cavalcante *et al*., 2020). Alkaloids exert anthelmintic activity by acting on the central nervous system, interfering swith nitrate production and leading to homeostatic disturbances and worm paralysis. Polyphenols interfere with energy production in parasitic worms' by releasing oxidative phosphorylation (Salhan *et al*., 2011). Flavonoids denature worms protein and cause death (Ulya *et al*., 2014). One of the polyphenolic compounds, such as tannins, binds to proteins and influences development towards the infective larval stage. Terpenoids cause decreased absorption of nutrients in worms, resulting in hemolysis and death (Jackson & Miller, 2006).

Conclusion

Methanol extract of *A. raddianum* and *K. arborea* leaves at 500 µg/mL has anthelmintic activity against *C. elegans*. The antihelmintic activity is supposed to come from the identified phytochemical content of the two extracts obtained by ultrasonic extraction. The effectiveness category of the samples could not be determined because there were still no criteria governing the effectiveness of plant extract. Further research, such as optimisation of effective doses or isolation of specific compounds, is necessary to ensure the anthelmintic potential of both plants.

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