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Research Article

Phytochemical Analysis, Antimalarial Properties, and Acute Toxicity of Aqueous Extracts of Trisamo and Jatu-Phala-Tiga Recipes

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Drug resistance remains a significant problem that threatens antimalarial drug treatment. Hence, the challenge is to find new effective antimalarial drugs. Based on our previous study, aqueous extracts of trisamo (TSM) and jatu-phala-tiga (JPT) had good *in vitro* antimalarial activities, and these recipes contain multiple beneficial pharmacological effects that could be useful for malaria therapy. Therefore, this study aimed to investigate the antimalarial activity and toxicity of the aqueous extracts of TSM and JPT in mouse models. The aqueous extractions were carried out using the decoction method. Compound identification was conducted using LC-QTOF-MS analysis. The antimalarial activities of TSM and JPT at doses 200, 400, and 600 mg/kg were evaluated against *Plasmodium berghei* ANKA infection using a four-day suppressive test. The toxic effects of oral administration of the extracts at 2 g/kg dose were determined using an acute toxicity test. The chemical constituents of TSM contained 83 compounds, whereas JPT contained 84 compounds. All doses of the extracts exhibited a significant suppression ($p < 0.05$) of the parasite compared to the negative control in a four-day test. The maximum activities were observed at 600 mg/kg dose with 67.02% suppression for TSM and 79.34% for JPT, followed by 400 mg/kg dose (57.63% for TSM and 64.79% for JPT) and then 200 mg/kg dose (52.35% for TSM and 54.46% for JPT). In addition, there were no significant differences ($p < 0.05$) in the RBC, MCV, and MCH levels of mice receiving JPT extract compared to the uninfected control. The WBC level of mice receiving 400 and 600 mg/kg of TSM, and 200 and 400 mg/kg of JPT, was significantly ($p < 0.05$) lower than the infected control, and the extracts did not significantly prevent the loss of platelets. For the acute toxicity test, there were no signs of toxicity or deaths in mice, and there were no differences in the histology, weight, or enzyme biochemistry of the liver and kidney between the extract and vehicle groups. However, the platelet count in the extract-treated mice was significantly higher than that in the control group. In conclusion, this study suggests that aqueous extracts of TSM and JPT have potent antimalarial activities and could be promising as new candidates for antimalarial drug development.

1. Introduction

Malaria remains one of the most serious illnesses in tropical and subtropical regions. It is a vector-borne disease caused by obligate intracellular *Plasmodium* parasites and is transmitted through the bite of infected female Anopheles mosquitoes [1]. Five species of Plasmodium, *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale*, and *P. knowlesi*, can cause diseases in humans [2]. Malarial paroxysm is a distinct clinical feature of the disease caused by the rupture of infected red blood cells (RBCs) [2]. Common symptoms include fever, chills, and headaches, whereas the major

complications of severe malaria include cerebral malaria, pulmonary edema, acute renal failure, severe anemia, bleeding, liver injury, and death [2]. According to the World Malaria Report of 2022, the estimated number of deaths globally was 619,000, and 247 million cases were reported [1]. The African region of the World Health Organization (WHO) has the highest malaria burden, accounting for approximately 95% of the global cases [1]. However, the case incidence has dropped since 2000 before increasing in 2020 due to a service interruption during the COVID-19 pandemic. Although effective treatment is a key factor in combating this disease, drug resistance poses a significant risk to the control and elimination of malaria [3]. After the emergence of chloroquine resistance in the 1960s, the WHO recommended artemisinin-based combination therapy (ACT) as the first-line treatment for uncomplicated malaria [4], which included artemether-lumefantrine (AL), artesunate-amodiaquine (AS-AQ), artesunate-mefloquine (AS-MQ), artesunate-sulfadoxine-pyrimethamine (AS-SP), dihydroartemisinin-piperaquine (DHA-PPQ), and artesunate-pyronaridine (AS-PY) combinations [3, 4]. The various treatments were determined based on the areas of resistance. Currently, the emergence of partial artemisinin resistance is of great concern and has been observed in countries in the WHO African region and the Greater Mekong Subregion (GMS) [1]. A treatment failure rate of >10% for AL in Burkina Faso and Uganda and for DHA-PPQ in Burkina Faso has been observed [1]. In GMS, mutations associated with SP resistance have been observed; hence, the failure of AS-SP could be of concern [1]. In addition, a high prevalence of mutations associated with partial artemisinin resistance was found in Myanmar and Thailand, and high rates of DHA-PPQ plus primaquine treatment failure were found in Sisaket Province, Thailand, which led the province to change its first-line drug to AS-PY in 2020 [1]. Novel strategies are needed to eradicate malarial parasites to overcome the emergence of drug-resistant parasites. Utilizing traditional medicine is one of several interesting ideas, especially polyherbal or herbal recipes, owing to their positive effects as a result of synergistic interactions [5]. Our previous study on the *in vitro* antiplasmodial properties of aqueous and ethanolic extracts of ten herbal traditional recipes reported that aqueous extracts of trisamo (TSM) and jatu-phala-tiga (JPT) exhibited good antimalarial activities [6]. Both are traditional herbal recipes that have been used for several centuries in Thailand [7]. The TSM is composed of three *Terminalia* species: *Terminalia bellirica* (Gaertn.) Roxb., *Terminalia chebula* (Roxb. ex DC.), and *Terminalia arjuna* (Roxb. ex DC.). Trisamo means "three" (tri-) fruits of *Terminalia* species (-samo), and these plants belong to the family *Combretaceae* [7]. The common names of *T. bellirica*, *T. chebula*, and *T. arjuna* are beleric myrobalans, chebulic myrobalans, and arjuns, respectively [7]. TSM is indicated for promoting good general health and relieving abdominal bloating and is also used as an antipyretic, expectorant, and rejuvenator [8]. Several biological benefits of the ingredients in the TSM recipe have been reported which include antipyretic, antibacterial, antioxidant, anti-inflammatory, antihyperglycemic, anticlastogenic, immunomodulatory, analgesic, radioprotective, gastrointestinal motility-promotion, cardioprotective, antiaging cytoprotective, anticancer, antidiabetic, wound-healing, and antinociceptive properties [8–11]. The meaning of JPT corresponds to the benefits of four fruits: Jatu means "four," phala means "fruits," and tiga means "benefits" or "usefulness." The four fruit ingredients include *Phyllanthus emblica* Linn. (*P. emblica*), *T. bellirica*, *T. chebula*, and *T. arjuna*. Indian gooseberry or *P. emblica* belongs to the family *Euphorbiaceae* and is commonly used in Ayurvedic systems for its many beneficial characteristics such as antidiabetic, antimicrobial, anti-inflammatory, and antiaging properties [7, 12]. JPT is well known for its antioxidant activity, and it is used as an antipyretic, laxative, stomachic, colon cleanser, detoxifying agent, health promotion agent, and rejuvenator in Thai traditional medicine [13, 14]. Scientific evidence has revealed that JPT has antimutagenic, cardioprotective, radioprotective, hepatoprotective, anti-inflammatory, and antiobesity properties [15, 16]. In addition, the TSM and JPT recipes consist of numerous secondary metabolites such as flavones, alkaloids, phenols, tannins, coumarin, terpenoids, glycosides, and saponins [17–19]. Several classes of phytoconstituents from natural products are responsible for their antimalarial activity [20]. Alkaloids, including terpenoidal, quinolone, and isoquinoline alkaloids, were identified with promising antimalarial activity [21]. The antimalarial action of plant flavonoids is believed to act by inhibiting fatty acid biosynthesis and the influx of L-glutamine-myoinositol in the infected red blood cells [22]. Terpene and coumarin derivatives have been reported to have potent antimalarial activities [23, 24]. Regarding the abovementioned, the phytoconstituents deposited in the TSM and JPT recipes may provide great potential for antimalarial activities. Thus, this study aimed to investigate the antimalarial activity and toxicity of TSM and JPT in a mouse model.

2. Materials and Methods

2.1. Management and Preparation of TSM and JPT Recipes. *T. bellirica*, *T. chebula*, *T. arjuna*, and *P. emblica* were bought at a Thai pharmacy store in the southern Thai province of Nakhon Si Thammarat's Muang District. The morphological identification of plants was confirmed by a botanist, and the deposited specimens SMD074002003 (*T. bellirica*), SMD070006007 (*T. chebula*), SMD070006002 (*T. arjuna*), and SMD209003007 (*P. emblica*) were at Walailak University in Thailand's School of Medicine's Department of Medical Sciences. The fruits were dried for 3 days in an oven (Memmert Model SFE 600, Schwabach, Germany) after being washed with tap water. Each fruit was ground using a herb grinder (Taizhou Jincheng Pharmaceutical Machinery Co., Ltd., Model: SF, Jiangsu, China). The TSM and JPT recipes were prepared according to Thai herbal pharmacopeia [7, 25]. The TSM recipe was prepared by mixing *T. bellirica*, *T. chebula*, and *T. arjuna*, in a 1 : 1 : 1 ratio, and the JPT recipe was prepared by mixing *T. bellirica*, *T. chebula*, *T. arjuna*, and *P. emblica* in a 1 : 1 : 1 : 1 ratio.

2.2. Aqueous Extraction Method. Aqueous extractions of TSM and JPT were performed using the decoction method [26, 27]. For each recipe, 60 g of plant material suspended in 600 mL of water was extracted by boiling for 30 min. Then, filtration through filter paper (Whatman, Buckinghamshire, England) was used to separate the liquid from the marc. Subsequently, the marc was re-extracted twice by boiling in 600 mL of water for 30 min. The rotary evaporator (Rotavapor, Buchi, China) was used to concentrate the combined filtrate at 45 rpm and 45°C. Then, the extract was dried at −89°C in a freeze-drying apparatus (Martin Christ, Germany). The crude extract was weighed, and the yield was determined as follows:

$$\text{Percentage yield} = \frac{\text{weight of crude extract}}{\text{initial weight of herbal recipe}} \times 100. \quad (1)$$

2.3. Compound Identification Using Liquid Chromatography-Quadrupole Time-of-Flight Mass Spectrometry (LC-QTOF-MS) Analysis. Chromatographic separations were accomplished according to our previous study [6]. The temperature in the column was fixed at 25°C. The mobile phase A was made up of 0.1% formic acid in water, and mobile phase B was made up of acetonitrile. A flow rate of the mobile phase was 0.20 mL/min, and the injection volume was 2 μL. The MS conditions involved an electrospray ionization (ESI) probe in negative mode with a scanning range of 100–1,200 m/z. Agilent MassHunter Workstation Software V8 was used to process the data. Compound identification was based on the similarity score, which was achieved by matching the retention times and mass data of an unknown compound to the reference spectra in a METLIN mass spectra library (Agilent Technologies). A similarity score of >90% was employed to identify the compounds deposited in the extracts.

2.4. Animals and Management. Male ICR mice that were 6–8 weeks old were obtained from Nomura Siam International Co., Ltd., in Bangkok, Thailand. All mice were acclimatized for 1 week under strictly hygienic laboratory conditions. Housing settings included cycling light and dark for 12 hours each cycle, with a regulated room temperature of 23 ± 2°C and humidity levels of 50–70%. Mice were given unlimited access to food pellets and clean drinking water.

2.5. Testing for Antimalarial Activity. The antimalarial activity was evaluated using Peters' 4-day suppressive test [28]. The rodent malaria parasite, *P. berghei* ANKA strain, was provided by Thomas F. McCutchan and obtained from BEI Resources, NIAID, NIH. Inoculation was initiated by intraperitoneal (IP) injection of 0.2 mL of infected blood into the donor mice. Once the parasitemia level reached 20–30%, blood was collected to infect recipient mice. To evaluate the *in vivo* antimalarial activity against early infection, 40 mice received IP injection of 0.2 mL of 1×10^7 *P. berghei* infected cells, whereas five mice were injected with 0.2 mL of normal saline solution as uninfected controls. Forty infected mice were randomly divided into eight groups (five mice per group), which included negative (received phosphate-buffered saline (PBS)) and positive (received 25 mg/kg chloroquine) control groups, and six experimental groups (received TSM or JPT). Aqueous extracts of TSM and JPT were administered at doses 200, 400, and 600 mg/kg. Oral administration was started at 3 h and then at 24, 48, and 72 h post-infection. On day 4, all mice were anesthetized by inhalation of 2% isoflurane in oxygen and euthanized immediately after blood collection by cardiac puncture. Thin blood smears were made from blood samples to determine the percentage of parasitemia. In addition, blood samples were used to determine changes in hematological parameters, including red blood cell (RBC) count, hemoglobin (HGB), hematocrit (HCT), mean cell volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), platelet (PLT) count, and white blood cell (WBC) count. Hematological analysis was carried out with an automatic AU480 chemistry analyzer (Beckman Coulter, USA). Parasitemia was monitored by Giemsa-stained thin blood smears, which were then viewed under a light microscope (Olympus CX31, Model CX31RBSFA, Tokyo, Japan) with oil immersion (100× magnification). The following formula was used to obtain the parasitemia and suppression percentages:

$$\begin{aligned} \%\text{parasitemia} &= \frac{\text{number of parasitised red cells}}{\text{number of total red blood cells}} \times 100, \\ \%\text{suppression} &= \frac{(\text{mean parasitemia in negative group} - \text{mean parasitemia in experimental group})}{\text{mean parasitemia in negative group}} \times 100. \end{aligned} \quad (2)$$

2.6. Acute Toxicity Test. This test was performed in accordance with the Organization for Economic Cooperation and Development (OECD) guideline No. 425 [29] according to a previously described method [6, 26, 30]. Three groups of five mice each were formed from a total of fifteen mice. PBS was used as the vehicle control for Group I. TSM and JPT were administered at a dose of 2 g/kg to Groups II and III, respectively. All mice were weighed before receiving the extract or PBS. To assess the toxicity of the extracts, 2 g/kg of the extract was administered after the mice had fasted for 3 h. The mice were observed immediately after feeding and then carefully observed for 30 min. Behavioral changes, signs of toxicity, and mortality were observed twice daily for 14 days. On day 14, all mice were weighed, anesthetized with 2%

isoflurane, and euthanized through cardiac puncture. Blood samples were collected from the heart for hematological and biochemical analyses. The liver and kidneys were removed and weighed to determine the relative organ weights. Relative organ weight was calculated using the following formula; thereafter, the organs were used for histopathological analysis.

$$\text{Relative organ weight} = \frac{\text{organ weight}}{\text{body weight}} \times 100. \quad (3)$$

2.7. Hematological, Biochemical, and Histopathological Assessment in Acute Toxicity Test. Blood was collected into two types of tubes including EDTA and serum clot activator tubes. Blood in EDTA tubes was used for the hematological analysis (RBC, HGB, HCT, MCV, MCHC, MCH, PLT, and WBC), whereas serum were used for evaluation of renal and hepatic functions, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), and creatinine. Blood samples were analyzed using an AU480 chemistry analyzer (Beckman Coulter, USA). For histopathological assessment, the liver and kidneys were fixed in 10% formalin, and hematoxylin and eosin staining was performed as previously described [31–33].

2.8. Statistical Analysis. SPSS for Microsoft Windows (version 17.0; IBM, Armonk, NY, USA) was used to conduct the statistical analyses. The mean ± standard error of the mean (SEM) is used to express all data. The data were examined for normality of distribution before being subjected to a one-way analysis of variance (ANOVA), with a significance level of $p < 0.05$.

3. Results

3.1. Percentage Yield of TSM and JPT Recipes. The percentage yield of the aqueous extracts of JPT (40.12) was slightly higher than that of TSM (39.62). The TSM appeared as a dark brown solid, and JPT was a light brown crumbly solid.

3.2. Compound Composition of TSM and JPT Detected by LC-QTOF-MS Analysis. The compounds in the TSM and JPT extracts were tentatively identified using LC-QTOF-MS analysis. Tables 1 and 2 show the compounds found in the aqueous extracts of TSM and JPT, respectively. The chemical constituents of TSM contained 83 compounds, whereas JPT contained 84 compounds. Figures 1 and 2 show the peak chromatograms of TSM and JPT, respectively.

3.3. Effects of the Extracts on Percentage Parasite Suppression. Percentage parasitemia and effects of crude extracts on the percentage suppression of *P. berghei* infection are shown in Table 3. The standard drug administered at a concentration of 25 mg/kg eliminated 100% of the blood-stage parasites. Administration of TSM and JPT extracts exhibited significant ($p < 0.05$) dose-dependent percentage parasite suppression compared to the negative control, with mean suppression percentage ranges 52.35–67.02% for TSM and 54.46–79.34% for JPT. In addition, percentage suppression at all doses of TSM was significantly lower than that in the chloroquine group ($p < 0.05$), whereas the percentage suppression at 600 mg/kg JPT showed no significant difference ($p < 0.05$) compared to chloroquine administration.

3.4. Effects of the Extracts on Hematological Changes in 4-Day Suppressive Test. The results of the hematological changes are shown in Figure 3. The indices of uninfected mice were used to represent hematologic reference values at the normal levels. Hematological alterations between normal and infected controls showed significant differences in RBC, MCV, and MCH levels ($p < 0.05$), while hemoglobin, hematocrit, and MCHC levels were not significantly different among all groups. Mice administered a standard drug demonstrated significantly higher RBC levels ($p < 0.05$), but MCV and MCH levels were significantly lower ($p < 0.05$) than those in the infected controls. TSM administered at a dose of 400 mg/kg revealed a significant decrease in RBC ($p < 0.05$), but JPT administration did not show a significant decrease in RBC compared with uninfected mice and those administered chloroquine. The differences between MCV and MCH levels showed that mice receiving JPT at all doses exhibited significantly ($p < 0.05$) lower levels than the infected control, and no difference was observed when compared to the chloroquine group. The MCV and MCH levels in the TSM group were significantly ($p < 0.05$) higher than those in the uninfected and chloroquine groups. Administration of 400 mg/kg TSM resulted in a significant increase in MCV, compared to 400 and 600 mg/kg of JPT, and all doses of TSM resulted in a significant difference in the MCH level compared to 400 mg/kg of JPT. The platelet counts of infected mice and mice that received the extracts were significantly ($p < 0.05$) lower than those of the normal control. Compared to the infected control, only the positive control group showed a significant increase in platelet counts ($p < 0.05$). In addition, chloroquine also produced a significant ($p < 0.05$) decrease in the WBC count compared to that in infected mice. The extracts did not prevent a significant ($p < 0.05$) loss of WBC compared with chloroquine. TSM (400 and 600 mg/kg) and JPT (200 and 400 mg/kg) were significantly decreased when compared to the infected control.

3.5. Clinical Observations, Analysis of Bodyweight, and Organs' Weights in Acute Toxicity Test. Oral administration of TSM or JPT at a dose of 2 g/kg in mice did not produce any significant changes in clinical signs compared to the vehicle control. There were general physical and behavioral appearances such as bright eyes, erect ears, normal body posture, and grooming. Signs of toxicity, such as diarrhea, tremors, convulsions, ataxia, or unusual behaviors, were not observed throughout 14 days. No mortality occurred during the experiment; therefore, the mean lethal dose (LD_{50}) of the aqueous extract of TSM and JPT administered via the oral route was higher than 2 g/kg. When compared to the vehicle control, the actual body weight, percentage of body weight change, and relative organ weights of the liver and kidney of

Table 1: Tentative identification of the chemical constituents from TSM by LC-QTOF-MS analysis.

No.	M/Z	RT (min)	Compounds	Formula	Molecular weight (g/mol)
1	283.2638	1.536	(+)-Isostearic acid	$C_{18}H_{36}O_2$	284.2711
2	181.0718	1.812	D-Sorbitol	$C_6H_{14}O_6$	182.0790
3	195.0507	1.837	D-Mannose	$C_6H_{12}O_7$	196.0580
4	383.1194	1.912	Acetyl-maltose	$C_{14}H_{24}O_{12}$	384.1266
5	173.0456	2.037	Shikimic acid	$C_7H_{10}O_5$	174.0529
6	205.0355	2.037	(R)-2-Hydroxybutane-1,2,4-tricarboxylate	$C_7H_{10}O_7$	206.0428
7	355.0312	2.789	(+)-Chebulic acid	$C_{14}H_{12}O_{11}$	356.0384
8	169.0145	3.403	Gallic acid	$C_7H_6O_5$	170.0217
9	243.0512	3.541	1-O-Galloylglycerol	$C_{10}H_{12}O_7$	244.0584
10	651.1193	3.879	Chrysoeriol 4′,7-diglucuronide	$C_{28}H_{28}O_{18}$	652.1266
11	325.0564	4.092	Fertaric acid	$C_{14}H_{14}O_9$	326.0636
12	235.0245	4.242	2-Hydroxy-3-carboxybenzalpyruvate	$C_{11}H_8O_6$	236.0319
13	191.0349	4.280	5,7-Dihydroxy-4-methylcoumarin	$C_{10}H_8O_4$	192.0422
14	265.0352	4.480	2-O-p-Coumaroyltartronic acid	$C_{12}H_{10}O_7$	266.0426
15	669.0937	4.606	Myricetin 3,7-diglucuronide	$C_{27}H_{26}O_{20}$	670.1008
16	299.0404	4.944	Mumefural	$C_{12}H_{12}O_9$	300.0477
17	313.0562	5.082	Salicyl acyl glucuronide	$C_{13}H_{14}O_9$	314.0636
18	469.0044	5.157	Sanguisorbic acid dilactone	$C_{21}H_{10}O_{13}$	470.0117
19	181.0144	5.345	2-Hydroxyisophthalic acid	$C_8H_6O_5$	182.0217
20	313.0562	5.621	Salicyl phenolic glucuronide	$C_{13}H_{14}O_9$	314.0634
21	285.0612	5.846	Uralenneoside	$C_{12}H_{14}O_8$	286.0684
22	359.0979	6.523	6′-Methoxypolygoacetophenoside	$C_{15}H_20O_{10}$	360.1052
23	541.0258	6.999	Punicacortein D	$C_{48}H_{28}O_{30}$	1084.0660
24	1083.0581	7.024	Punicalagin	$C_{48}H_{28}O_{30}$	1084.0653
25	347.0770	7.162	Alpha-(1,2-dihydroxyethyl)-1,2,3,4-tetrahydro-7-hydroxy-9-methoxy-3,4-dioxocyclopenta[c] [1]benzopyran-6-acetaldehyde	$C_{17}H_{16}O_8$	348.0842
26	483.0776	7.324	1,2′-di-O-galloylhamamelofuranose	$C_{20}H_20O_{14}$	484.0848
27	403.1240	7.625	Oleoside 11-methyl ester	$C_{17}H_{24}O_{11}$	404.1313
28	220.0616	7.751	Methyl dioxindole-3-acetate	$C_{11}H_{11}NO_4$	221.0689
29	321.0253	7.951	Digallate	$C_{14}H_{10}O_9$	322.0325
30	183.0297	8.126	Methyl 2,4,6-trihydroxybenzoate	$C_8H_8O_5$	184.0370
31	467.1189	8.452	Leucodelphinidin 3-O-alpha-L-rhamnopyranoside	$C_{21}H_{24}O_{12}$	468.1261
32	219.0293	8.552	Purpurogallin	$C_{11}H_8O_5$	220.0367
33	635.0885	9.003	3-O-galloylhamamelitannin	$C_{27}H_{24}O_{18}$	636.0958
34	295.0456	9.304	cis-Coutaric acid	$C_{13}H_{12}O_8$	296.0528
35	651.0831	9.454	Amlaic acid	$C_{27}H_{24}O_{19}$	652.0903
36	477.0671	9.479	Quercetin 3′-O-glucuronide	$C_{21}H_{18}O_{13}$	478.0743
37	633.0744	10.006	Pterocaryanin B	$C_{27}H_{22}O_{18}$	634.0813
38	785.0837	10.970	Sanguiin H1	$C_{34}H_{26}O_{22}$	786.0908
39	515.1912	11.133	Spicatin	$C_{27}H_{32}O_{10}$	516.1983
40	371.0976	11.734	Dihydroferulic acid 4-O-glucuronide	$C_{16}H_{20}O_{10}$	372.1049
41	249.0399	12.035	2-hydroxy-6-oxo-6-(2-hydroxyphenoxy)-hexa-2,4-dienoate	$C_{12}H_{10}O_6$	250.0472
42	247.0247	12.235	7-deshydroxypyrogallin-4-carboxylic acid	$C_{12}H_8O_6$	248.0320

Table 1: Continued.

No.	M/Z	RT (min)	Compounds	Formula	Molecular weight (g/mol)
43	447.0927	12.436	1,2,6,8-tetrahydroxy-3-methylanthraquinone 2-O-b-D-glucoside	$C_{21}H_{20}O_{11}$	448.0999
44	239.0557	12.561	(1R,6R)-6-hydroxy-2-succinylcyclohexa-2,4-diene-1-carboxylate	$C_{11}H_{12}O_6$	240.0629
45	600.9885	12.724	Diellagilactone	$C_{28}H_{10}O_{16}$	601.9956
46	465.1027	13.238	(−)-epicatechin 7-O-glucuronide	$C_{21}H_{22}O_{12}$	466.1100
47	197.0455	13.313	3,4-O-dimethylgallic acid	$C_9H_{10}O_5$	198.0527
48	937.0934	13.338	Punicafolin	$C_{41}H_{30}O_{26}$	938.1004
49	953.0890	13.664	Isoterchebin	$C_{41}H_{30}O_{27}$	954.0963
50	787.0992	14.628	1,2′,3,5-tetra-O-galloylhamamelofuranose	$C_{34}H_{28}O_{22}$	788.1064
51	609.1454	14.904	Luteolin 6-C-glucoside 8-C-arabinoside	$C_{27}H_{30}O_{16}$	610.1527
52	431.0976	14.966	Isovitexin	$C_{21}H_{20}O_{10}$	432.1048
53	300.9989	15.217	Ellagic acid	$C_{14}H_6O_8$	302.0061
54	421.0772	15.831	Isomangiferin	$C_{19}H_{18}O_{11}$	422.0844
55	491.0822	15.994	Isorhamnetin 4′-O-glucuronide	$C_{22}H_{20}O_{13}$	492.0895
56	357.1183	16.469	Phlorisobutyrophenone 2-glucoside	$C_{16}H_{22}O_9$	358.1256
57	955.1049	16.633	Chebulinic acid	$C_{41}H_{32}O_{27}$	956.1120
58	355.1027	17.071	1-O-2′-hydroxy-4′-methoxycinnamoyl-b-D-glucose	$C_{16}H_{20}O_9$	356.1100
59	261.0403	17.422	2-Acetyl-5,8-dihydroxy-3-methoxy-1,4-naphthoquinone	$C_{13}H_{10}O_6$	262.0475
60	435.0930	17.497	Taxifolin 3-arabinoside	$C_{20}H_{20}O_{11}$	436.1001
61	207.0660	18.725	Sinapyl aldehyde	$C_{11}H_{12}O_4$	208.0732
62	259.0243	18.988	Urolithin D	$C_{13}H_8O_6$	260.0316
63	243.0655	19.226	3-Desmethyl-5-deshydroxyscleroin	$C_{14}H_{12}O_4$	244.0727
64	331.0817	19.552	2′,3,5-trihydroxy-5′,7-dimethoxyflavanone	$C_{17}H_{16}O_7$	332.0889
65	461.0723	20.529	3-Methylellagic acid 8-rhamnoside	$C_{21}H_{18}O_{12}$	462.0796
66	287.0557	21.180	3′,4′,5,7-tetrahydroxyisoflavanone	$C_{15}H_{12}O_6$	288.0630
67	217.0503	21.305	Piperic acid	$C_{12}H_{10}O_4$	218.0576
68	355.0450	22.182	Grevilline C	$C_{18}H_{12}O_8$	356.0525
69	573.0875	23.886	Mangiferin 6′-gallate	$C_{26}H_{22}O_{15}$	574.0947
70	303.0508	23.987	Pratenol B	$C_{15}H_{12}O_7$	304.0580
71	461.1086	25.941	Rhamnetin 3-rhamnoside	$C_{22}H_{22}O_{11}$	462.1159
72	285.0403	27.494	Luteolin	$C_{15}H_{10}O_6$	286.0476
73	147.0449	27.720	Trans-cinnamic acid	$C_9H_8O_2$	148.0522
74	301.0348	27.795	Hieracin	$C_{15}H_{10}O_7$	302.0421
75	567.1134	28.834	Chrysophanol 8-(6-galloylglucoside)	$C_{28}H_{24}O_{13}$	568.1206
76	329.0300	28.948	2,8-di-O-methylellagic acid	$C_{16}H_{10}O_8$	330.0373
77	695.4003	31.203	Glucosyl passiflorate	$C_{37}H_{60}O_{12}$	696.4074
78	613.1187	31.278	6-cinnamoyl-1,2-digalloylglucose	$C_{29}H_{26}O_{15}$	614.1259
79	329.2329	33.307	9S,10S,11R-trihydroxy-12Z-octadecenoic acid	$C_{18}H_{34}O_5$	330.2402
80	287.2223	35.186	9,10-dihydroxy-hexadecanoic acid	$C_{16}H_{32}O_4$	288.2295
81	503.3372	35.262	(3 beta, 19 alpha)-3,19,23,24-tetrahydroxy-12-oleanen-28-oic acid	$C_{30}H_{48}O_6$	504.3444
82	273.0402	35.449	1,3,6-trihydroxy-5-methoxyxanthone	$C_{14}H_{10}O_6$	274.0474
83	343.0456	36.565	Aflatoxin GM1	$C_{17}H_{12}O_8$	344.0529

Table 2: Tentative identification of the chemical constituents from JPT by LC-QTOF-MS analysis.

No.	M/Z	RT (min)	Compounds	Formula	Molecular weight (g/mol)
1	333.0589	1.719	2-(beta-D-glucosyl)-sn-glycerol 3-phosphate	$C_9H_{19}O_{11}P$	334.0663
2	191.0560	1.794	Quinic acid	$C_7H_{12}O_6$	192.0633
3	181.0721	1.819	D-sorbitol	$C_6H_{14}O_6$	182.0793
4	209.0304	1.932	Galactaric acid	$C_6H_{10}O_8$	210.0377
5	191.0204	1.970	Glucaric acid lactone	$C_6H_8O_7$	192.0277
6	361.0413	2.045	2-O-galloylgalactaric acid	$C_{13}H_{14}O_{12}$	362.0486
7	355.0311	2.809	(+)-chebulic acid	$C_{14}H_{12}O_{11}$	356.0383
8	169.0146	3.410	Gallic acid	$C_7H_6O_5$	170.0218
9	243.0510	3.548	1-O-galloylglycerol	$C_{10}H_{12}O_7$	244.0582
10	311.0409	3.699	cis-caffeoyl tartaric acid	$C_{13}H_{12}O_9$	312.0484
11	343.0306	3.786	5-O-galloyl-1,4-galactarolactone	$C_{13}H_{12}O_{11}$	344.0379
12	325.0566	3.899	Fertaric acid	$C_{14}H_{14}O_9$	326.0639
13	651.1197	3.949	Chrysoeriol 4′,7-diglucuronide	$C_{28}H_{28}O_{18}$	652.1268
14	191.0349	4.300	5,7-dihydroxy-4-methylcoumarin	$C_{10}H_8O_4$	192.0422
15	235.0246	4.325	2-hydroxy-3-carboxybenzalpyruvate	$C_{11}H_8O_6$	236.0320
16	933.0620	4.350	2-O-galloylpunicalin	$C_{41}H_{26}O_{26}$	934.0690
17	265.0354	4.488	2-O-p-coumaroyltartronic acid	$C_{12}H_{10}O_7$	266.0428
18	669.0933	4.676	Myricetin 3,7-diglucuronide	$C_{27}H_{26}O_{20}$	670.1007
19	299.0408	4.826	Mumefural	$C_{12}H_{12}O_9$	300.0481
20	469.0045	5.202	Sanguisorbic acid dilactone	$C_{21}H_{10}O_{13}$	470.0117
21	181.0142	5.352	2-Hydroxyisophthalic acid	$C_8H_6O_5$	182.0215
22	483.0783	5.603	1,2′-di-O-galloylhamamelofuranose	$C_{20}H_20O_{14}$	484.0854
23	313.0561	5.628	Salicyl phenolic glucuronide	$C_{13}H_{14}O_9$	314.0634
24	234.0407	5.753	3,4-dihydro-7-methoxy-2-methylene-3-oxo-2H-1,4-benzoxazine-5-carboxylic acid	$C_{11}H_9NO_5$	235.0481
25	285.0611	5.853	Uralenneoside	$C_{12}H_{14}O_8$	286.0683
26	403.1238	6.191	Oleoside 11-methyl ester	$C_{17}H_{24}O_{11}$	404.1310
27	359.0984	6.505	6′-methoxypolygoacetophenoside	$C_{15}H_{20}O_{10}$	360.1056
28	1083.0578	7.031	Punicalagin	$C_{48}H_{28}O_{30}$	1084.065
29	541.0258	7.081	Punicacortein D	$C_{48}H_{28}O_{30}$	1084.066
30	347.0770	7.231	Alpha-(1,2-dihydroxyethyl)-1,2,3,4-tetrahydro-7-hydroxy-9-methoxy-3,4-dioxocyclopenta[c] [1]benzopyran-6-acetaldehyde	$C_{17}H_{16}O_8$	348.0842
31	220.0614	7.795	Methyl dioxindole-3-acetate	$C_{11}H_{11}NO_4$	221.0686
32	321.0251	7.945	Digallate	$C_{14}H_{10}O_9$	322.0323
33	1083.1151	8.159	Putranjivain A	$C_{46}H_{36}O_{31}$	1084.122
34	467.1191	8.409	Leucodelphinidin 3-O-alpha-L-rhamnopyranoside	$C_{21}H_{24}O_{12}$	468.1263
35	219.0295	8.559	Purpurogallin	$C_{11}H_8O_5$	220.0368
36	635.0882	9.023	3-O-galloylhamamelitannin	$C_{27}H_{24}O_{18}$	636.0955
37	295.0453	9.336	cis-Coutaric acid	$C_{13}H_{12}O_8$	296.0525
38	651.0832	9.462	Amlaic acid	$C_{27}H_{24}O_{19}$	652.0905
39	477.0671	9.486	Quercetin 3′-O-glucuronide	$C_{21}H_{18}O_{13}$	478.0742
40	633.0744	10.013	Pterocaryanin B	$C_{27}H_{22}O_{18}$	634.0812
41	785.0834	10.990	Sanguiin H1	$C_{34}H_{26}O_{22}$	786.0904
42	515.1912	11.165	Spicatin	$C_{27}H_{32}O_{10}$	516.1984
43	371.0975	11.742	Dihydroferulic acid 4-O-glucuronide	$C_{16}H_{20}O_{10}$	372.1047
44	935.0773	11.766	1-O-galloylpedunculagin	$C_{41}H_{28}O_{26}$	936.0843
45	249.0399	12.067	2-hydroxy-6-oxo-6-(2-hydroxyphenoxy)-hexa-2,4-dienoate	$C_{12}H_{10}O_6$	250.0472
46	247.0246	12.243	7-deshydroxypyrogallin-4-carboxylic acid	$C_{12}H_8O_6$	248.0320
47	447.0926	12.443	1,2,6,8-tetrahydroxy-3-methylanthraquinone 2-O-b-D-glucoside	$C_{21}H_{20}O_{11}$	448.0998
48	239.0556	12.568	(1R,6R)-6-hydroxy-2-succinylcyclohexa-2,4-diene-1-carboxylate	$C_{11}H_{12}O_6$	240.0629
49	600.9885	12.731	Diellagilactone	$C_{28}H_{10}O_{16}$	601.9956
50	197.0456	13.320	3,4-O-dimethylgallic acid	$C_9H_{10}O_5$	198.0528
51	937.0937	13.345	Punicafolin	$C_{41}H_{30}O_{26}$	938.1007
52	953.0890	13.734	Isoterchebin	$C_{41}H_{30}O_{27}$	954.0963
53	787.0990	14.723	1,2′,3,5-tetra-O-galloylhamamelofuranose	$C_{34}H_{28}O_{22}$	788.1064
54	431.0975	14.899	Isovitexin	$C_{21}H_2OO_{10}$	432.1047
55	300.9991	15.124	Ellagic acid	$C_{14}H_6O_8$	302.0064
56	421.0773	15.851	Isomangiferin	$C_{19}H_{18}O_{11}$	422.0844

Table 2: Continued.

No.	M/Z	RT (min)	Compounds	Formula	Molecular weight (g/mol)
57	491.0824	15.926	Isorhamnetin 4′-O-glucuronide	$C_{22}H_{20}O_{13}$	492.0897
58	463.0874	15.976	Quercetin 3-galactoside	$C_{21}H_{20}O_{12}$	464.0947
59	303.0503	16.577	(±)-taxifolin	$C_{15}H_{12}O_7$	304.0576
60	955.1041	16.628	Chebulinic acid	$C_{41}H_{32}O_{27}$	956.1113
61	355.1027	17.078	1-O-2′-hydroxy-4′-methoxycinnamoyl-b-D-glucose	$C_{16}H_{20}O_9$	356.1100
62	939.1095	17.204	1,2,3,4,6-pentakis-O-galloyl-beta-D-glucose	$C_{41}H_{32}O_{26}$	940.1166
63	435.0928	17.417	Taxifolin 3-arabinoside	$C_{20}H_{20}O_{11}$	436.0999
64	261.0402	17.429	2-Acetyl-5,8-dihydroxy-3-methoxy-1,4-naphthoquinone	$C_{13}H_{10}O_6$	262.0474
65	276.0507	17.579	2-phthalimidoglutaric acid	$C_{13}H_{11}NO_6$	277.0579
66	207.0659	18.720	Sinapyl aldehyde	$C_{11}H_{12}O_4$	208.0731
67	259.0242	19.083	Urolithin D	$C_{13}H_8O_6$	260.0316
68	461.0721	19.634	3-Methylellagic acid 8-rhamnoside	$C_{21}H_{18}O_{12}$	462.0793
69	287.0556	21.163	3′,4′,5,7-tetrahydroxyisoflavanone	$C_{15}H_{12}O_6$	288.0629
70	217.0502	21.313	Piperic acid	$C_{12}H_{10}O_4$	218.0575
71	303.0508	24.019	Pratenol B	$C_{15}H_{12}O_7$	304.0580
72	673.2124	24.370	Premithramycin A2′	$C_{33}H_{38}O_{15}$	674.2196
73	461.1084	25.961	Rhamnetin 3-rhamnoside	$C_{22}H_{22}O_{11}$	462.1157
74	285.0401	27.539	Luteolin	$C_{15}H_{10}O_6$	286.0474
75	147.0449	27.740	trans-Cinnamic acid	$C_9H_8O_2$	148.0522
76	301.0352	27.890	Hieracin	$C_{15}H_{10}O_7$	302.0424
77	295.0970	28.654	1-(4-hydroxy-3-methoxyphenyl)-5-(4-hydroxyphenyl)-1,4-pentadien-3-one	$C_{18}H_{16}O_4$	296.1043
78	329.0301	28.967	2,8-di-O-methylellagic acid	$C_{16}H_{10}O_8$	330.0373
79	695.4004	31.235	Glucosyl passiflorate	$C_{37}H_{60}O_{12}$	696.4074
80	613.1187	31.310	6-cinnamoyl-1,2-digalloylglucose	$C_{29}H_{26}O_{15}$	614.1260
81	287.2224	35.156	9,10-dihydroxy-hexadecanoic acid	$C_{16}H_{32}O_4$	288.2296
82	503.3374	35.294	(3 beta, 19 alpha)-3,19,23,24-tetrahydroxy-12-oleanen-28-oic acid	$C_{30}H_{48}O_6$	504.3445
83	273.0399	35.519	1,3,6-trihydroxy-5-methoxyxanthone	$C_{14}H_{10}O_6$	274.0472
84	343.0459	36.572	Aflatoxin GM1	$C_{17}H_{12}O_8$	344.0531

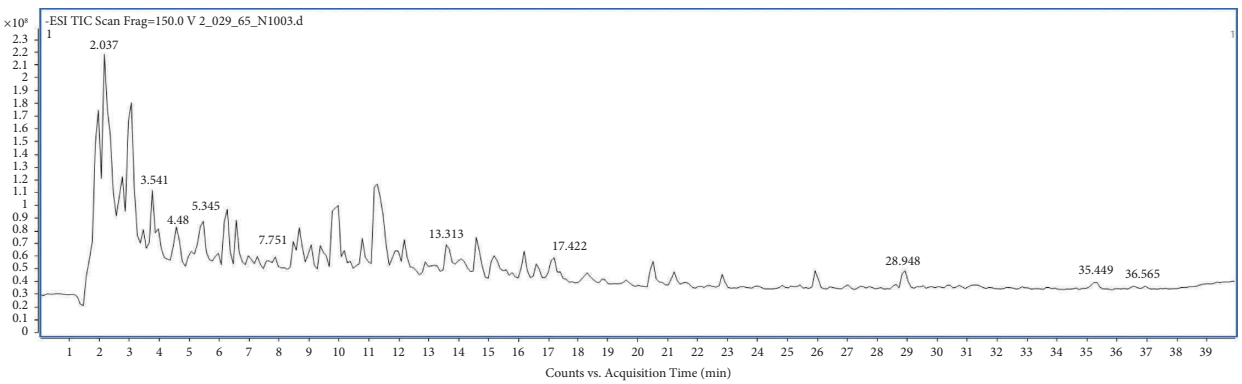

Figure 1: Full-scan chromatogram of TSM recipe.

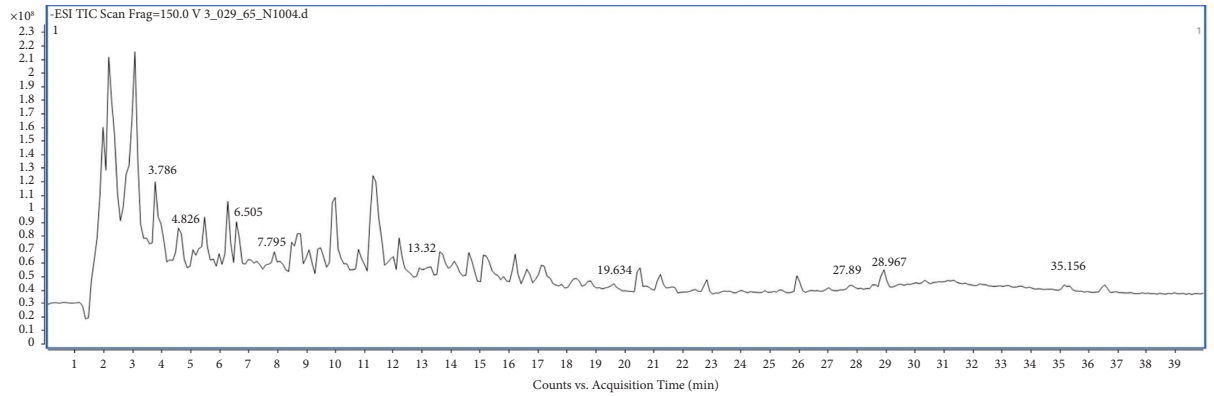

Figure 2: Full-scan chromatogram of JPT recipe.

Table 3: Percentage parasitemia and suppression of TSM and JPT recipes.

Group	Dose (mg/kg)	% parasitemia	% suppression
Not infected + PBS	—	—	—
Infected + PBS	—	18.93 ± 0.81	—
Infected + CQ	25	0[a]	100[a]
Infected + TSM	200	9.02 ± 0.72[a,b]	52.35 ± 3.82[a,b]
	400	8.02 ± 0.77[a,b]	57.63 ± 4.09[a,b]
	600	6.24 ± 0.57[a,b]	67.02 ± 2.99[a,b]
Infected + JPT	200	8.62 ± 1.70[a,b]	54.46 ± 8.97[a,b]
	400	6.67 ± 0.72[a,b]	64.79 ± 3.83[a,b]
	600	3.91 ± 1.11[a,c,h]	79.34 ± 5.86[a,c,h]

Data are presented as mean ± SEM ($n = 5$ per group). Differences were considered statistically significant at $p < 0.05$. [a]compared with the negative control group receiving PBS, [b]compared with the positive control group receiving CQ, [c]compared with TSM 200 mg/kg, [d]compared with TSM 400 mg/kg, [e]compared with TSM 600 mg/kg, [f]compared with JPT 200 mg/kg, [g]compared with JPT 400 mg/kg, and [h]compared with JPT 600 mg/kg.

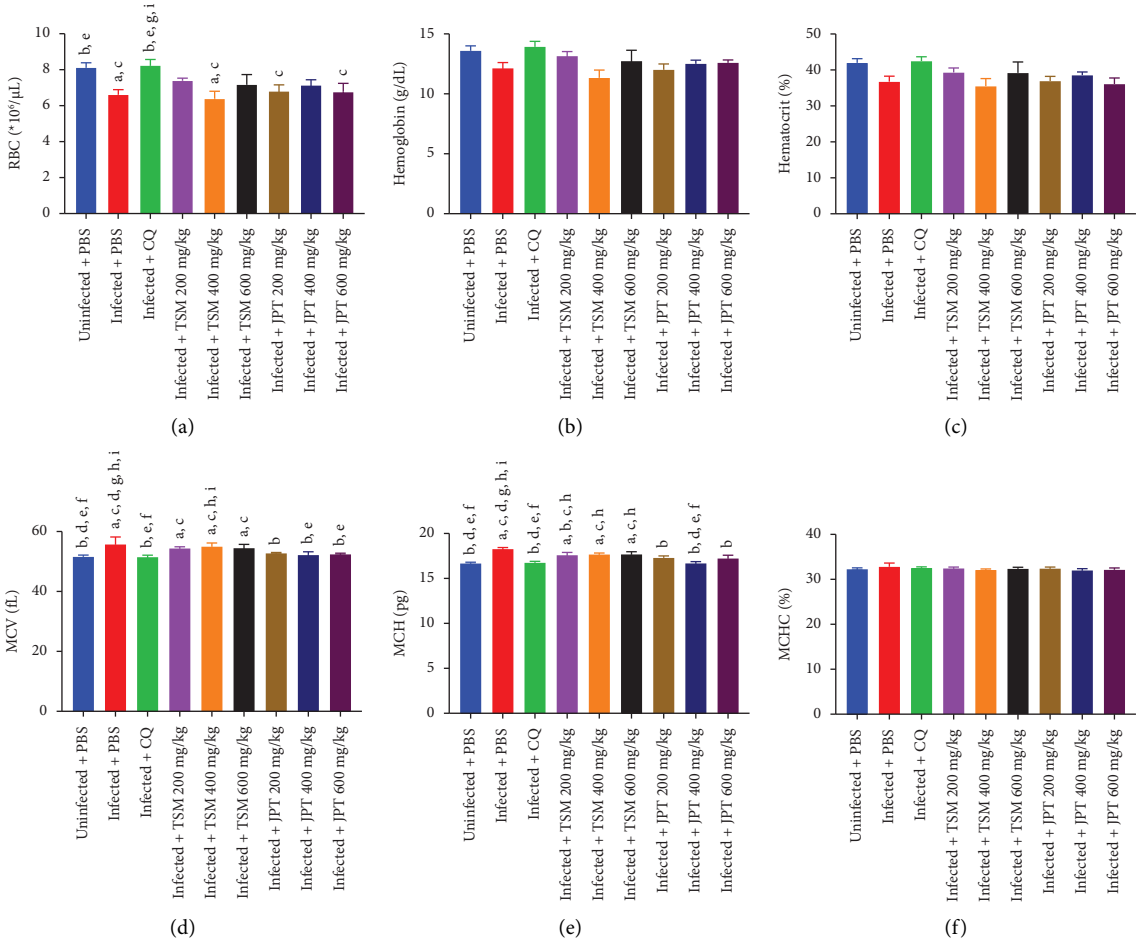

Figure 3: Continued.

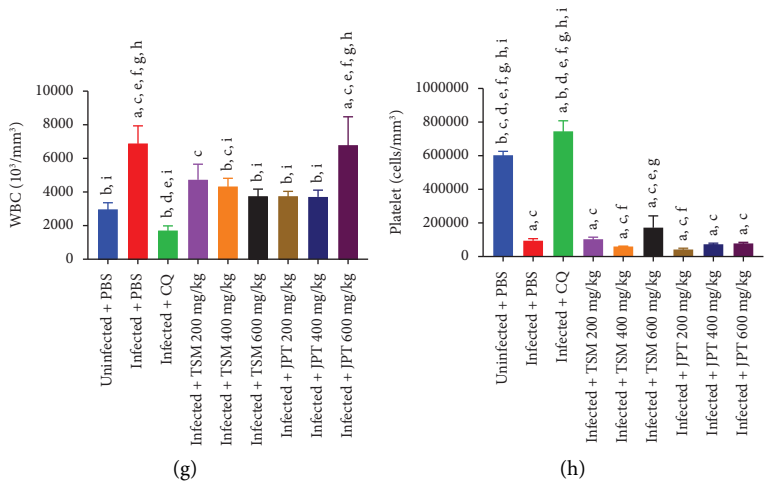

Figure 3: Effects of TSM and JPT on hematological parameters in the 4-day suppressive test: (a) RBC count, (b) hemoglobin levels, (c) hematocrit levels, (d) MCV levels, (e) MCH levels, (f) MCHC levels, (g) WBC count, and (h) platelet count. Data are presented as the mean ± SEM ($n = 5$ per group). There were statistically significant differences at $p < 0.05$, [a]compared with uninfected mice, [b]compared with negative control group receiving PBS, [c]compared with the positive control group receiving CQ, [d]compared with TSM 200 mg/kg, [e]compared with TSM 400 mg/kg, [f]compared with TSM 600 mg/kg, [g]compared with JPT 200 mg/kg, [h]compared with JPT 400 mg/kg, and [i]compared with JPT 600 mg/kg.

mice administered that the extracts showed no significant difference ($p > 0.05$) (see in Table 4).

3.6. Effects of the Extracts on Hematological and Biochemical Changes in Acute Toxicity Test. Hematological results revealed that the platelet counts of the TSM and JPT groups were significantly higher than that of the vehicle control group (see Figure 4). Biochemical parameters of the liver and kidney function tests are presented in Table 5. No significant differences in BUN, CREA, AST, ALT, or ALP levels were observed among the groups.

3.7. Effects of TSM and JPT on Histopathology in Acute Toxicity Test. Figure 5 shows the histopathological examination of the liver and kidneys in the acute toxicity test at a dose of 2 g/kg. Figures 5(a) and 5(b) show the normal structure of the liver and kidney histology, respectively, which were obtained from mice in the control group. In comparison, liver and kidney sections showed no differences between the control and mice treated with TSM and JPT. Liver sections (Figures 5(a), 5(c), and 5(e)) revealed normal hepatocytes without hepatic congestion, inflammatory cell infiltration, or sinusoidal dilatation. Kidney sections (Figures 5(b), 5(d), and 5(f)) showed unchanged glomeruli and renal tubules without vascular congestion.

4. Discussion

As antimalarial drug resistance has been a major problem in malaria control, effective vaccines are unavailable. Therefore, new treatments are urgently needed. Our previous report showed that aqueous extracts from TSM and JPT have potent antiplasmodial activity against *P. falciparum* [6]. As a result, the current study sought to assess the antimalarial properties and acute toxicity of aqueous extracts of TSM and JPT in mouse models.

For *in vivo* antimalarial testing, the antimalarial activities of TSM and JPT were investigated at 200, 400, and 600 mg/kg using a 4-day suppressive test. The highest average percentage parasite suppression of 600 mg/kg JPT was 79.34%, and the extracts at all doses significantly suppressed parasite growth compared with the infected control. However, only JPT at 600 mg/kg showed no significant difference ($p < 0.05$) compared to chloroquine, which may imply that the effect of JPT at 600 mg/kg is similar to that of chloroquine. The biological properties of plant extracts are known to be mediated by phytocomponents [34]. Based on the LC-MS analysis, our findings are consistent with those of previous studies. Chebulinic acid, gallic acid, ellagic acid, quinic acid, and luteolin were found in the fruits of *T. bellirica*, *T. chebula*, and *T. arjuna* extracts [8, 35, 36]. In addition, the phytocomponent of *P. emblica* was reported to have tannins such as chebulic acid, gallic acid, and punicalagin; flavonoids such as luteolin and quercetin derivatives; polyphenolics such as ellagic acid; and phenolics such as chebulinic acid [12, 37–39]. Ellagic acid has been reported to possess antioxidant, anti-inflammatory, antimutagenic, antiproliferative, and antimalarial properties [40, 41]. Chebulinic acid has been reported to have numerous biological activities, including antidiabetic, antifibrotic, anti-inflammatory, antitumor, antiatherogenic, antioxidant, antiulcer, hepatoprotective, and antiviral properties [42]. Quinic acid has an important antibacterial effect [43]. Accordingly, the compound described above, or the other compounds present in TSM and JPT might exert antimalarial activities through individual or synergistic effects.

Table 4: Body weight and organ weight in acute toxicity test.

Group	Body weight (g)		% increase of body weight	Relative weight (g)	
	Day 0	Day 14		Liver	Kidney
PBS	37.71 ± 0.56	41.68 ± 0.87	10.54 ± 1.51	6.96 ± 0.73	1.89 ± 0.12
TSM	35.35 ± 1.06	38.41 ± 1.31	8.68 ± 1.02	5.74 ± 0.32	1.73 ± 0.80
JPT	35.62 ± 0.74	38.78 ± 0.90	8.88 ± 1.37	5.82 ± 0.47	1.89 ± 0.92

All values are expressed as mean ± SEM. There were no statistically significant differences at $p < 0.05$.

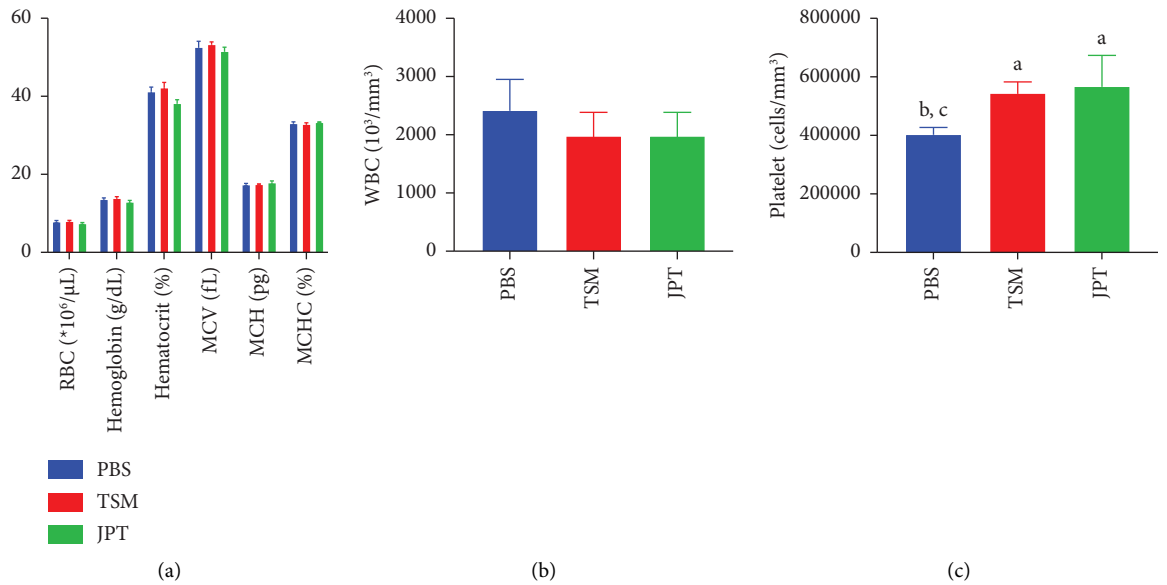

Figure 4: Effects of the extracts on hematological changes in acute toxicity test: (a) RBCs and related parameters, (b) WBC count, and (c) platelet count. All values are expressed as mean ± SEM ($n = 5$ per group). There were statistically significant differences at $p < 0.05$, [a]compared with negative control, [b]compared with 2 g/kg of TSM extract, and [c]compared with 2 g/kg of JPT extract.

Table 5: Biochemical profiles of the liver and kidney function in acute toxicity test.

Group	BUN (mg/dL)	CREA (mg/dL)	AST (U/L)	ALT (U/L)	ALP (U/L)
PBS	24.60 ± 0.40	0.16 ± 0.00	113.60 ± 13.72	32.00 ± 1.97	119.60 ± 18.20
TSM	24.20 ± 1.43	0.16 ± 0.01	116.40 ± 16.46	36.40 ± 6.02	117.40 ± 15.78
JPT	21.60 ± 0.81	0.18 ± 0.01	92.80 ± 8.52	29.80 ± 2.35	104.80 ± 7.34

All values are expressed as mean ± SEM. There were no statistically significant differences at $p < 0.05$.

In addition, the antimalarial activity of the extracts in this study showed that the percentage parasite suppression of JPT was higher than that of TSM. The reason for this could be the extra ingredient from *P. emblica* in the JPT recipes. *P. emblica* is important in traditional medicinal systems, and its various pharmacological benefits have been reported, including antimicrobial, antioxidant, anti-inflammatory, antipyretic, antitusive, antiatherogenic, anticancer, antidiabetic, antiaging, cardioprotective, gastroprotective, nephroprotective, neuroprotective, chemopreventive, analgesic, and immunomodulatory properties [44]. Furthermore, JPT and its components have been reported to exhibit strong antioxidant activity [45]. Based on previous evidence, we suggest that *P. emblica* improves the antimalarial property of the extract.

Furthermore, this study investigated the effects of the extracts on hematological parameters during malaria infection because hematological abnormalities are considered a characteristic of malaria, especially RBCs [46]. The differences in RBC, MCV, and MCH between the uninfected and infected groups were significant, and chloroquine improved these blood parameters to normal ranges compared to the uninfected control. The reduction of RBCs in infected mice may be caused by the destruction or sequestration of RBCs, or reduction of RBC production in the bone marrow [9]. The significant increases in MCV and MCH were indicative of malaria-induced macrocytic anemia [47, 48]. The RBC count in the 400 mg/kg TSM group was significantly lower than that in the uninfected control. The MCV and MCH of all TSM extract doses differed significantly from

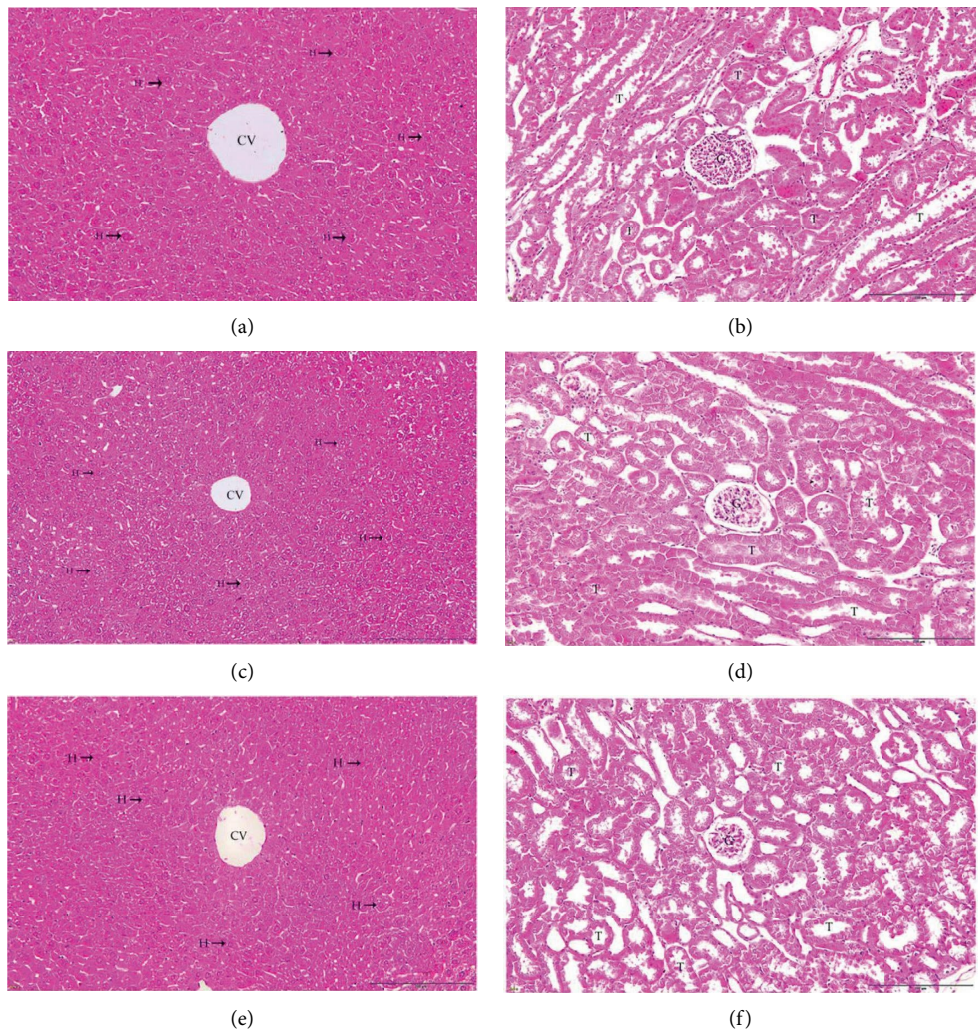

FIGURE 5: Histopathological micrograph of the liver and kidney of mice in the acute toxicity test. All images were acquired at 20× magnification. Bar = 20 μm; CV: central vein; H hepatocyte; T renal tubule; G glomerulus. (a) Liver histology of control mice; (b) kidney histology of control mice; (c) liver histology of TSM-treated mice; (d) kidney histology of TSM-treated mice; (e) liver histology of JPT-treated mice; (f) kidney histology of JPT-treated mice.

those of the uninfected control. However, the RBC count, MCV, and MCH of mice that received JPT showed no significant differences compared to the uninfected control. This finding implies that only the JPT extract can prevent malaria-induced macrocytic anemia. The ability to improve RBC and related parameters may be due to the inhibitory effects of the drug and its extracts on parasite growth. WBC and related parameters play an important role in infectious diseases [46]. WBC responds to infectious agents, and thrombocytopenia is a common feature of Plasmodium infection, which is associated with several mechanisms, such as endothelial damage and isolated platelet consumption [46]. The platelet count of mice in the infected control group was significantly reduced, but the WBC count was increased, when compared with the uninfected control group. The extracts from TSM and JPT showed a significant and dramatic decrease in platelet count compared to the normal control and chloroquine. This could mean that extracts at doses 200, 400, and 600 mg/kg did not maintain platelet homeostasis during malaria infection. In terms of WBC parameters, TSM reduced the WBC count in a dose-dependent manner. This finding may indicate that a decrease in the WBC count is associated with a reduction in the percentage of parasites. Interestingly, 600 mg/kg JPT resulted in the highest WBC count and the highest percentage parasite suppression among the extracts. This result implies that the antimalarial activity of JPT at this dose may be attributed to the activation of immune cells, which is consistent with a previous study [12]. *P. emblica*, an ingredient in the JPT recipe, has been reported to exhibit the ability to enhance immunity [12]. Consequently, JPT extract not only exhibited stronger antimalarial activity but also exhibited greater maintenance of RBC parameters than the crude extract from TSM.

Although natural products have been used to treat several diseases since ancient times, the negative effects of plant products must be considered. According to the results of the toxicity study, there were no deaths or physical or

behavioral changes for 14 days, thereby indicating that the LD_{50} of TSM and JPT was greater than 2 g/kg. TSM and JPT were classified as relatively low acute toxicity hazards in Category 5 according to the international system of chemical classification [49]. The effects of the extracts on changes in body weight and organ weight are sensitive indicators for general health status and organ damage in animals [50, 51]. We found no significant changes in body weight or organ weight when compared with the vehicle control at the endpoint. In addition, this study focused on the negative effects of the extracts on the hematological markers, biochemical enzymes, and pathology of the liver and kidney. For all blood parameters, only the platelet count was significantly increased in mice treated with TSM and JPT compared to the vehicle control. This result implies that the extract at a dose of 2 g/kg may have an effect on platelet enhancement activity, suggesting that this effect may be beneficial for improving thrombocythemia in blood diseases such as malaria. To ensure the safety of the extracts, the biochemical enzymes and pathology of the liver and kidney were assessed. The liver and kidneys play a dominant role in drug metabolism and elimination after ingestion. The liver is well known as the primary organ for parasite development in the pre-erythrocytic stages, resulting in stiffness of the infected liver cells [52]. Acute kidney injury is a well-known significant organ dysfunction caused by malaria infection, and hematological abnormalities are regarded as a key feature of malaria infection [46, 53]. There were no significant changes in biochemical enzymes and histology of the liver and kidneys. This finding implies that the extracts were not associated with nephrotoxicity or hepatotoxicity caused by herbal medicine. Regarding the results of the toxicity test, aqueous extracts of TSM and JPT showed clear evidence that the extracts were considered safe in mice when administered at 2 g/kg, and these extracts may provide great choices for antimalarial drug candidates because they are safe for major cells that can be damaged by the Plasmodium parasite, such as hepatocytes and RBCs.

5. Conclusions

This study demonstrates that aqueous extracts of TSM and JPT exert potent antimalarial activities against *P. berghei* and are considered safe for oral administration. Therefore, TSM and JPT should be considered as an alternative treatment for malaria. Further experiments should be conducted to test the antimalarial activity in nonhuman primates and in clinical trials.

Data Availability

The data associated with this study are included within the published article. Additional files are available from corresponding authors upon request.

Ethical Approval

The animal study protocol was approved by the Institutional Review Board (or Ethics Committee) of Walailak University, National Research Council of Thailand (NRCT) (protocol number: WU-ACUC-65049).

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

A.P., P.C., and C.P. designed the research studies. A.P., P.C., W.P., A.K., and C.P. carried out the experiments. A.P., P.C., and C.P. analyzed data. A.P., P.C., and C.P. reviewed statistical analysis. A.P., P.C., and C.P. drafted the original manuscript. P.C., A.W.S, and C.P. reviewed and edited the manuscript. All authors read and approved the final version of the manuscript.

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