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Inhibition of Spore Germination of *Phakopsora pachyrhizi* Using Crude Extracts of *Amaranthus spinosus*

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Abstract

The effectiveness of methanolic and *n*-hexane crude extracts of *Amaranthus spinosus* to inhibit the spore germination of *Phakopsora pachyrhizi,* a causal agent of soybean rust disease was studied. Both methanolic and *n*-hexane crude extracts inhibited spore germination at concentrations of 0.1% to 5.0%. Methanolic extract of the roots at a concentration of 2.5% inhibited 55% of spore germination, and this result was similar when higher concentration at 5% of methanolic and *n*-hexane extracts of the flowers was used. The presence of alkaloids, flavonoids, tannins, saponins, and terpenoids in the extract may be responsible for the inhibition. The extract was potential to be utilized as a botanical fungicide.

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Keywords: Botanical fungicide, *Amaranthus spinosus*, soybean rust disease, *Phakopsora pachyrhizi,* spore germination

Introduction

Soybean rust disease caused by *Phakopsora pachyrhizi* Syd.& P.Syd. is one of the most destructive foliar diseases of soybean in soybean-growing area of the world. Yield losses from 10% to 80% have been reported in the tropical and subtropical areas including Asia, Africa, and America, especially when environment conditions support disease development [1-5]. Severe infected crops defoliate and mature rapidly than healthy crops. Fungicides are effective in controlling the disease, however, their application is not a cost-effective way and not considered environmental friendly [3,4]. Application of natural fungicides extracted from plant materials can be used as an alternative control of this disease.

Amaranthus spinosus L. is well known as an invasive weed. Amaranthus is adopted from the Greek “amarantos” which means “unfading”, also known as spiny amaranth, spiny pigweed, and prickly amaranth [6]. This fast growing weed is difficult to be eradicated from the environment. However, potential benefits of this weed extract have been reported such as antimalarial properties,

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hepatoprotective and antioxidant activity [7,8]. The extract also poses antibacterial properties to both strains of gram-positive and gram-negative bacteria, along with the yeasts and dermatophytes. The weed especially flower extract contains secondary metabolites including flavonoids, coumarins, terpenoids, tannins, saponins, steroids, cardiac glycosides, and iridoids [6,9].

Antimicrobial activities of Amaranthus extracts to inhibit the gram-positive of *Staphylococcus aureus* and *Bacillus* spp., the gram-negative of *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosae*, *Proteus mirabilis*, and *Klebsiella pneumonia* were reported by Maiyo *et al*. [9]. However, a pathogenic fungus *Candida albicans* was resistant to the leave extract. Methanolic extract of this weed inhibited fungal growth of *Fusarium* sp., *Aspergillus* sp., and *Alternaria* sp. [6]. Recently, Yusnawan [10] reported that methanolic root extract of spiny amaranth collected from East Java, Indonesia inhibited more than 50% of spore germination of *Puccinia arachidis*, the causal agent of peanut rust disease. However, the antifungal activity of this weed extract originated from Indonesia to inhibit spore germination of *P. pachyrhizi* has not been conducted yet. Therefore, this study aimed to determine the effectiveness of roots, leaves and flowers of spiny amaranth extracted with methanol and *n*-hexane to inhibit the spore germination of *P. pachyrhizi*.

Materials and Methods

Fresh whole plant was collected from Kendalpayak research station, Malang, East Java,Indonesia. The plant was washed thoroughly with tap water to remove adhering materials. Roots, leaves and flowers were separated and dried. The dried samples were ground to form fine particles. Moisture content of each sample was measured before extraction. The samples were stored in air-tight containers before used.

Two different polarities of solvents, i.e. methanol and *n*-hexane were used to extract active ingredients of the roots, leaves, and flowers. Maceration of each sample was conducted for 18 hours after shaking the suspension on an orbital shaker at 100 rpm for 4 hours. The supernatant was concentrated with a vacuum rotary evaporator. The concentrated extracts were weighed and made up to a known volume. The extracts were stored at 4^{o}C in the dark for further use [10].

The activity of the extracts to inhibit spore germination was conducted in a three-factor of completely randomized design. Polarity of the solvents, bioparts of the weed (roots, leaves, and flowers) and four concentrations (0.1%, 1%, 2.5%, and 5%) were the first, second, and third factors, respectively. Infected leaves containing mature urediospores of *P. pachyrhizi* were harvested from crops grown in a greenhouse. The infected leaves were incubated in petridishes for two days. The humidity at around 95% was maintained inside the petridishes by placing wet cotton layers. Spores were collected and suspended in sterile water. Inhibition of spore germination was conducted using the extracts. Phosphate buffer was used as a control [10].

The methanolic and hexane extracts were subjected to phytochemical screening to determine the presence of secondary metabolites, i.e. alkaloids, flavonoids, tannins, saponins, and terpenoids. Specific colour reaction after being reacted with respective reagents was recorded. Mayer and Wagner reagents, Mg and HCl, $FeCl_3$ and gelatin, Liebermann-Burchard reagent and H_2SO_4 were used to detect alkaloids, flavonoids, tannins, and terpenoids [11,12].

Secondary metabolites in the extracts were separated using silica gel plates. Mobile phases to separate alkaloids, flavonoids, saponins, and terpenoids were methanol:chloroform (0.5:9.5 v/v), chloroform:methanol (9:1 v/v), *n*-hexane:acetone (4:1 v/v), and *n*-hexane:ethyl acetate (2:8 v/v) [12,13,14]. The extracts (5 µl) were spotted on the plates and eluted using the suitable mobile phases.