Evaluation of anti-malarial activity of *Orostachys japonicus* (Crassulaceae) against 3D7, K1 strains, and field isolates (Madang, Papua New Guinea) of *Plasmodium falciparum* and identification of the active principle

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**Abstract** — The present study was undertaken to investigate the anti-malarial activity of a native plant of Korea, *Orostachys japonicus* (*O. japonicus*). *O. japonicus* extract and its constituents were tested for their inhibitory effects on the growth of 3D7 and K1 strains of *Plasmodium falciparum* (P. falciparum). Among the five organic solvent-soluble portions separated from the crude extract, dichloromethane-soluble (DcM-soluble) portion exhibited the highest activity. DcM-soluble portion was further analyzed for its chemical composition and three major compounds were identified, among which 3-(benzylamino)-4-ethyl-6-phenyl-1,2,4-triazin-5-one was revealed to be the most active compound. The compound induced no significant hemolysis, nor did it possess any toxicity to human cells. The compound was also effective in inhibiting the growth of *P. falciparum* field isolates from Madang, Papua New Guinea. When tested in vivo against *Plasmodium berghei*, however, the compound poorly inhibited plasmodial growth. Based on this observation and chemical structure analysis, it was conjectured that the compound derives its anti-plasmodial activity from its action against *P. falciparum* M18 aspartyl aminopeptidase (PfM18AAP). The highly promising anti-malarial activity exhibited by its major active compound led to the conclusion that *O. japonicus* holds a great deal of promise as a candidate for the source of a new anti-malarial drug. Further studies are needed to investigate the active compound’s mechanism of action and its safety to human health.

**Keywords** — anti-plasmodial activity; plant extract; *Orostachys japonicus*; *Plasmodium falciparum*; field isolates; natural compound

I. INTRODUCTION

PREVALENCE of malaria throughout the world is without doubt one of the most urgent and serious concerns in the fields of public health today. In 2004, it was estimated that approximately 3.2 billion people were exposed to the risks of malarial infection and that some 350-500 million people contracted the disease, with at least a million deaths incurred annually [1].

Among various types of malaria, infection by *Plasmodium falciparum* (*P. falciparum*) is the most dangerous form of malaria, with the highest rates of complications and mortality [2], and takes its biggest toll on young children of age 5 years or below [2, 3]. Over the last few decades, many attempts have been made to develop an effective vaccine against malaria caused by *P. falciparum* and other *Plasmodium* species, but there are currently no licensed vaccines against malaria [3, 4]. The absence of vaccines thus leads to a greater need for a powerful and effective antimalarial drug that could be readily relied on in cases of severe malarial infections.

In light of the global trend of multi-resistant *P. falciparum* emergence, a great deal of attention has been given to the chemotherapeutic treatment of malaria in the last ten years. Currently, the World Health Organization (WHO) recommends the use of artemisinin-based combination therapy (ACT) as the standard form of treatment for uncomplicated *P. falciparum* malaria so as to reduce the possibilities of drug resistance development [5]. Despite such precautionary measures taken, however, signs of resistance to artemisinin have already been reported in some regions of Asia [6-8], which pose a grave threat for the control of malaria. In light of such concerns regarding drug resistance, the need for the development of novel anti-malarial drugs is rendered all the more important.

In search of novel active ingredients that could be developed into such new anti-malarial drugs, many researchers have turned to the plant kingdom, which still remains a rich source of pharmacologically active compounds. Because constituents of plant extracts can have synergistic effects as a result of various interactions among their constituents, plant extracts could be more effective than products composed of single compounds [9, 10]. Besides the obvious examples of quinine, extracted from Cinchona trees [11], and artemisinin, isolated from *Artemisia annua* [12], there have been numerous instances of plant extracts exhibiting high anti-malarial activities [13-17]. In the past search for active plant extracts, the ethnopharmacological strategy (i.e. drawn from the traditional knowledge of plants known for their therapeutic
effects) has proven to be especially effective, the progress of which has been summarized in a review provided by Willcox et al. [18, 19]. Such progress, coupled with the fact that approximately 80% of the world’s population still depends on traditional medicinal products as a part of primary health care [20, 21], underscores the importance of continuing to search the traditional sources for active plant constituents with promising anti-malarial activities.

The situation of malaria in Korea, albeit now down to a near-eradication level, used to be a concern in the pre-modern era [22]. The treatment strategies for malaria, which was known as “hakjil” in traditional Korean, mainly consisted of administering a combination of different herbal plant extracts [23]. In a well-known, centuries-old encyclopedia of traditional Korean medicine, titled “Dongui Bogam”, there are numerous references to various indigenous Korean herbal plants with therapeutic effects on malaria patients [24]. One such plant that was well noted in the Dongui Bogam for its potent anti-malarial activities is Orostachys japonicus [24], more commonly known as “Bawisol” in the Korean language.

O. japonicus is a perennial herbaceous plant that belongs to the Crassulaceae family, and grows mainly in Korea and Japan. Various types of biological activities (including anti-inflammatory, anti-ulcerogenic, anti-oxidant, etc.) of O. japonicus extracts have been tested in several studies before [25-27], but according to our literature review, no previous study has been conducted to experimentally evaluate its anti-malarial activity.

The current study thus aimed to evaluate for the first time the anti-plasmodial activity of O. japonicus extract and its constituents against P. falciparum, using both in vitro and in vivo assays. A major active compound was identified and isolated, after which its hemolytic and cytotoxic activities were examined in order to establish its selectivity and safety. Lastly, the isolated compound’s potential as a novel active principle for anti-malarial drugs was tested on P. falciparum field isolates from Madang, Papua New Guinea.

II. METHODS

A. Plant Material Preparation

O. japonicus whole plant was collected at Mt. Gwanak, Seoul in late September, 2011. The collected sample was verified to be O. japonicus by the Research Institute for Agriculture and Life Science, Seoul National University. A voucher specimen was deposited at the same institute and was assigned a label of OJ-01.

B. Crude Extraction Preparation

O. japonicus whole plant was dried in a ventilated room (30 °C), cut into slices and pulverized. After pulverization, 200 g of dried O. japonicus was extracted with 95% ethanol (EtOH) for 3 h three times. O. japonicus EtOH extract (OJ-EtOH extract) was further concentrated by means of rotary evaporation at 40 °C.

C. Organic Solvent Fractionation

The concentrate of OJ-EtOH extract was suspended in distilled water and was sequentially partitioned into n-hexane- (hexane), dichloromethane- (DeM), ethylacetate- (EtOAc), n-butanol- (BuOH), and water-soluble (H2O) portions for subsequent bioassays. Each organic solvent-soluble portion weighed 5.29, 12.67, 3.9, 27.9, and 11.3 g respectively. All portions were once again concentrated by rotary evaporation at 40 °C and were stored away in a freezer at -20 °C until later use.

D. GC-MS Analysis

DeM-soluble portion separated from OJ-EtOH extract was subjected to GC-MS analysis. It was analyzed by a GC-MS system that consists of a Shimadzu 2010 series (Shimadzu, Kyoto, Japan) GC equipped with an AOC-20S automatic liquid sampler and interfaced directly to a QP2010 mass selective detector controlled via an accompanying data system. A 30 mm DB-5MS capillary column (0.25 mm id, 0.25 μm film) was used. With respect to GC temperatures, the following program was adhered to: held at 50 °C for 5 min, heated at a temperature ramp of 4 °C/min up to 300 °C and maintained at the temperature for 30 min. The injection volume was 1 μL in splitless mode. Helium served as a carrier gas with a 1 mL/min flow rate. The temperature of the injector was maintained at 250 °C. A solvent delay of 5 min was maintained throughout the procedures. Tuning was performed using the autotune feature with perfluorotributylamine (PFTBA). The electron multiplier voltage was kept at 1,400 V. All data were obtained by collecting the full-scan mass spectra within the scan range of 200-550 amu. The GC-MS interface line and MS inlet temperature was 250 °C, and the ion-source temperature was 280 °C. Chemical constituents of DeM-soluble portion were identified through comparison of mass spectra of each peak with those of authentic samples in the NIST mass spectra library of compounds.

E. Preparative HPLC

DeM-soluble portion was injected into a reversed-phase preparative HPLC system. A Lichrospher RP-18 column (2.5x25 cm; 12–15 μ, Merck packing system) served as the stationary phase. The mobile phase was composed of methanol–water (50:50) in isocratic elution with a flow rate of 20 ml/min (Armen pump). Three major compounds (Compound 1, Compound 2, and Compound 3) identified in GC-MS analysis were collected (Büchi fraction collector). The experimental procedures described here were repeated a total of three times. In each round, the remaining fractions in which one of the three major compounds was absent were pooled together, yielding as a result Compound 1, Compound 1-depleted fraction, Compound 2, Compound 2-depleted fraction, Compound 3, and Compound 3-depleted fraction. Each major compound-depleted fraction was rechecked by GC-MS analysis to ensure the absence of the major compound that was intended to be absent.
F. P. falciparum Culture

According to a culture protocol described previously by Trager and Jensen [28], P. falciparum parasites (chloroquine-susceptible 3D7 strain and chloroquine-resistant K1 strain) were cultured in O2- human erythrocytes and maintained in an incubator with a gaseous phase of 90% N2, 5% O2 and 5% CO2, at a 1% hematocrit level in complete medium at 37 °C. The complete medium comprised RPMI 1640 medium containing 1-glutamine and 25 mM HEPES buffer (Gibco, NY, USA), 0.5% AlbuMAX I (Gibco), 0.225% NaHCO3 (Gibco), 10 µg/mL of gentamicin (Gibco) and 50 µg/mL of hypoxanthine (Sigma, St. Louis, USA). As described in a previous protocol [29], the parasite ring stage was synchronized using 5% sorbitol and the late trophozoite and schizont stages were induced by incubation for additional 24 h.

G.HRP2-based ELISA against Cultured P. falciparum

The inhibitory effects of test samples (OJ-EtOH extract, organic solvent-soluble portions from OJ-EtOH extract, and pure compounds isolated) on the invasion of human erythrocytes by P. falciparum merozoites were evaluated by HRP2-based enzyme-linked immunosorbent assays (HRP2-based ELISA). Standard 96-well microculture plates (Costar, NY, USA) were coated with 190 µL of parasites (0.25% in final parasitemia) in parasitized red blood cells (pRBCs) rich in trophozoite and schizont stages) in 1.0% hematocrit. Various concentrations of test samples were then prepared with rich in trophozoite and schizont stages) in 1.0% hematocrit. % in final parasitemia) in parasitized red blood cells (pRBCs, hRBCs) in suspension (1% hematocrit) served as drug-free control (i.e. 0% RBC lysis) and phosphate-buffered saline (PBS) as a negative control (i.e. 0% RBC lysis) respectively. The RBC lysis percentage was calculated as follows: \([\frac{(OD_{450} \text{ of sample-treated well} - OD_{450} \text{ of hRBCs})}{(OD_{450} \text{ of sample-treated well} - OD_{450} \text{ of hRBCs})} \times 100\]. The 50% inhibitory concentration (IC50) values of test samples against P. falciparum in vitro were determined by nonlinear dose-response curve fitting.

H.In Vitro Hemolysis Assays

Hemolytic activity of Compound 1 was evaluated. Hemolysis assays were conducted using Compound 1 isolated from DcM-soluble portion, according to a protocol described previously by Robert et al. [30]. Red blood cells (RBCs) suspensions (10% in PBS (v/v)) were incubated under agitation at 25 °C for 1 h, with the pure compound solutions (final concentration = 100 µg/mL and dimethyl sulfoxide < 1%). Mixtures were centrifuged at 25 °C at 10,000 × g and the absorbance was measured at 550 nm using a microplate spectrophotometer (BioTek, Winooski, United States). 1% Triton X-100 (v/v) served as a positive control (i.e. 100% RBC lysis) and phosphate-buffered saline (PBS) as a negative control (i.e. 0% RBC lysis) respectively. The RBC lysis percentage was calculated as follows: \([\frac{(OD_{450} \text{ of Compound 1} - OD_{450} \text{ of PBS})/(OD_{450} \text{ of 1% Triton X-100} - OD_{450} \text{ of PBS})}{(OD_{450} \text{ of sample-treated well} - OD_{450} \text{ of hRBCs})} \times 100\]. The compound was tested in triplicates.

I. In Vitro Cytotoxicity Assays

The cytotoxicity of Compound 1 was assessed using WI-38 cells. WI-38 cells, which are from a diploid human cell culture line made up of fibroblasts, were maintained in continuous culture in DMEM medium (Bio Whittaker) at 37 °C in an atmosphere of 5.5% CO2. The medium was supplemented with 10% heat-inactivated fetal bovine serum, 1% L-glutamine (200 mM), penicillin (100 UI/mL), and streptomycin (100 µg/mL).

Compounds 1 was dissolved in DMSO (Sigma) to yield a concentration of 5 mg/mL. 96-well microculture plates (Costar, NY, USA) were seeded with 200 µL of medium containing 8,000 WI-38 cells in suspension. After incubation of 24 h, cells were treated with eight dilutions of Compound 1, at final concentrations of 0.5, 1, 5, 10, 25, 50, 100, and 150 µg/mL (final DMSO concentration ≤ 1%). After incubation of additional 48 h, WST-1 tetrazolium salt that served as a cytotoxicity indicator was added. After 1 h, absorbance was measured at 450 nm using a microplate spectrophotometer (BioTek, Winooski, United States). The absorbance measured correlates directly with the number of viable cells. Each condition was repeated in triplicates. Camptothecin (Sigma-Aldrich) served as positive control and non-treated cells as the negative control. IC50 values were determined by linear regression from the set of 8 concentrations of the compound.

J. In Vivo Anti-plasmodial Assays

The anti-plasmodial activity of Compound 1 was evaluated in vivo using mice infected with Plasmodium berghei ANKA. BALB/c mice (7-8 weeks old, 25 ± 3 g) were divided into three test groups: Compound 1-, chloroquine- (positive control), and PBS-treated (negative control) groups. A total of 9 mice were
included in each test group. A test protocol based on the 4-day suppressive test [31] was followed. According to the protocol, pRBCs were collected from an infected mouse and dissolved in PBS and the mice in test groups were infected by injecting a 0.2 mL suspension (approximately \(1.0 \times 10^6\) parasites) intraperitoneally. From day 1 post-infection to day 4 post-infection, 100 mg/kg of Compound 1 was administered to each mouse each day. The mice in the positive control group received chloroquine (5 mg/kg), and those in the negative control group only received 0.2 mL of PBS. At day 4 post-infection, blood samples from all the tested mice were inspected by microscopy. Percent inhibition by Compound 1 was calculated as follows: 100 – [(mean parasitemia in Compound 1-treated group)/(mean parasitemia in PBS-treated group)] \(\times 100\). These experimental procedures were approved by the Ethics Committee of the Centers for Disease Control and Prevention, Korea and were designed in accord with the internationally recognized ethical guidelines.

K. HRP2-based ELISA against \(P. falciparum\) Field Isolates

The inhibitory effects of Compound 1 isolated from DcM-soluble portion on \(P. falciparum\) growth were further examined by conducting HRP2-base ELISA on 53 \(P. falciparum\) field isolates collected from a region in Madang, Papua New Guinea (without culture adaptation). The 53 blood samples tested were obtained from \(P. falciparum\)-infected patients who were hospitalized at Madang General Hospital, Madang Province, Papua New Guinea. The confirmation of \(P. falciparum\) infection was achieved by thin and thick blood smears, and polymerase chain reaction. Prior to blood sample collection, informed consent was sought from all participating patients, in accord with relevant regulations set by the National Department of Health, Papua New Guinea and the Ethics Committee of the Centers for Disease Control and Prevention, Korea.

L. Data Analysis

All statistical analyses were carried out using a software GraphPad Prism version 6 [32].

III. RESULTS AND DISCUSSION

A. Chemical Composition of DcM-soluble Portion

Through the GC-MS analysis, a total of 15 peaks were identified in the DcM-soluble portion. DcM-soluble portion was found to be composed of three major compounds (% area \(\geq\) 10 %) and 12 other constituents of unknown chemical identities. The three identified major compounds were 3-(benzylamino)-4-ethyl-6-phenyl-1,2,4-triazin-5-one (named Compound 1), 3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one (named Compound 2), and 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one (named Compound 3), arranged in the decreasing order of percentage area. The three major compounds accounted for 53.29, 13.85 and 11.37 % of the DcM-soluble portion respectively. The rest of the information regarding the chemical composition of DcM-soluble portion is given in Table I.

<table>
<thead>
<tr>
<th>Peak</th>
<th>% area</th>
<th>RT, min</th>
<th>Compound</th>
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<td>1</td>
<td>53.29</td>
<td>77.239</td>
<td>3-(benzylamino)-4-ethyl-6-phenyl-1,2,4-triazin-5-one&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
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</tr>
<tr>
<td>3</td>
<td>11.37</td>
<td>51.292</td>
<td>2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>6.29</td>
<td>42.416</td>
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</tr>
<tr>
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</tr>
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<tr>
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</tr>
<tr>
<td>15</td>
<td>0.28</td>
<td>5.331</td>
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</tr>
</tbody>
</table>

<sup>a</sup> Retention time
<sup>b</sup> Major compounds

The three major compounds identified through GC-MS were then isolated by preparative HPLC to evaluate their anti-plasmodial activities individually. Furthermore, each time one of the three major compounds was isolated from DcM-soluble portion, the remaining fractions were pooled together for subsequent activity assays, in order to find out to which extent each major compound contributed to the overall anti-plasmodial activity of DcM-soluble portion. The three resulting mixtures, each of which lacked in Compound 1 (C1), Compound 2 (C2), and Compound 3 (C3) respectively, were dubbed C1-, C2-, and C3-depleted fractions. Each of these fractions was once again subjected to GC-MS analysis to ensure the absence of the compound that was supposed to be absent.

B. Anti-plasmodial Activity of Major Compounds and Major compound-depleted Fractions

The anti-plasmodial activities of the three major compounds and three major compound-depleted fractions are presented in Table II. As for the pure compounds, the WHO guidelines are stricter in such a way that only those with IC50 \(\leq\) 1 \(\mu\)g/mL are considered to be highly active [33, 34]. Based on these standards, Compound 2 and 3 were judged to have either moderate or low activity. Compound 2 had IC50 values higher than 15 \(\mu\)g/mL against both 3D7 and K1 strains. Similarly, Compound 3 was found to have IC50 values higher than 5 \(\mu\)g/mL against both strains. None of the two compounds exhibited anti-plasmodial activities significant enough to be noted. However, Compound 1, which was identified in the present study as the largest constituent of the DcM-soluble portion, showed an excellent anti-plasmodial activity. Its demonstrated IC50 values were 0.308 and 0.303 \(\mu\)g/mL against
3D7 and K1 strains respectively. Dose-response curves for all three major compounds are given in Fig. 1, 2, and 3.

The claim that Compound 1 seemed to be the main active compound in OJ-EtOH extract was further corroborated by the results observed from three major compound-depleted fractions. Depletion of Compound 2 or 3 from the original DcM-soluble portion at most led to a 1.6-fold increase in IC50 values. In contrast, removal of Compound 1 resulted in 15.2- and 12.8-fold increases in IC50 values against 3D7 and K1 strains respectively. These results clearly show that Compound 1 most significantly contributes to the overall anti-plasmodial activity of the original DcM-soluble portion, and OJ-EtOH extract as well. A more quantitative measure of the extent to which Compound 1 contributes to the overall activity of OJ-EtOH extract was made through a formula described by Deharo and Ginsburg [35]: the level of contribution of Compound 1 was estimated to be 90.2%. Based on these results, it was concluded that Compound 1 is the main active compound in OJ-EtOH extract. The chemical structure of Compound 1 is illustrated in Fig. 4.
C. Hemolytic and Cytotoxic Activities of Compound 1

No significant hemolytic activity was observed with Compound 1 as the RBC lysis percentage of Compound 1 was determined to be less than 1%. This is a good indicator that the anti-plasmodial effects of Compound 1 are not induced by the hemolysis of RBCs, but by the actual action against *P. falciparum* parasites.

The cytotoxicity of Compound 1 against WI-38 cells was found out to be of an insignificant magnitude, with a determined IC\textsubscript{50} value higher than 100 µg/mL. The positive control camptothecin exhibited an IC\textsubscript{50} value of 0.027 ± 0.013 µg/mL. In summary, these results indicate that the highly promising activity of Compound 1 does not come at the expense of toxicity to human cells and thus imply the very high selectivity of Compound 1 in its action against *P. falciparum*.

D. In Vivo Anti-plasmodial Activity of Compound 1

Since such excellent anti-plasmodial activities were consistently observed with Compound 1 throughout all the *in vitro* assays, it was natural to assume that similar results would be obtained from *in vivo* assays as well. However, to much surprise of the authors, Compound 1 failed to induce any significant level of *P. berghei* growth inhibition in vivo. Detailed results of *in vivo* anti-plasmodial bioassays are presented in Table III.

![Chemical structure of Compound 1](image)

Fig. 4 Chemical structure of Compound 1

E. Anti-plasmodial Activity of Compound 1 against *P. falciparum* Field Isolates

IC\textsubscript{50} value of Compound 1 against 53 *P. falciparum* field isolates collected in Madang, Papua New Guinea were determined, and then compared with IC\textsubscript{50} values against 3D7 and K1 strains. Chloroquine also served as a reference for comparison. The results are presented in Table IV. In Fig. 5, the median of IC\textsubscript{50} values against the 53 field isolates are shown with interquartile ranges.

![Distribution graph of IC\textsubscript{50} values against *P. falciparum* field isolates (median shown with interquartile ranges)](image)

Fig. 5 Distribution graph of IC\textsubscript{50} values against *P. falciparum* field isolates (median shown with interquartile ranges)

Over the course of four days, Compound 1 was only able to inhibit the parasitemia growth by a mere 12.94%, whereas chloroquine exhibited a 94.96% inhibition rate. Despite the fact that Compound 1 was administered at a 20-fold higher dose as compared to chloroquine, its inhibitory effects against *P. berghei* were surprisingly poor. One possible explanation for this phenomenon was that Compound 1 acts at a target site that is specific to *P. falciparum* only and thus does not show any significant inhibitory activity against other *Plasmodium* species.
Further studies are needed in the future in order to verify the degradation of host cells’ proteins, without which the enzyme is crucial to the survival of P. falciparum growth. In the study, Kusch et al. synthesized reclones of PfM18AAP and subjected them to enzymatic inhibition assays using chemical constituents of Balanites aegyptiaca, among which 3-(benzylamino)-4-ethyl-6-phenyl-1,2,4-triazin-5-one, a structural derivate of 6-phenyl-2(H)-1,2,4-triazin-5-one, a structural derivate of 3-(benzylamino)-4-ethyl-6-phenyl-1,2,4-triazin-5-one and has been demonstrated to be biologically active against Plasmodium species in previous studies. After a thorough review of the database, one such chemical that fulfilled both of the above-mentioned criteria was found: 6-phenyl-2(H)-1,2,4-triazin-5-one, a structural derivate of 3-(benzylamino)-4-ethyl-6-phenyl-1,2,4-triazin-5-one. In a previous study by Kusch et al. [36], 6-phenyl-2(H)-1,2,4-triazin-5-one was identified as the active principle in the seeds of Balanites aegyptiaca, which was experimentally shown to be a highly effective inhibitor of in vitro P. falciparum growth. In the study, Kusch et al. demonstrated that the antimalarial activity of 6-phenyl-2(H)-1,2,4-triazin-5-one resulted from its inhibitory effects on a parasitic enzyme that is specific to P. falciparum. Named P. falciparum M18 aspartyl aminopeptidase (PfM18AAP), the enzyme is crucial to the survival of P. falciparum within human erythrocytes, playing a pivotal role in the degradation of host cells’ proteins, without which the growing parasite cannot survive [37]. In the study, Kusch et al. synthesized reclones of PfM18AAP and subjected them to enzymatic inhibition assays using chemical constituents of Balanites aegyptiaca, among which 6-phenyl-2(H)-1,2,4-triazin-5-one exhibited the highest activity [36]. The authors attributed its demonstrated inhibitory activity to the formation of metal chelates between the compound and the cocatalytic zinc metal ions of PfM18AAP [36]. Based on these grounds, a hypothesis is hereby proposed that 3-(benzylamino)-4-ethyl-6-phenyl-1,2,4-triazin-5-one also works through a similar mechanism of action and thus inhibits the growth of P. falciparum by acting against PfM18AAP. Further studies are needed in the future in order to verify the conjecture and establish a firmer understanding of the compound’s mechanism of action that underlies its highly promising inhibitory activities against P. falciparum growth.

IV. CONCLUSIONS

O. japonicus ethanol extract exhibited a very promising anti-plasmodial activity in vitro. Its main active compound, identified as 3-(benzylamino)-4-ethyl-6-phenyl-1,2,4-triazin-5-one, showed not only an excellent inhibitory activity on P. falciparum growth, but also high selectivity and low cytotoxicity to human cells. These results support the development of the compound as an active principle in novel anti-malarial drugs.

Although 3-(benzylamino)-4-ethyl-6-phenyl-1,2,4-triazin-5-one poorly inhibited P. berghei growth in vivo, the phenomenon might be attributed to the compound’s specific target site that is only present in P. falciparum. In line with such observations, it was hypothesized that the compound derives its anti-plasmodial activity from its action against P. falciparum M18 aspartyl aminopeptidase, an enzyme specific to P. falciparum. In the future, further studies are required to investigate the compound’s exact mechanism of action and to further verify its safety.

REFERENCES


