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RESEARCH ARTICLE

In vitro antiplasmodial and toxicological activities of *Vittaria anguste-elongata* Hayata extracts

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Abstract

Background: *The Vittaria anguste-elongata* Hayata fern is a common species that grows on the trunks of palm trees. On the other hand, the phytochemical and pharmacological activities of this specie have not been investigated to a great extent. **Objective:** In this study, we reported the invitro antiplasmodial and toxicological activities from a variety of this specie's extract. **Method:** Methanol was used to extract the aerial part of the specie, and then a liquid-liquid extraction was done using *n*-hexane, dichloromethane, and ethyl acetate). Assays for antiplasmodial activity and toxicity were carried out with two different strains of *Plasmodium falciparum* (3D7 and W2), as well as the brine shrimp lethality test. **Result:** The antiplasmodial activities demonstrated that the dichloromethane extract had the most potential antiplasmodial activities among the extracts with an IC₅₀ value of 13.65±0.06 µg/mL. On the other hand, the toxicological activities performed on *Artemia salina* demonstrated that *n*-hexane, dichloromethane, and ethyl acetate were all classified as toxic. **Conclusion:** These findings provide a foundation for future research into isolating and analysing the biological activity of secondary metabolites found in the extract.

Introduction

Ferns (Polypodiophyta) play a crucial role in both human and forest ecosystems. In forest ecosystems, they play a role in humus formation and protect the soil from erosion, while in human life, they have the potential as vegetables, handicrafts, ornamental plants, and traditional medicine ingredients (Cao *et al.*, 2017). Studies of the phytochemistry of these plants indicate that they have pharmacological properties. It has been reported to possess pharmacological activity, including anti-inflammatory, antinocyseptic, antibacterial, and antimalarial properties (Szypuła *et al.*, 2021). According to Odediran and colleagues, *Nephrolepis biserrata* and *N. undulata* extracts from Nigeria are effective against chloroquine-sensitive *Plasmodium berghei* in mice (Odediran *et al.*, 2022). According to the ethnopharmacological data collected and analysed by Nugraha and colleagues in their research component, 58 species of ferns, including *Vittaria anguste-elongata* hayata, have been used to treat various diseases, including skin diseases (Nugraha *et al.*, 2020).

V. anguste-elongata Hayata is one of the ferns that has not been extensively studied but is widely distributed in Indonesia and can be used for therapeutic materials and knee pain treatment (Sekar *et al.*, 2011). Furthermore, Sathish and colleagues found that this plant has the potential to be antimicrobial. Moreover, the species exhibited cytotoxicity against two human cancer cell lines, lung carcinoma (NCI-H460) and central nervous system carcinoma (SF-268) (Wu *et al.*, 2005; Sathish *et al.*, 2014).

In this study, the extraction of the species with varying polarity is described. In addition, we described the novel anti-plasmodium activity and toxicity of the extracts. The molecular chemotypes formerly derived from this specie were also discussed in relation to the purported pharmacological properties of the plant.

Methods

V. anguste-elongata Hayata was collected from the trunk of an oil palm tree in the Riau province, Indonesia. This plant was identified by the Head of the Botany laboratory at the department of Biology, Riau University. The specie's aerial parts were dredged in the open air, away from direct sunlight. A total of 3kg of *V. elongata* was macerated by soaking in methanol for 72 hours, and the extract was filtered every 24 hours within 72 hours. The sample was sonicated for one hour before filtering. The macerate was collected, and the solvent was evaporated using a rotary evaporator to produce a viscous methanol extract. The viscous methanol extract was partitioned with n-hexane and then with dichloromethane (DCM), ethyl, and water to obtain n-hexane, DCM, ethyl acetate, and water extracts.

In vitro anti-plasmodial assays

Various concentrations of the extracts were used to assess the anti-plasmodium activity against chloroquine-sensitive (3D7) and chloroquine-resistant (W2) strains of *Plasmodium falciparum*. In Hendra's earlier study, this experiment was done in triplicate (Hendra *et al.*, 2021; Hendra *et al.*, 2022).

Toxicity assay

The Brine Shrimp Lethality test (BSLT) was utilised to determine the extracts' toxicity level. The assay was conducted thrice, and the data were reported as

mean, and standard deviation. By plotting the median mortality percentage against the log of concentration, we were able to determine the concentration (IC₅₀) at which fifty per cent of the population died directly as a result of exposure to the extracts (Hendra *et al.*, 2021; Hendra *et al.*, 2022).

Results

In vitro evaluations were performed to determine the antiplasmodial activity against chloroquine-sensitive 3D7 and chloroquine-resistant W2 strains of *P. falciparum*, as well as the toxicity by using the brine shrimp lethality analysis. At a concentration of 100µg/mL, the dichloromethane extract inhibited the growth of *P. falciparum* by 66.11±0.08%. Whereas the n-hexane, ethyl acetate, and water extracts showed inhibition of less than 50%. Each of the four extracts exhibited activity against *P. falciparum* in a dose-dependent manner. The IC₅₀ value for the extracts was found to be 0.0056±0.0011 µg/mL, which indicates that they had a lower activity level compared to artemisinin. In addition, the extracts were subjected to a brine shrimp lethality test in order to determine their level of toxicity, and the results showed that the extracts exhibited a wide range of toxicity levels, as seen in Table I. The extract of n-hexane showed the highest level of toxicity, followed by dichloromethane, ethyl acetate, and water.

Table I: *In vitro* Anti-plasmodial activity and toxicity of *V. anguste-elongata* Hayata extracts

Sample	Anti-plasmodia (IC ₅₀ ; µg/mL)	Toxicity (LC ₅₀ ; µg/mL)
n-Hexane extract	>100	62.47±0.4
Dichloromethane extract	13.65±0.06	163.87±0.2
Ethyl acetate extract	>100	839.20±0.3
Water extract	>100	>1000
Artemisinin (Control)	0.0056 ± 0.0011	-

Discussion

In this study, the methanol extract was subjected to a solvent-solvent partition using a modified Kupchan partition. The Kuphan partition is a simple and effective way to begin purification. This is done by separating the total methanol extract from four large (non-polar to polar) extracts, which are simplified mixes of different polarities using only partitions made from non-miscellaneous solvents. Extracts of compounds with different polarities were analysed for their antiplasmodia and toxicity properties, suggesting that there may be significant differences in distribution between the extracts (Hendra *et al.*,

2020). It was also discovered that the four extracts had varying degrees of activity against both chloroquine-sensitive 3D7 and chloroquine-resistant W2 strains of *P. falciparum* (Table I). Based on their IC₅₀ values, the extracts' activities were classified as either highly active (IC₅₀ < 5µg/ml), promising (5µg/ml < IC₅₀ < 15µg/ml), moderate (15 µg/ml < IC₅₀ < 50µg/ml), or poorly active (IC₅₀ >50g/ml) in accordance with the World Health Organisation (WHO) guidelines and the fundamental requirements of antiparasitic drug discovery (Fidock *et al.*, 2004). Since the n-hexane, ethyl acetate, and water extracts were rated as having low antiplasmodial activity, the

dichloromethane extract was deemed to have high potential. This might be due to presence of phytochemicals within the extract. However, the phytochemical study of this specie has not yet been intensively studied. Wu and colleagues reported that there are 32 compounds from dichloromethane and ethyl acetate extracts, including vittrerin (dihydrostilbenoids), apigenin, and amentoflavone (Wu *et al.*, 2005). Apigenin inhibited *P. berghei* growth in mice by 69.74% at concentrations of 70mg/kg/day in a dose-dependent manner, with significant suppression of parasitemia (Amiri *et al.*, 2018). In addition, amentoflavone isolated from the leaves of *Allanblackia monticola* was reported to inhibit *P. falciparum* FcM29 with IC₅₀ value of 54.2µM (Azebaze *et al.*, 2007).

In order to determine the overall level of the extracts' toxicity, a lethality test was performed on brine shrimp using the extracts. The analysis is an uncomplicated and highly efficient method for determining the potential toxicity of bioactive substances or extracts. The ability for test samples to kill shrimps, a relatively straightforward zoological organism, is the foundation of this theory (*Artemia salina*). The LC₅₀ value for the plant extracts was found to be less than 1000µg/ml, as stated by Meyer and the colleagues. On the other hand, the LC₅₀ value for the plant extracts was found to be less than 30µg/ml for the shrimp, indicating that the plant extracts were extremely toxic for the shrimp (Meyer *et al.*, 1982). The findings demonstrated varying degrees of toxicity across all of the extracts (Table I). According to the findings, n-hexane, dichloromethane, and ethyl acetate are all considered to be toxic, whereas water extract was found to be non-toxic. There was a significant link between the toxicity of BSLT and its ability to kill cancer cells, specifically those of the nine KB cell line (human nasopharyngeal carcinoma), as well as other tumours and *in vivo* murine leukaemia. The BSLT is able to identify compounds with strong anticancer activity; however, its predictive ability to differentiate between compounds with strong, moderate, and weak anticancer activity is limited. As a result, the BSLT is capable of conducting a speedy screening for powerful cytotoxins and achieving a higher level of cancer discrimination. This study found that the methanol extract from this species possessed cytotoxicity against gastric and nasopharynx carcinoma cell lines, which is in agreement with a report that had been published previously (Wu *et al.*, 2005; Karimi *et al.*, 2010).

Conclusion

The findings of this research project have shown that an extract of *V. anguste-elongata* Hayata possesses potentially useful anti-plasmodial activity. The antiplasmodial activity of the dichloromethane extract was found to be significant. These findings provide a foundation for additional research into isolating secondary metabolites found in the extract and analysing their biological activity.

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