Malaria is a global health concern that threatens many countries. Plasmodium sp. may facilitate human transmission and is currently relatively resistant to chloroquine. The people in Nusa Tenggara, Indonesia, have traditionally employed the cortex of Black Pulai (Alstonia spectabilis) as a treatment for malaria for a considerable period. The objective of this study is to analyze the phytochemical composition of the 96% ethanol extract of A. spectabilis cortex (ASCE) and evaluate the antimalarial properties of tablets derived from A. spectabilis cortex extract (ASCT). A metabolite profile analysis was conducted on ASCE utilizing a UPLC-QToF-MS/MS method. An antimalarial test was conducted on ASCT on mice (Mus musculus) infected with Plasmodium berghei, and the blood smears of the mice were analyzed along with liver tissue damage. The results showed that administering ASCT could reduce the percentage of parasitemia in mice as well as the average liver damage score. This situation is feasible due to the presence of significant compounds in the ASCE that are anticipated to function as antiplasmodium agents, including villalstonine, vincadifformine, and pleiocarpamine. From these findings, one can infer that ASCT, which includes ASCE as its active component, has the potential to serve as a preferred antimalarial medication.

Keywords: Alstonia Spectabilis; Antimalarial; Metabolite Profiling; Tablet.
condition is because Indonesia has a heterogeneous climate and is vulnerable to regional and global climate change, thereby increasing the risk of transmission via mosquito vectors.

This disease really threatens people’s health, especially for poor people living in remote areas, besides that it can also reduce productivity, cause economic losses, and increase the mortality of babies, children and adults. The WHO characterizes malaria elimination as the cessation of local transmission, signifying a reduction in the occurrence of malaria cases to zero in specific geographical regions. However, over the past few decades, there has been a growing resistance of Plasmodium parasites to antimalarial quinine derivatives, particularly chloroquine, rendering the drug ineffective.

The increasing resistance of Plasmodium to the chloroquine, and there are also side effects of chloroquine such as gastrointestinal disorders, headaches, blurred vision, insomnia, and exacerbation of psoriasis encourages researchers to continue looking for new and more effective antimalarial drugs. Plants have become one of the most important sources in the search for potential new antimalarial drugs in the traditional medicine of various ethnicities throughout the world. The indigenous people of Nusa Tenggara, Indonesia, traditionally employ Black Pulai (Alstonia spectabilis) as an antimalarial remedy. This plant has demonstrated robust inhibitory activity against the P. falciparum 3D7 strain, as confirmed through in vitro testing in initial research.

The great benefits contained in *A. spectabilis* have great potential for development to be used as a medicinal product so that it is more easily accepted by patients, but the difficulty of penetrating active substances from natural ingredients into the body due to differences in lipid solubility and molecular size is an inhibiting factor for drug molecules to pass through biological membranes thereby interfering with systemic absorption. One solution to form a dosage form that has good stability is tablets, therefore further investigation has been conducted to formulate tablets using a 96% ethanol extract derived from the cortex of *A. spectabilis*.

This study aims to assess the phytochemical composition of *A. spectabilis* cortex extract (ASCE) utilizing ultra-high-performance liquid chromatography quadrupole time-of-flight mass spectrometry (UPLC-QToF-MS/MS). Additionally, it seeks to evaluate the antimalarial properties of tablets derived from *A. spectabilis* cortex extract (ASCT) by conducting experiments on mice infected with *P. berghei*.

### MATERIALS

**Plant material**

*A. spectabilis* cortex was obtained from the Nusa Tenggara area of Indonesia and has been determined at the UPT Materia Medica, Batu, Indonesia.

**Chemical material**

Merck (Darmstadt, Germany) supplied ethanol 96%, formic acid, dichloromethane, methanol, and wright eosin, while Smart-Lab Indonesia (Tangerang, Indonesia) provided acetonitrile H-1010. Imedco (Tangerang, Indonesia) was the source for ket-A-100, xylazine 2%, and hydroxychloroquine sulfate.

**ASCT**

ASCT was formulated and provided to us by PT. Agaricus Sido Makmur Sentosa, Malang, Indonesia). ASCT was formulated using wet granulation, with the active ingredient being 96% ethanol extract of *A. spectabilis* cortex. Each ASCT is equivalent to 100 g of simplicia. The excipients used as constituents of the tablet formula were lactose, microcrystalline cellulose, sodium starch glycolate, silica hydrate, magnesium stearate, copovidine, and methylparaben.

**Parasite culture**

The *P. berghei* culture used is the ANKA strain, which was obtained from the Health Animal Clinic Research and Diagnostic Laboratory, Malang, Indonesia.

**Animal subject**

The test animals used were male Swiss mice (*Mus musculus*) weighing 20-25 g and 2-3 months old. These mice were obtained from the Biosciences Institute, Brawijaya University and have passed the ethical suitability test from the Brawijaya University Research Ethics Commission with ethical suitability letter No: 156-KEP-UB-2023.
METHOD

Extraction

Roughly 500 g of powdered *A. spectabilis* cortex underwent ultrasonic extraction with 96% ethanol through three ten-minute cycles, utilizing a Soltec Sonica 5300EP S3. The resulting solution was filtered and dried at 50 °C using a Heidolph G3 rotary evaporator. After this procedure, 25 g of the 96% ethanolic extract was acquired and subsequently subjected to additional testing and analysis, in this study, metabolite profiling was carried out 16.

Metabolite profiling

Metabolite profiling was conducted using a UPLC-QToF-MS/MS instrument at the Forensic Laboratory Center of the Indonesian National Police Criminal Investigation Agency. A 96% ethanolic extract was generated through solid-phase extraction (SPE) using dichloromethane and methanol as solvents. Subsequently, 5 L of the extract was evenly injected into an ACQUITY UPLC® H-Class System (Waters, USA) equipped with an MS Xevo G2-S QToF detector (Waters, USA). The separation of samples occurred on an ACQUITY BEH C18 column (1.7 μm × 2.1 mm × 50 mm) with acetonitrile + water and 0.05% formic acid + 0.05% formic acid as mobile phases. The flow rate was maintained at 0.2 mL/minute. MassLynx 4.1 was employed to process the UPLC-QToF-MS/MS analysis results, generating chromatogram data and m/z spectra for each detected peak. The identified compounds were then validated using online databases, including MassBank (https://massbank.eu/MassBank), ChemSpider (http://www.chemspider.com), and PubChem (https://pubchem.ncbi.nlm.nih.gov).

Antimalarial activity test

The procedure for testing the antimalarial activity of ASCT followed the standard Peter’s Test Method 17. This research used a completely randomized design (CRD) through an experiment with four treatment groups, where each treatment consisted of three mice. Normal control group (without any induction), negative control group (mice induced by *P. berghei*), positive control group (mice induced by *P. berghei* + hydroxychloroquine sulfate 0.2 mg/20 gBW mice/day), and treatment group (mice induced by *P. berghei* + ASCT at a dose of 5.46 mg/20 gBW of mice/day).

Mice were acclimatized for 7 days before

the *P. berghei* inoculation process. Inoculation was carried out by administering 1x10⁷/0.1 ml of suspension intraperitoneally to donor mice that had a parasitemia percentage of more than 20%. Donor mice blood was taken and infected as much as 1x10⁷/0.1 ml into treated mice intraperitoneally.

Antimalarial activity test on mice according to treatment groups. Treatment is given if all test mice show parasite growth with parasitemia ± 1% after 24 h post-infection. The test material was administered using a gastric probe once a day for 3 days.

The observation of parasitemia rates was carried out for 7 days to evaluate the effectiveness of ASCT. Thin blood smear preparations are made carrying out liver damage scoring, necrosis, and degeneration. Blood and liver preparations were scrutinized using a light microscope (Nikon Eclipse type Eii) assisted by an Optilab microscope camera. The examination involved observing 10 different fields of view at a magnification of 1000 times for blood preparations. In the case of liver preparations, the examination was conducted in 5 fields of view with a magnification of 400 times.

RESULTS AND DISCUSSION

Metabolite profiling

In order to determine the specific compounds responsible for the antimalarial activity of ASCE, the UPLC-QToF-MS/MS instrument was utilized to identify secondary metabolite compounds. The total ion chromatogram (TIC) is visible in (Figure 1). In the interim, the percent area, m/z, retention time (RT), compound name and molecule formula values are displayed in (Table 1).

Following the metabolite profiling conducted through UPLC-QToF-MS/MS, accelerated solvent extraction produced a collection of 37 compounds. Among these, 8 were unidentified, and 29 were recognized or known compounds. In the process of metabolite profiling, not every peak in TIC can be identified among all compounds that have been detected. An indication of this is the presence of unknown compounds, which are those that are not included in the database. These compounds may consist
of undetected impurities or degradants by the instrument, as well as novel compounds that have not yet been added to the database. The unidentified compounds with elevated concentrations are of particular significance. Several “major” or “dominant” compounds are identified through the examination of these metabolite compounds \(^{18,19}\). The percent area indicates that these compounds are more abundant in the sample compared to other compounds. In the ASCE, the major compounds were the alkaloid villalstonine (44.6101 %), vincadifformine (22.2509 %), and pleiocarpamine (10.2855). Every known major compound possesses antimalarial properties.

**Antimalarial activity test**

The analysis of parasitemia percentage revealed the growth of *P. berghei*, which was inoculated into mice, commencing on day 1 (D1). The assessment of parasite growth involved calculating the number of erythrocytes infected with ring, trophozoite, and schizont stages per thousand erythrocytes, multiplied by 100%. Table 2 presents the results of parasitemia growth before any treatment. It’s noteworthy that malaria is transmitted by the hemoprotozoa *P. berghei* to small rodents. As an anti-malarial drug screening model, *P. berghei* is extensively utilized, owing to its status as a malaria parasite that exclusively infects mammals apart from humans. The in vivo evaluation of this parasite was justified on the grounds that its structure, physiology, and life cycle are remarkably similar to those of human malaria \(^{20}\).

In Table 2, it is presented that the growth of parasites in the ASCT group showed that ASCT had good results in inhibiting the growth of parasitemia compared to the negative control and the chloroquine groups. In Figure 2, illustrates the distinctions in the features of regular and infected erythrocytes. Normal erythrocytes exhibit a yellowish hue and lack a nucleus. In contrast, erythrocytes infected with *P. berghei* appear paler, dotted, and larger in size compared to normal erythrocytes. \(^{21}\).

The results of Table 3 and Figure 3 show that the ASCT group gave a lower liver damage scoring value compared to the negative group, both from necrosis and degeneration scoring. Degeneration is an early sign of liver damage due to toxins, which is temporary (reversible), and cells can still recover or return to normal if toxin exposure is stopped. Degeneration is distinguished by alterations in the cell cytoplasm, marked by an augmentation in cell fluid and

![Fig. 1. TIC of ASCE](image-url)
Table 1. Prediction of compounds in ASCE

<table>
<thead>
<tr>
<th>No.</th>
<th>RT (minute)</th>
<th>% Area</th>
<th>m/z</th>
<th>Molecular Formula and Structure</th>
<th>Compound Name</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>3.475</td>
<td>0.2421</td>
<td>154.0269</td>
<td>C₇H₆O₄</td>
<td>2,3-Dihydroxybenzoic acid</td>
</tr>
<tr>
<td>2</td>
<td>3.693</td>
<td>0.4022</td>
<td>393.1633</td>
<td>C₁₆H₂₇NO₁₀</td>
<td>6R)-3,5-Dideoxy-5-[(7-oxoheptanoyl)amino]-6-[(1R,2R)-1,2,3-trihydroxypropyl]-á-L-threo-hex-2-ulopyranosonic acid</td>
</tr>
<tr>
<td>3</td>
<td>4.747</td>
<td>1.2172</td>
<td>356.1741</td>
<td>C₁₄H₃₀N₄O₂Cl₂</td>
<td>4-Ethyl-N-[3-(1-piperazinyl)propyl]-2-morpholinecarboxamide dihydrochloride</td>
</tr>
<tr>
<td>4</td>
<td>5.325</td>
<td>22.2509</td>
<td>338.2000</td>
<td>C₂₁H₂₆N₂O₂</td>
<td>Vincadifformine</td>
</tr>
<tr>
<td>5</td>
<td>5.718</td>
<td>0.3906</td>
<td>644.3731</td>
<td>C₄₁H₄₈N₄O₃</td>
<td>Macralstonidine</td>
</tr>
<tr>
<td>6</td>
<td>6.007</td>
<td>3.7115</td>
<td>336.1836</td>
<td>C₂₁H₂₄N₂O₂</td>
<td>Catharanthine</td>
</tr>
<tr>
<td>No.</td>
<td>MW</td>
<td>LogP</td>
<td>Fragrance</td>
<td>Molecular Formula</td>
<td></td>
</tr>
<tr>
<td>-----</td>
<td>-------</td>
<td>-------</td>
<td>------------</td>
<td>-------------------</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>322.1686</td>
<td>6.204</td>
<td>10.2855</td>
<td>C_{20}H_{22}N_{2}O_{2}</td>
<td>Pleiocarpamine</td>
</tr>
<tr>
<td>8</td>
<td>660.3675</td>
<td>7.258</td>
<td>44.6101</td>
<td>C_{41}H_{48}N_{4}O_{4}</td>
<td>Villalstonine</td>
</tr>
<tr>
<td>9</td>
<td>674.3829</td>
<td>7.982</td>
<td>1.2543</td>
<td>C_{42}H_{50}N_{4}O_{4}</td>
<td>1-Benzyl-3-[[4'-(4-[4-(hydroxymethyl)phenyl]-6-[[2-(1-pyrrolidinylmethyl]-1-pyrrolidinyl]methyl]-1,3-dioxan-2-yl]-3-biphenyl]methyl]urea</td>
</tr>
<tr>
<td>10</td>
<td>680.3099</td>
<td>8.355</td>
<td>0.0796</td>
<td>C_{40}H_{44}N_{2}O_{8}</td>
<td>(6aS)-1,2,3,9,10-Pentamethoxy-6-methyl-8-(4-[[5S]-6-methyl-5,6,7,8-tetrahydro[1,3]dioxolo[4,5-g]isoquinoline-5-yl]methyl]phenoxy)-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinoline</td>
</tr>
<tr>
<td>11</td>
<td>418.2260</td>
<td>8.439</td>
<td>0.3998</td>
<td>C_{26}H_{30}N_{3}O_{3}</td>
<td>Bis(ù-piperidinylacetyl)dibenzofuran</td>
</tr>
<tr>
<td>12</td>
<td>528.2628</td>
<td>8.769</td>
<td>0.3726</td>
<td>C_{26}H_{36}N_{8}O_{3}S</td>
<td>N-(4-(4,6-Di(piperidin-1-yl)-1,3,5-triazin-2-yl)piperazin-1-ylsulfonyl)phenyl)acetamide</td>
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</tbody>
</table>
13 8.924 0.0518 498.2525 \( \text{C}_{24} \text{H}_{34} \text{N}_8 \text{O}_2 \text{S} \) N-\{4-[(4,6-Bis(ethylamino)-1,3,5-triazin-2-yl]amino\}methyl)cyclohexyl[methyl]-8-quinolinesulfonamide

14 9.171 0.2327 676.3161 \( \text{C}_{42} \text{H}_{48} \text{N}_2 \text{O}_2 \text{S}_2 \) 2,5-Bis(2-ethylhexyl)-3,6-bis(5-phenyl-2-thienyl)-2,5-dihydropyrrolo[3,4-c]pyrrole-1,4-dione

15 9.410 0.0585 484.2364 \( \text{C}_{30} \text{H}_{32} \text{N}_2 \text{O}_4 \) Dipentyl 2,2'-biquinoline-4,4'-dicarboxylate

16 9.669 0.9600 674.3460 \( \text{C}_{33} \text{H}_{30} \text{N}_5 \text{O}_5 \) N-\{(4-Azidophenyl)(methyl)carbamoyl\}-S-\{(4S,5R,6E,8E)-1-carboxy-4-hydroxy-6,8-nonadecadien-5-yl\}-L-cysteinylglycine

17 9.958 0.0404 542.2783 \( \text{C}_{33} \text{H}_{38} \text{N}_2 \text{O}_5 \) 1-[3-(Dimethylamino)propyl]-4-[hydroxy(4-isobutoxy-3-methylphenyl)methylene]-5-(3-phenoxyphenyl)-2,3-pyrrolidinedione

18 10.196 0.0059 692.3562 \( \text{C}_{40} \text{H}_{52} \text{O}_{10} \) (5bS,8R,11R,11aR,12S,13R,13aR)-13a-\{(1R)-1-Acetoxyethyl\}-8-hydroxy-5b,9,11a-trimethyl-8-[(1Z)-2-phenyl-1-propen-1-yl]-1,3,5a,5b,6,7,7a,8,11,11a,11b,12,13,13a-tetradecahydrophenanthro[2,1-c]oxepine-11,12,13-triyl triacetate
<table>
<thead>
<tr>
<th>No.</th>
<th>Retention Time (min)</th>
<th>RI</th>
<th>Molecular Formula</th>
<th>Molecular Weight</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>10.401</td>
<td></td>
<td>C₁₇H₁₉NO₃</td>
<td>285.1367</td>
<td>D-Morphine</td>
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<tr>
<td>20</td>
<td>10.682</td>
<td></td>
<td>C₁₈H₁₈N₂O₄</td>
<td>326.1263</td>
<td>1,4-Bis([2-hydroxyethyl]amino)-9,10-anthraquinone</td>
</tr>
<tr>
<td>21</td>
<td>10.879</td>
<td></td>
<td>C₂₁H₂₂N₂O₃</td>
<td>350.1628</td>
<td>Perakine</td>
</tr>
<tr>
<td>22</td>
<td>10.992</td>
<td></td>
<td>C₁₈H₂₇N₆O₂Cl</td>
<td>394.1887</td>
<td>1-[(1-(3-Benzodioxol-5-yl)methyl)-1H-tetrazol-5-yl]propyl]-4-ethylpiperazine hydrochloride</td>
</tr>
<tr>
<td>23</td>
<td>11.829</td>
<td></td>
<td>C₁₈H₃₉N₂O₃</td>
<td>317.2934</td>
<td>Phytosphingosine</td>
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<td>24</td>
<td>11.954</td>
<td></td>
<td>C₁₉H₁₄N₂O₃</td>
<td>318.1006</td>
<td>N-[(E)-(3-Nitrophenyl)methylene]-4-phenoxyaniline</td>
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<tr>
<td>25</td>
<td>12.159</td>
<td></td>
<td>C₁₈H₁₂N₂O₂</td>
<td>288.0902</td>
<td>Pyrenediamide</td>
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<tr>
<td>26</td>
<td>12.482</td>
<td></td>
<td>C₃₃H₄₁NO₁₄</td>
<td>695.4094</td>
<td>Hexadecyl 3-O-[(6R)-5-acetamido-3,5-dideoxy-6-[(1R,2R)-1,2,3-trihydroxypropyl]-â-D-threo-hex-2-uloxyranosyl]-â-D-galactopyranoside</td>
</tr>
</tbody>
</table>
Table 2. % growth of parasitemia

<table>
<thead>
<tr>
<th>Group</th>
<th>% parasitemia growth</th>
<th>% parasitemia growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>0.00</td>
<td>0%</td>
</tr>
<tr>
<td>Negative Control</td>
<td>90.00</td>
<td>26%</td>
</tr>
<tr>
<td>Positive Control (Chloroquine 0.2 mg/20 gBW mice/day)</td>
<td>92.00</td>
<td>15%</td>
</tr>
<tr>
<td>ASCT 5.46 mg/20 gBW of mice/day</td>
<td>95.00</td>
<td>14%</td>
</tr>
</tbody>
</table>

swelling. However, the cell nucleus can maintain its integrity as long as the cell does not undergo severe injury. This degeneration occurs due to oxidation disorders, which cause the buildup of protein deposits in the cytoplasm, so that the cytoplasm appears cloudy. Parenchymatous degeneration represents the least severe form of degeneration and is considered reversible. Continued parenchymatous degeneration can cause hydrophic degeneration. Hepatocytes that experience hydrophilic degeneration will swell and appear as vacuoles filled with water in the cytoplasm. The cause of persistent damage to hepatocytes can be tearing of the plasma membrane and changes in the nucleus, so that hepatocytes experience necrosis. In addition, necrosis can occur as a result of reactive chemical metabolites binding to nucleophilic proteins within hepatocytes or the presence of metabolites with free chains capable of covalently binding to proteins and unsaturated fatty acids in cell membranes. This process leads to lipid peroxidation and membrane damage, ultimately culminating in hepatocyte death.

The results of blood observations and liver damage showed that the ASCT group with dose 5.46 mg/20gBW of mice/day could be an
alternative herbal antimalarial treatment. In this study, it was proven that ASCT had the ability to inhibit the growth of the *P. berghei* parasite in the blood of mice better than the positive control chloroquine. This is thought to be the way antimalarial drugs work in inhibiting the growth of parasites in the blood of mice so that they can reduce the percentage of parasitemia during the three days of observation.

The ASCE contains several antimalarial compounds, one of which is alkaloids, where alkaloid compounds inhibit parasite growth by blocking parasite growth through intracellular transport of choline. In addition to alkaloid compounds as antimalarials, namely by inhibiting the detoxification process of parasite hemorrhoids through vacuoles, food becomes a non-toxic malaria pigment 24.

Villalstonine is one of the compounds contained in ASCE. This compound has demonstrated diverse biological activities, encompassing anticancer, antiamoebic, and

<table>
<thead>
<tr>
<th>Group</th>
<th>Necrosis</th>
<th>Degeneration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>0.7</td>
<td>0.8</td>
</tr>
<tr>
<td>Negative Control</td>
<td>4.0</td>
<td>3.8</td>
</tr>
<tr>
<td>Positive Control (Chloroquine 0.2 mg/20 gBW mice/day)</td>
<td>1.3</td>
<td>2.0</td>
</tr>
<tr>
<td>ASCT 5.46 mg/20 gBW of mice/day</td>
<td>2.5</td>
<td>2.1</td>
</tr>
</tbody>
</table>

Fig. 2. Normal erythrocytes and erythrocytes infected with *P. berghei*
antimalarial properties. In antimalarial potential, villalstonine has 1/15\textsuperscript{th} potency compared to chloroquine as single compound in fighting malaria and strong antiplasmodial activity against the multidrug-resistant K1 strain of \textit{P. falciparum} with an IC\textsubscript{50} value of 0.27 \textmu M\textsuperscript{25, 26}. Vincadifformine and Pleiocarpamine are also compounds found in ASCE. Vincadifformine is recognized for its antimalarial biological activity against \textit{P. falciparum}, exhibiting efficacy on the A FcM strain (a chloroquine-resistant strain with an IC\textsubscript{50} for chloroquine of approximately 230 ng/mL) and the Nigerian strain (a chloroquine-sensitive strain with a chloroquine IC\textsubscript{50} of about 41 ng/mL)\textsuperscript{27}. In contrast, the compound Pleiocarpamine demonstrates antimalarial activity as evidenced by test results utilizing an antiplasmodial assay with the induction of \textit{P. falciparum} strain K1 and in vivo testing involving mice induced by \textit{P. berghei}\textsuperscript{28}.

**CONCLUSION**

Based on these findings, it is possible to conclude that ASCT with ASCE as an active ingredient has an antimalarial effect by lowering the proportion of parasitemia in mice as well as the average liver damage score. In ASCE, several key chemicals, including villalstonine, vincadifformine, and pleiocarpamine, are predicted to be responsible for this effect.

**ACKNOWLEDGMENT**

The authors express their gratitude to PT. Agaricus Sido Makmur Sentosa for providing the ASCT in this study.

**Conflict of Interest**

The authors declare that there is no conflict of interest.
Funding Sources
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