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Research Article

Study of Secondary Metabolites of Azolla Microphylla as Candidates for Aedes Aegypti Mosquito Repellents

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Abstract

Dengue fever (DHF) is a disease that becomes an epidemic in an area every year. Prevention of the spread of DHF has been widely carried out as well as for vector bites. The use of repellent as one way to protect humans from mosquito bites. Alternative to the use of chemicals through the use of plants to repel mosquitoes need to be introduced. Mosquito repellents using plant-based ingredients have the advantage of being easily decomposed in nature. The aim of this study is to determine the content of secondary metabolites that can be used as mosquito repellents through multilevel extraction and to test the effectiveness of secondary metabolite content on the repellent power of Aedes aegypti mosquitoes. This study is an experimental study using 3 types of solvents with different levels of polarity. The results of the study obtained were positive for flavonoids with ethyl acetate solvent, positive for tepenoids and tannins with ethanol solvent. The positive compounds from the Azolla extract have the potential to repel mosquitoes.

Keywords: azolla microphylla, secondary metabolites, aedes aegypti.

INTRODUCTION

Dengue fever (DHF) is a disease caused by the bite of the Aedes aegypti mosquito and is one of the biggest health problems in Indonesia. DHF is caused by a virus that has 4 common serotypes (DEN-1, DEN-2, DEN-3, DEN-4) and until now there has been no effective vaccine to provide protection.(Sembiring & BT Hasan, 2020). Preventive measures against the

spread of dengue fever have been widely implemented as well as to prevent vector bites from dengue fever. The use of repellents as one way to protect humans from mosquito bites is widely implemented, but most commercial mosquito repellents contain chemicals such as N, N-diethyl-metatoluamide (DEET), Allethrin N, N-diethyl mendelic acid amide and dimethyl phthalate. These chemical mosquito repellents have been identified as unsafe for health and must be used with caution because of the effects they cause, such as allergies, dermatitis, and cardiovascular and neurological side effects. The use of chemical mosquito repellents can also have a negative impact on the natural ecosystem, causing resistance and adverse effects on non-target organisms.(Asadollahi et al., 2019).

Alternative use of natural materials as a substitute for chemicals to repel or repel mosquitoes needs to be introduced. Mosquito repellents use plant-based materials so that they are easily decomposed (bio-degradable) in nature, do not pollute the environment and are relatively safe for humans and livestock, because residues (remaining substances) are easily lost. Some plants in certain parts such as leaves, flowers, seeds, stems, rhizomes or tubers have natural insecticide content(Aseptianova et al., 2017).

Several studies have been conducted on several plants that have the potential to be mosquito repellents, such as mint leaves, galangal tubers, sambiloto, babadotan, avocado leaves, bay leaves, red shoots, zodia leaves.(Aseptianova et al., 2017), lemongrass(Vitaningtyas et al., 2019), betel leaf(Sembiring & BT Hasan, 2020), orange, clove, ginger, citronella, eucalyptus(Sritabutra & Soonwera, 2013). The plant is known to contain active compounds such as flavonoids, saponins, tannins, alkaloids, and terpenoids.(Aseptianova et al., 2017)which can be used as a natural mosquito repellent.

In addition to land plants, materials from aquatic plants such as Azolla can also be used. The potential of Azolla plants as an alternative bio-insecticide has been studied by(Ravi et al., 2018),(Ravi et al., 2020)with high mortality results in both eggs, larvae and adult mosquitoes. Azolla's ability is inseparable from the chemical compounds contained in it such as Diethyl Phthalate, 1-Nonadecene, Neophytadiene, Hexadecanoic acid, methyl ester(Ravi et al., 2018). Research conducted by(Ratnasari & Sungging Pradana, 2019)also shows that Azolla contains flavonoids, tannins and saponins. The use of Azolla as a natural mosquito repellent has not been widely used so that research is needed related to the active substances in the Azolla plant as a mosquito repellent development.

Mosquito repellents made from natural ingredients are currently not widely available and research on these ingredients is still limited to the laboratory scale. In an effort to increase the diversity of mosquito repellents, especially those derived from natural ingredients, ongoing research is needed to obtain maximum results.

METHOD

a. Collection and Preparation of Azolla microphylla

*Azolla microphylla*taken from cultivators in Central Java. The Azolla plants that have been obtained are then cleaned with running water and the extraction process is carried out.

b. Extraction of Azolla microphylla with Polarity Pelarut Multilevel

Extraction begins with the preparation of dry simplisia powder. The material is washed clean with water then ovened for 48 hours at a temperature of 50°C then blended and sieved with mesh no. 20(Boesri et al., 2015) (Macdonald et al., 2016). Powder from the material was taken 2.5 kg and soaked with 6 L of n-hexane solution in a glass container and left for 48 hours while stirring occasionally, then filtered until the residue and filtrate were obtained.The filtrate was evaporated and a thick n-hexane extract (non-polar) was obtained. The residue was soaked again in 6 L of Ethyl acetate (EtoAc) solution for 48 hours, then filtered and the residue and filtrate were obtained.

The filtrate was evaporated and a thick ethyl acetate extract (semi-polar) was obtained. The residue was soaked again with 6 L of 70% ethanol (EtOH) for 48 hours and filtered to produce the residue and filtrate. The filtrate was evaporated and a thick ethanol extract (polar) was obtained. The residue was soaked again with a mixture of ethanol and DMSO with a ratio of 1:1 for 48 hours, then filtered and the residue and filtrate were produced. The filtrate was evaporated and a liquid extract of EtOH:DMSO (1:1, v/v) (very polar) was obtained.(Nogata et al., 2006) (Praise Astuti Wahyuningsih et al., 2021).

c. Metabolite Screening TestSecondary

1. Flavonoid Examination

The filtrate solution of the test extract as much as 5 ml was added with Mg powder as much as 0.05 mg and 1 ml of concentrated HCl then vigorous shaking was carried out. The results of shaking if there is a color change to red, yellow, or orange indicates the presence of flavonoids.(Larasati & Putri, 2023).

2. Alkaloid Examination

The test solution of 2 ml was evaporated on a porcelain cup. The residue produced was then dissolved with 5 ml of 2 N HCl. The solution obtained was divided into 3 test tubes. The first tube was added with 3 drops of 2N HCl which served as a blank. The second tube was added with 3 drops of Wagner reagent and the third tube was added with 3 drops of Mayer reagent. The reddish brown precipitate formed in the second tube and the white precipitate in the third tube indicated the presence of alkaloids(Larasati & Putri, 2023).

3. Saponin Examination

Ethanol extract of 0.5 ml was put into a test tube, 10 ml of hot water was added, cooled and then vigorously shaken vertically for 10 seconds and 1 drop of 2N HCl was added. The formation of foam as high as 1-10 cm which is stable for ± 10 minutes indicates the presence of saponins.(Larasati & Putri, 2023).

4. Tannin Examination

2 ml of the test extract solution is reacted with several drops of 10% iron (III) chloride solution. If a color change occurs to dark blue or greenish black, this indicates the presence of tannin.(Larasati & Putri, 2023).

5. Terpenoid Examination

1 gram of test extract was added with hot etOH (ethyl alcohol), filtered and heated to dryness. The extract was added with 1 ml of diethyl ether and homogenized and 1 drop of H_2SO_4 and 1 drop of anhydrous CH_3COOH were added. If the test solution changes color to red or purple, it indicates the presence of terpenoids.(Prasetyo, 2021).

RESULT AND DISCUSSION

A. Extraction Results

The results of extraction with graded solvents using n-hexane, ethyl acetate and ethanol (EtOH) showed results in the form of thick extracts with the weight of each extract being 213.6 grams for n-Hexane, 130 grams for ethanol and 221.5 grams for ethyl acetate. The extraction results will then be mixed with DMSO to carry out a challenge test with mosquitoes.

Figure 1. Results of thick extract of Azolla microphylla

Extraction of the sample begins with maceration using 3 solvents that have different levels of polarity, namely n-Hexane, ethanol and ethyl acetate. The use of solvents with different levels of polarity aims to obtain secondary metabolite compounds/substances with different levels of polarity. For example, macerate with nonpolar solvents will extract non-polar compounds, macerate with semipolar solvents will extract semipolar compounds and macerate with polar solvents will extract polar compounds(Praise Astuti Wahyuningsih et al., 2021). This is also in accordance with research(Pratiwi et al., 2022). Secondary metabolite compounds will be maximally extracted using multi-stage extraction using solvents of different polarities.

B. Secondary Metabolite Screening Test

Secondary metabolite screening tests were conducted on flavonoids, alkaloids, saponins, tannins and terpenoids. The results obtained for flavonoids were positive in ethyl acetate solvent, terpenoids and tannins were positive in ethanol solvent. In general, the results of secondary metabolite compound screening are in table 1.

Table 1. Results of Secondary Metabolite Compound Screening Tests

Types of solvents	Flavonoid	Alkaloid	Saponins	Tannin	Terpenoid
N-Hexane	-	-	-	-	-
Ethyl Acetate	+	-	-	-	-
Ethanol	-	-	-	+	+

Based on the results of testing the secondary metabolite compounds/substances from Azolla microphylla extract, positive results were obtained for flavonoids, terpenoids and tannins. Positive flavonoid results are indicated by the formation of a red-orange color, positive terpenoids are indicated by a brownish-red color, and positive tannins are indicated by the presence of blackish-green deposits.(Afifah Rukmini, 2020). The three compounds are active compounds that can be used as mosquito repellents. This is in accordance with the statement(Asadollahi et al., 2019) (Nyawira Wangai et al., 2020)which states that several active compounds such as flavonoids, saponins, tannins, alkaloids and terpenoids can be used as natural mosquito repellents.

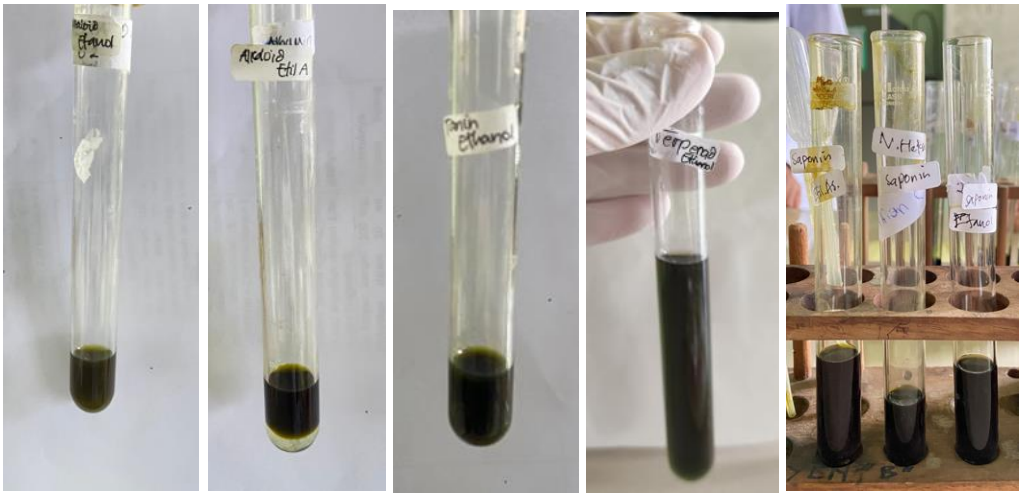

Figure 2. Some results of secondary metabolite compound screening tests.

CONCLUSION

Secondary metabolite compounds can be found in various plants, both land and water. Azolla microphylla contains metabolite compounds that can be used as mosquito repellent candidates such as flavonoids, tannins and terpenoids. The results of the secondary metabolite compound content can be developed through further research by looking at the levels of each compound.

Bibliography

Afifah Rukmini. (2020). Skrining Fitokimia Familia Piperaceae. Jurnal Biologi Dan Pembelajarannya (JB&P), 7(1), 28–32. https://doi.org/10.29407/jbp.v7i1.14805

Asadollahi, A., Khoobdel, M., Zahraei-Ramazani, A., Azarmi, S., & Mosawi, S. H. (2019). Effectiveness of plant-based repellents against different Anopheles species: A systematic review. Malaria Journal, 18(1), 1–20. https://doi.org/10.1186/s12936-019-3064-8

Aseptianova, A., Fitri Wijayanti, T., & Nurina, N. (2017). Efektifitas Pemanfaatan Tanaman Sebagai Insektisida Elektrik Untuk Mengendalikan Nyamuk Penular Penyakit Dbd. Bioeksperimen: Jurnal Penelitian Biologi, 3(2), 10. https://doi.org/10.23917/bioeksperimen.v3i2.5178

Boesri, H., Heriyanto, B., Susanti, L., & Handayani, S. W. (2015). The Repellency Some of Extract Plants Againts Aedes Aegypti Mosquitoes Vector of Dengue Fever. Jurnal Vektora, 7(2), 79–84.

Larasati, D., & Putri, F. M. S. (2023). Skrining Fitokimia dan Penentuan Kadar Flavonoid Ekstrak Etanol Limbah Kulit Pisang (Musa acuminata Colla). Jurnal Mandala Pharmacon Indonesia, 9(1), 125–131. https://doi.org/10.35311/jmpi.v9i1.330

Macdonald, O., Adeboye, O., Ngozi, R., Olatunbosun, S., & Author, C. (2016). Fractionation and Identification of Bioactive Constituents From Sapium Ellipticum (Hochst) Leaf Extract. Animal Research International, 13(3), 2492–2503. www.zoo-unn.org

Nogata, Y., Sakamoto, K., Shiratsuchi, H., Ishii, T., Yano, M., & Ohta, H. (2006). Flavonoid composition of fruit tissues of citrus species. Bioscience, Biotechnology and Biochemistry, 70(1), 178–192. https://doi.org/10.1271/bbb.70.178

Nyawira Wangai, L., Kimani Kamau, K., Munyekenye, G., Nderu, D., Maina, E., Gitau, W., Murigi, M., Kamau, S., Njuguna, M., Gichuki, J., & Otieno, F. (2020). Efficacy of Plant-based Repellents Against Anopheles Mosquitoes: A Systematic Review. Biomedical Sciences, 6(3), 44. https://doi.org/10.11648/j.bs.20200603.11

Prasetyo, B. F. (2021). Uji Aktivitas Antioksidan dan Daya Hambat Enzim Tirosinase Ekstrak

Etanol Azolla filiculoides Lam. Jurnal Sains Farmasi & Klinis, 8(1), 53. https://doi.org/10.25077/jsfk.8.1.53-59.2021

Pratiwi, S. W., Anggraeni, A., & Bahti, H. H. (2022). Chimica et Natura Acta. Chimica et Natura Acta, 10(2), 66–71.

Puji Astuti Wahyuningsih, S., Ratnasari Ekawati, E., & Darmanto, W. (2021). Evaluation of the Bioactivity of MeOH:DMSO (1:1, v/v) Lime Peel Extract on Methicillin-Resistant Staphylococcus aureus. Journal of Hunan University（Natural Sciences）, 48(5).

Ratnasari, E., & Sungging Pradana, M. (2019). The Effectiveness of Azolla pinnata in Inhibiting the Growth of Salmonella typhi. Jurnal Biota, 5(1), 2528–262. http://jurnal.radenfatah.ac.id/index.php/biota

Ravi, R., Rajendran, D., Oh, W. Da, Mat Rasat, M. S., Hamzah, Z., Ishak, I. H., & Mohd Amin, M. F. (2020). The potential use of Azolla pinnata as an alternative bio-insecticide. Scientific Reports, 10(1), 1–9. https://doi.org/10.1038/s41598-020-75054-0

Ravi, R., Zulkrnin, N. S. H., Rozhan, N. N., Yusoff, N. R. N., Rasat, M. S. M., Ahmad, M. I., Ishak, I. H., & Amin, M. F. M. (2018). Chemical composition and larvicidal activities of Azolla pinnata extracts against Aedes (Diptera:Culicidae). PLoS ONE, 13(11), 1–18. https://doi.org/10.1371/journal.pone.0206982

Sembiring, F. K., & BT Hasan, R. S. (2020). the Effectiveness Test of Betel Leaf Extract Toward the Development of Aedes Aegypti Sp Larvae. Biospecies, 13(1), 1–7. https://doi.org/10.22437/biospecies.v13i1.8386

Sritabutra, D., & Soonwera, M. (2013). Repellent activity of herbal essential oils against Aedes aegypti (Linn.) and Culex quinquefasciatus (Say.). Asian Pacific Journal of Tropical Disease, 3(4), 271–276. https://doi.org/10.1016/S2222-1808(13)60069-9

Vitaningtyas, Y., Agustiningrum, M. Y. D., Shella, S., Prisilia, C., & Putri, C. E. T. (2019). Pengolahan Serai Sebagai Tanaman Obat Pengusir Nyamuk Bersama Anak-Anak Di Pemukiman Pemulung Blok O Yogyakarta. ABDIMAS ALTRUIS: Jurnal Pengabdian Kepada Masyarakat, 2(1), 14–23. https://doi.org/10.24071/aa.v2i1.2124.