Pharmacogn. Res., 2023; 15(4):733-750.
https://www.phcogres.com

Original Article

Evaluation of Selectivity Index and Phytoconstituents Profile of Various Extracts from the Stem of *Strychnos lucida* R. Br. as Anti-malarial

Uswatun Khasanah[1,*], Alvan Febrian Shalas[1], Bachtiar Rifai Pratita Ihsan[1], Lung Ayu Asti Wulandari[2], Nadela Cintya Nurtyas[2], Waril[2], Iin Alipiani[2], Ulfa Malihatus Sholiha[2]

[1]Department of Pharmacy, Faculty of Medicine, Brawijaya University, Malang, INDONESIA.
[2]Undergraduate Program of Pharmacy, Department of Pharmacy, Faculty of Medicine, Brawijaya University, Malang, INDONESIA.

ABSTRACT

Introduction: *Strychnos lucida* R. Br. or Songga was empirically used as an anti-malarial and immunostimulant in the Tetun tribe, Indonesia. However, almost all plants from the genus Strychnos contain the alkaloid toxic compound strychnine. **Objectives:** The aims of this study are to determine *in vitro/in vivo* anti-malarial activity, to analyze the selectivity index, and to measure the phytoconstituents of various extracts (water, ethanol, ethyl acetate, n-hexane) from *S. lucida* stem. **Materials and Methods:** *In vitro* anti-malarial study was conducted against *Plasmodium falciparum* 3D7-chloroquine-sensitive, and *in vitro* cytotoxic was performed against Monkey kidney Vero cell. *Plasmodium berghei* ANKA-chloroquine sensitive infected malaria mice were used as a model for evaluation of *in vivo* anti-plasmodial. Phytoconstituents profile was determined using Thermo scientific LC-HRMS, and the *m/z* data was analyzed using Compound Discoverer software with mzCloud MS/MS Library. **Results:** Water (W), Ethanol (E), and Ethyl Acetate (EA) extract exhibited more potential *in vitro/in vivo* anti-malarial activity than n-hexane (H) extract (IC_{50} 2.48±0.09; 2.45±0.02; 2.90±0.07; 7.64±0.30 µg/mL, respectively). The selectivity index of water, ethanol, ethyl acetate, and n-hexane extract are 211.47, 78.46, 249.62, and 32.39, respectively). According to the LC-HRMS profile, water and ethanol extract mainly contain alkaloid and phenolic compounds. Ethyl acetate and n-hexane mostly contain terpenoids and fatty acids. **Conclusion:** According to the result, we conclude that ethanol and water extract from *S. lucida* R. Br. display potential anti-malarial. Alkaloids and phenolic compounds probably have the most contribution to their anti-malarial activity.

Keywords: *Strycnos lucida* R. Br., Anti-malarial, Selectivity index, Vero cell, LC-HRMS.

Correspondence:
Mrs. Apt. Uswatun Khasanah, M.Farm.
Department of Pharmacy, Faculty of Medicine, Brawijaya University, Malang, INDONESIA.
Email: uswah_apt@ub.ac.id

Received: 05-06-2023;
Revised: 07-07-2023;
Accepted: 26-07-2023.

INTRODUCTION

Malaria is an infectious disease that has become a major project in the Sustainable Development Goals (SDGs) program. In 2024, the Ministry of Health in Indonesia targets to increase the elimination of malaria cases in 405 cities, especially in the provinces of Maluku, West Papua, East Nusa Tenggara North Maluku, and Papua.[1] The use of medicinal plants by locals in malaria-endemic areas is still common. Some of the plants used empirically to treat malaria symptoms in Indonesia are stem barks of *Alstonia spectabilis, Strychnos lucida, Fatuoa pilosa*, roots of *Calotropis gigantea, Neoalsomitra podagrica*, the whole plant of *Cleome rutidosperma, Physalis angulata, Alstonia scholaris*, leaves of *Melia azedarach*, and *Jatropha curcas*.[2,3] From the results of ethnobotanical research, *Strychnos lucida* or Songga and *Jatropha curcas* are most often mentioned and used by the Tetun people on the island of Timor.[2] The stem of *Strychnos lucida* R. Br. also shows antioxidant, anti-bacterial, and anti-cancer activity.[4] However, genus *Strychnos* contains strychnine which is a toxic compound. Clinical manifestations of strychnine toxicity include liver, kidney injury, and tonic extensor convulsions.[5-8] Based on these data, it is required to study the anti-malarial activity and cytotoxic of various extracts of *Strychnos lucida* R. Br. stem both *in vitro* and *in vivo*. Secondary metabolites screening is also required to identify the phytoconstituents that contribute to its anti-malarial activity.

OPEN ACCESS

DOI: 10.5530/pres.15.4.078

Publishing Partner : EManuscript Tech. [www.emanuscript.in]

MATERIALS AND METHODS

Plant material and extraction

Strychnos lucida R. Br. stem was obtained from Dompu, Bima district, West Nusa Tenggara, Indonesia. The stems were harvested when the plants were 7-9 years old, with a stem diameter of 5-10

cm. The sample was identified by botanist and a plant voucher specimen was saved with code 787/IPH.06/HM/VII/2020 in Purwodadi Botanical Garden, East java, Indonesia. The stems are dried, powdered, and then stored in an airtight container. *Strychnos lucida* R. Br. stem powder was macerated using organic solvents of 80% ethanol, ethyl acetate, and n-hexane for 72 hr. Some stem powder is boiled with distilled water for 15 min (starting when the water starts to boil). The ratio of *S. lucida* stem powder and solvent is 1:10. The organic solvent from liquid extract was removed using a rotary evaporator and oven at temperature 400C, in addition the water extract was dried using freeze dryer. The dried extract was stored at 2-6°C for further procedure.

Culture of *Plasmodium falciparum* 3D7-chloroquine sensitive

Plasmodium falciparum 3D7-chloroquine sensitive was obtained from the Institute of Tropical Disease (ITD), Airlangga University, Surabaya, Indonesia. The *Plasmodium falciparum* culture method refers to Trager and Jensen with some modifications.[9] The parasite was cultured on human blood group O+ in complete media. Complete media consisted of RPMI 1640 supplemented with 0.05 g hypoxanthine, 5.96 g HEPES, 50 g/mL gentamicin, 2.1 g $NaHCO_3$, and 10% human O+ serum. Parasite cultures were stored in a petri dish and incubated at 37°C. Parasite growth monitoring was conducted by completing thin blood smears and Giemsa staining. Percent parasitaemia was calculated under the microscope at 1000x magnification. Subculture was performed if the parasitaemia percentage had reached 6%.

In vitro anti-malarial assay

The culture stock was diluted with complete media and O+ human blood to reach the parasitaemia percent of 1%, and most of the parasites were in the ring stage. The anti-malarial activity procedure refers to Khasanah with modifications.[10] Each sample was weighed 10 mg and dissolved in 100 µL DMSO. Serial dilutions were completed for each sample to obtain five concentrations (100; 10; 1; 0.1; 0.01 µg/mL). The anti-malarial assay was conducted using 24 well microplates. Each well contained 500 µL sample solutions and 500 µL parasite cultures. Chloroquine Diphosphate (CQ) was used as a standard anti-malarial drug. The microplates were incubated for 48 hr at 37°C.

The slides from the anti-malarial assay were observed under a microscope with 1000x magnification and counted the number of parasite-infected erythrocytes per 1000 erythrocytes. Percent parasitaemia was calculated using the following formula:

$$\text{Growth percentage (\% growth)} = \text{\% Parasitemia} - D_0$$

$$\text{Percent of inhibition (\% inhibition)} = 100\% - \{Xe/Xc) \times 100\%\}$$

Note

- D_0 = Parasitemia percentage of infected red blood cell on day 0.
- Xe = Growth percentage of experimental groups.
- Xc = Growth percentage of negative control.

If values were calculated using Probit analysis using SPSS 26.

In vitro toxicity assay

Monkey kidney Vero cells was obtained from the Institute of Tropical Disease (ITD), Airlangga University, Surabaya, Indonesia. The culture procedure of monkey kidney Vero cell lines refers to Dwivedi with modification.[11] Monkey kidney Vero cells were grown in a flash containing complete media (RPMI, Penicillin-streptomycin 1%, Fungizone 0.5%, FBS 10%). Subculture was performed when the cells became 90% confluent by disaggregating the cells using trypsin phosphate into suspension. The culture stock was diluted using complete media to obtain a 1 x 10^5 cells/mL density. The cell suspension was seeded into 96-well microtiter plates and incubated for 24-48 hr until the cells were 90% confluent. After the cell was attached, the complete media was removed. One hundred microliters extract solutions were added into each well and incubated for 24 hr at 37°C.

Cytotoxic activity was determined using the MTT assay. One hundred microliters of MTT solution (5 mg/mL) were added to each well and incubated for 4 hr. After incubation, the MTT solution was removed, and DMSO was added to each well. The absorbance was recorded using an ELISA reader at a wavelength of 540 nm, and the percent viability was used to calculate the cytotoxic concentration CC_{50} value of each sample.

In vivo anti-malarial activity assay against *Plasmodium berghei* ANKA-chloroquine sensitive

In vivo anti-malarial procedure had been reviewed and approved by the ethical commission faculty of medicine, Brawijaya university, with a number 311/EC/KEPK/10/2021.

Plasmodium berghei ANKA-chloroquine sensitive was obtained from the parasitology laboratory, Faculty of Medicine, University of Brawijaya, Malang, Indonesia. Mus musculus Balb/C were obtained from the pharmacology laboratory of the Faculty of Medicine, Brawijaya University. The male mice were 2 months old and weighed 20-30 g. Mice were acclimatized for seven days and fed *ad libitum* before treatment. Experimental animals were divided into 14 groups: negative control (infectious mice and received CMC-Na solution), positive group (chloroquine 10 mg/kg BW), ethanol extract group (dose of 100; 10; 1 mg/kg BW), water extract group (dose of 100; 10; 1 mg/kg BW), ethyl acetate extract group (dose of 100; 10; 1 mg/kg BW), n-hexane extract group (dose of 100; 10; 1 mg/kg BW). Each group consisted of 5 mice.

Donor mice were inoculated with 0.2 mL frozen stock of *Plasmodium berghei* intraperitoneally. Observation of percent parasitaemia was performed every day through a tail blood smear.

Donor mice were sacrificed when the parasitemia percentage reached 10%. The number of erythrocytes was calculated using a hematocytometer, and each animal obtained 10^7 erythrocytes intraperitoneally.

The *in vivo* anti-malarial activity assay refers to the 4-suppressive daily test.[12] Each extract was dissolved in CMC-Na and administered orally for four days (day 0 – day 3). Thin blood smears from the tails were made for seven days (day 0 – day 6), then calculated percent parasitemia, parasitaemia growth percentage, and percent growth inhibition using the following formula:

$$Growth\ inhibition\ percentage\ =\ 100\% - \left(\frac{Xe}{Xc}\ x\ 100\%\right)$$

Note

Xe: Parasitaemia growth percentage of experimental group.

Xc: Parasitaemia growth percentage of control negative group.

Statistical analysis

Analysis of several data were performed using Statistical Product and Service Solution 26 (IBM SPSS 26). Probability test was conducted to determine IC_{50} from *in vitro* anti-malarial assay. In addition, one-way ANOVA was used to evaluate the significancy data of growth percentage and inhibition percentage each group with $p< 0.05$.

Phytoconstituents profiling using LC-HRMS

Each extract was weighed 10 mg and dissolved in 10 mL of the appropriate solvent. 80% aquadest and ethanol extract were dissolved in pro-HPLC aquadest, n-hexane and ethyl acetate extract in pro-HPLC methanol. The extract solution was filtered using a 0.22 µM membrane filter. Samples were injected into HPLC Thermo Scientific Dionex Ultimate 3000 RSLCano with this method:

Mobile phase: (A) formic acid 0.1% in water and (B) formic acid 0.1% in acetonitrile.

Stationary phase: Hypersil GOLD aQ 50 x 1 mm x 1.9 µM particle size.

The column temperature: 30°C.

Flow rate: 40 µL/min.

High-resolution mass spectrometer Thermo Scientific Q Exative is used as a detector in positive ion mode. The *m/z* data is processed using Compound Discoverer software with mzCloud MS/MS Library.

RESULTS

Extraction, *in vitro* anti-malarial and cytotoxic assay

Strycnos lucida R. Br. extraction process produces the following percent yield: water extract (2.40% w/w); ethanol extract 80% (3.67% w/w); ethyl acetate extract (0.69% w/w); n-hexane extract (0.59% w/w). In anti-malarial activity showed that water and ethanol extracts could inhibit the growth of parasites more potentially than ethyl acetate and n-hexane extracts (Tables 1 and 2). Ethanol and n-hexana extract exhibited potential cytotoxic against Vero cell monkey kidney. In addition, selectivity index from the highest was ethyl acetate extract, water extract, ethanol extract, and n-hexane extract, respectively (Table 1).

In vivo anti-malarial activity

Paracitemia percentage of each sample followed the dose dependent manner, higher concentration possed lower paracitemia percentage (Figure 1). Each extract inhibited the paracitemia growth, and attenuated the replication of *Plasmodium falciparum*, which is shown by inhibition percentage. Therefore, in this study, water extract exhibited lower paracitemia percentage than other extracts (Figure 1). There was no significant difference between the growth percentage of water extract group and chloroquine group (Table 2). The inhibition percentage of each extract ranged from 26.74% - 72.72%. Therefore, higher inhibition was shown by water extract at dose 100 mg/kgBW (Table 2).

Analysis of phytoconstituents profile

Phytoconstituents profiling was conducted to determine each extract's secondary metabolite compounds. The water extract mainly composed of 41.778% alkaloids and 17.504% phenolic derivates (Figure 2). Some of the phenolic compounds in the water extract were (1R,3R,4S,5S)-4-{[(2E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy}-1,3,5-trihydroxycyclohexane-1-carboxylic acid identified as cinnamic acid derivates and 4-methoxybenzaldehyde (Table 3).[13] The most abundant of alkaloid compounds in the aqueous extract were choline; 2,2,6,6-Tetramethyl-1-piperidinol (TEMPO) and betain (Table 3).

The ethanol extract contained alkaloids (39.934%), phenolic derivates (22.143%), and terpenoids (14.645%) (Figure 2). The phenolic compounds in the ethanol extract included apocynin, pyrogallol, myristicine, and caffeic acid (Table 4). Choline, TEMPO, Betaine and D-(+)-pipecolinic acid compounds had the highest percentages among other alkaloids (18.034; 6.769; 5.418; and 4.107%, respectively).

The phytoconstituents of ethyl acetate extract mainly composed of alkaloid (25.847%), terpenoids (22.859%), and fatty acid derivates (21.436%) (Figure 2). Strychnine, reserpine, nicotinamide, naphthoquinone, benzylamine, and piperidine derivatives were identified as alkaloid from ethyl acetate extract (Table 5).

Table 1: ***In vitro*** **anti-malarial and cytotoxic profiles of each extract.**

Samples	Anti-malarial (IC_{50} = µg/mL)	Cytotoxic (CC_{50} = µg/mL)	Selectivity index
Water extract	2.48±0.09	524.86±30.82	211.47
Et-OH extract	2.45±0.02	192.26±4.39	78.46
Ethyl acetat extract	2.90±0.07	724.27±136.18	249.62
n-hexana extract	7.64±0.30	247.27±2.21	32.39

Table 2: **Evaluation of** ***in vivo*** **anti-malarial from each extract.**

Groups	Concentration (mg/kgBW)	Growth percentage (%)	Inhibition percentage (%)
Water extract	1	1.47±0.26	59.70[b]
	10	1.35±0.15	62.90[b]
	100	0.99±0.12	72.72[b]
Et-OH extract	1	2.44±0.54[a]	33.06[b]
	10	2.42±0.25[a]	33.49[b]
	100	2.05±0.60[a]	43.74[b]
Ethyl acetat extract	1	2.50±0.44[a]	31.34[b]
	10	2.16±0.32[a]	40.63[b]
	100	2.07±0.21[a]	42.99[b]
n-hexana extract	1	2.67±0.40[a]	26.74[b]
	10	2.32±0.24[a]	36.32[b]
	100	2.24±0.31[a]	38.53[b]
Negative control group	NA	3.64±2.71[a]	NA
Positive (chloroquine) group	10	0.039±0.026	98.93

Notes: [a]growth percentage $p< 0.05$ (compare to chloroquin group); [b]inhibition percntage $p< 0.05$ (compare to chloroquin).

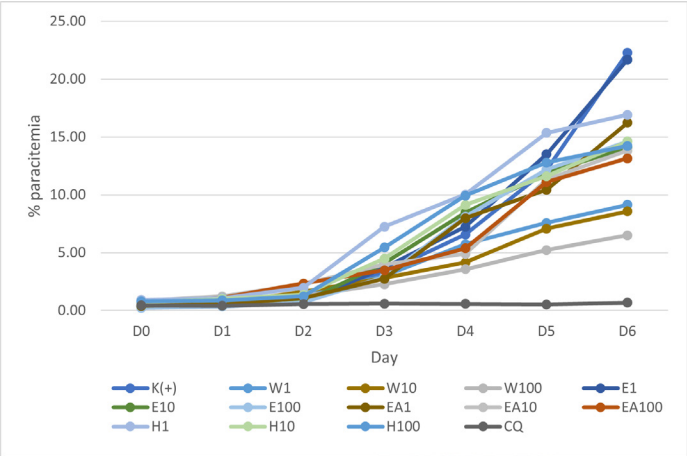

Figure 1: Paracitemia percentage of each extract and dose from D0 – D6.

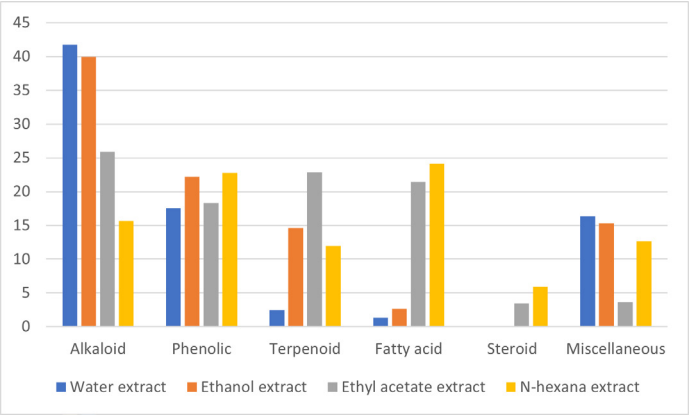

Figure 2: Percentage of secondary metabolites profile from each extract.

Table 3: Secondary metabolite profiles of water extract of *S. lucida* R. Br.

Compounds	Rt (Retention time)	Formula	Calculated Molecular weight	Area percentage (%)
Alkaloid				
Choline	1.065	$C_5H_{13}N\ O$	103.09966	15.958
2,2,6,6-Tetramethyl-1-piperidinol (C9H19NO)	12.35	$C_9H_{19}N\ O$	157.14635	8.247
Betaine	0.9	$C_5H_{11}NO_2$	117.07884	5.578
Nicotinamide	1.248	$C_6H_6N_2O$	122.04787	2.028
2-[amino(3-chloroanilino)methylene]malononitrile (C10H7ClN4)	0.874	$C_{10}H_7CLN_4$	218.03983	1.778
(15E)-15-ethylidene-18-(methoxycarbonyl)-17-methyl-12-oxo-10,17-diaz atetracyclo[12.3.1.0Âl,ÂaÂa.0â•´,â•a]octadeca-3(11),4,6,8-tetraen-17-ium-17-olate (C21H24N2O4)	5.506	$C_{21}H_{24}N_2O_4$	368.17293	1.698
N-{4-[(2R,3R)-3-(Hydroxymethyl)-4-methyl-5-oxo-2-morpholinyl]phenyl}-3-phenylpropanamide	6.409	$C_{21}H_{24}N_2O_4$	350.16249	0.851
5-(4-morpholinoanilino)-5-oxo-3-phenylpentanoic acid	5.052	$C_{21}H_{24}N_2O_4$	368.17293	0.780
5-({[3-chloro-5-(trifluoromethyl)-2-pyridyl]methyl} thio)-4-pentyl-4H-1,2,4- triazol-3-ol	0.978	$C_{14}H_{16}CLF_3N_4OS$	380.07143	0.724
4-(dimethylamino)-1,1-diphenylbut-3-en-2-one	1.047	$C_{18}H_{19}NO$	265.15199	0.707
EPK	6.194	$C_{16}H_{28}N_4O_6$	394.18852	0.565
Caprolactam	3.483	$C_6H_{11}NO$	113.08399	0.511
Dothiepin	6.158	$C_{19}H_{21}NS$	295.14147	0.433
DEET	11.722	$C_{12}H_{17}NO$	191.13071	0.342
N1-(3-Pyridylmethyl)-3-(3,4-dichlorophenyl) acrylamide	0.87	$C_{15}H_{12}Cl_2N_2O$	306.03465	0.270
3-(5-phenyl-1,3-oxazol-2-yl)-4-(trifluoromethyl) pyridine	0.879	$C_{15}H_9F_3N_2O$	290.06066	0.246
4-morpholinobenzoic acid	2.19	$C_{11}H_{13}NO_3$	207.08919	0.238
1H-indene-3-carboxamide	0.941	$C_{10}H_9NO$	181.05023	0.162
Rilpivirine	4.92	$C_{22}H_{18}N_6$	366.15724	0.159
α-Pyrrolidinopropiophenone	16.634	$C_{13}H_{17}NO$	203.13077	0.151
N-Cyclohexyl-N-methylcyclohexanamine	7.296	$C_{13}H_{25}N$	195.19838	0.146
N1-[4-(6-Methyl-1,3-benzothiazol-2-yl)phenyl]-2-cyclopentyl-2-phenylphenymide	5.298	$C_{27}H_{26}N_2OS$	426.17854	0.134
Isocytosine	1.246	$C_4H_5N_3O$	111.04321	0.072
Total				41.778
Phenolic				
(1r,3R,4s,5S)-4-{[(2E)-3-(3,4-dihydroxyphenyl) prop-2-enoyl]oxy}-1,3,5-trihydr oxycyclohexane-1-carboxylic acid	2.829	$C_{16}H_{18}O_9$	354.09462	6.082
2,3-Dihydroxybenzoic acid	2.374	$C_7H_6O_4$	154.02629	1.601
4-oxo-4,5,6,7-tetrahydrobenzo[b]furan-3-carboxylic acid	2.725	$C_9H_8O_4$	180.04181	1.756

Compounds	Rt (Retention time)	Formula	Calculated Molecular weight	Area percentage (%)
Caffeic acid (C9H8O4)	4.761	C_9H_8O4	180.04181	0.822
3,4-Dihydroxyphenylpropionic acid	6.144	$C_9H_{10}O_4$	164.04704	0.699
4-Methoxybenzaldehyde	1.712	$C_8H_8O_2$	136.05216	1.063
1S,3R,4S,5R)-3,5-bis({[(2E)-3-(3,4-dihydroxyphenyl) prop-2-enoyl]oxy})-1,4-dihydr oxycyclohexane-1-carboxylic acid	8.129	$C_{25}H_{24}O_{12}$	516.12621	0.763
7-Hydroxycoumarine	14.838	$C_9H_6O_3$	162.03131	0.399
(1aR,2E,4aR,6S,7S,7aR,8R,11aS)-1,1,3,6-Tetrameth yl-9-methylene-4-oxo-1,1a,4,5,6,7,7a,8,9,10,11,11a-dodecahydro-4aH-cyclopenta[a]cyclopropa[f][11] annulene-4a,7,8-triyl triacetate	8.941	$C_{26}H_{36}O_7$	460.2455	0.493
Bis(4-ethylbenzylidene)sorbitol	14.618	$C_{24}H_{30}O_6$	414.20358	0.358
4-(2,3-dihydro-1,4-benzodioxin-6-yl)butanoic acid	13.159	$C_{12}H_{14}O_4$	244.07076	0.340
3,5-di-tert-Butyl-4-hydroxybenzaldehyde	16.957	$C_{15}H_{22}O_2$	234.1617	0.331
(2S,4R,5S,6S,7R)-5,6,12,14-tetrahydroxy-4-(hydroxymethyl)-13-methoxy-3,8-dioxatricyclo[8.4.0.0Â?,â•·]tetradeca-1(14),1 0,12-trien-9-one	0.982	$C_{14}H_{16}O_9$	350.06087	0.299
4-methoxy-6-(prop-2-en-1-yl)-2H-1,3-benzodioxole	7.64	$C_{11}H_{12}O_3$	192.07842	1.051
6-Gingerol	13.037	$C_{17}H_{26}O_4$	276.17195	0.272
4-Hydroxy-6-methyl-2-pyrone	5.579	$C_6H_6O_3$	126.03154	0.267
Vanillin	6.14	$C_8H_8O_3$	152.04706	0.264
3,5-di-tert-Butyl-4-hydroxybenzoic acid	14.942	$C_{15}H_{22}O_3$	250.15654	0.174
Apocynin	2.103	$C_9H_{10}O_3$	166.06273	0.137
4-oxo-5-phenylpentanoic acid	8.51	$C_{11}H_{12}O_3$	192.07833	0.221
2-(Cyclohexylmethylidene)-1,2,3,4-tetrahydronaphth alen-1-one	1.265	$C_{17}H_{20}O$	240.14685	0.111
Total				17.504
Terpenoid				
4-(4-hydroxy-2,6,6-trimethyl-3-{[(2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy} cyclohef1-en-1-yl)butan-2-one	6.078	$C_{19}H_{32}O_8$	410.18325	1.251
(-)-Caryophyllene oxide	7.501	$C_{15}H_{24}O$	220.18242	0.413
Crotonic acid	1.251	$C_4H_6O_2$	86.03696	0.395
Diacetoxyscirpenol	6.895	$C_{19}H_{26}O_7$	388.14498	0.262
Loganin	5.506	$C_{17}H_{26}O_{10}$	407.17839	0.156
Total				2.478
Fatty acid				
Eicosatetraynoic acid	12.608	$C_{20}H_{24}O_2$	296.17699	1.114
Tetranor-12(S)-HETE	16.975	$C_{16}H_{26}O_3$	248.17731	0.260
Total				1.374
miscellaneous compounds (phthalate, sulphate, glucose, amino acid derivates)				

Compounds	Rt (Retention time)	Formula	Calculated Molecular weight	Area percentage (%)
Diisobutylphthalate	17.925	$C_{16}H_{22}O_4$	278.15105	7.285
Bis(2-ethylhexyl) phthalate	23.163	$C_{24}H_{38}O_4$	390.27641	0.143
n-Pentyl isopentyl phthalate	18.079	$C_{18}H_{26}O_4$	323.20915	1.082
D-(+)-Proline	0.968	$C_5H_9NO_2$	115.06326	6.808
Prolylleucine	1.39	$C_{11}H_{20}N_2O_3$	228.14696	0.168
Tributyl phosphate	16.525	$C_{12}H_{27}O_4P$	266.16431	0.557
D-(+)-Maltose	0.877	$C_{12}H_{22}O_{11}$	364.09748	0.303
Total				16.348

Table 4: Secondary metabolite profiles of ethanol extract of *Strychnos lucida* R. Br.

Compounds	Rt (Retention time)	Formula	Calculated Molecular weight	Area percentage (%)
Alkaloid				
Choline	1.061	C_5H_1NO	103.09975	18.034
2,2,6,6-Tetramethyl-1-piperidinol (TEMPO)	12.346	$C_9H_{19}NO$	157.14635	6.769
Betaine	0.885	$C_5H_{11}NO_2$	117.07889	5.418
EPK	6.202	$C_{16}H_{28}N_4O_6$	394.18889	0.715
D-(+)-Pipecolinic acid	1.246	$C_6H_{11}NO_2$	129.07877	4.107
Caprolactam	3.468	$C_6H_{11}NO$	113.08406	0.376
DEET	11.715	$C_{12}H_{17}O$	191.13075	0.238
11-piperidino-2,3-dihydro-1H-cyclopenta[4,5] pyrido[1,2-a]benzimidazole-4-carbonitrile	13.036	$C_{20}H_2N_4$	316.16478	0.231
Rilpivirine	4.944	$C_{22}H_{18}N_6$	366.15749	0.102
5-({[3-chloro-5-(trifluoromethyl)-2-pyridyl]methyl} thio)-4-pentyl-4H-1,2,4- triazol-3-ol	0.975	$C_{14}H_{16}ClF_3N_4OS$	380.07152	0.920
4-(dimethylamino)-1,1-diphenylbut-3-en-2- one	1.045	$C_{18}H_{19}NO$	265.1519	0.678
N-{4-[(2R,3R)-3-(Hydroxymethyl)-4-methyl-5-oxo-2-morpholinyl]phenyl}-3- phenylpropanamide	6.432	$C_{21}H_{24}N_2O_4$	350.16274	0.587
Dothiepin	6.173	$C_{19}H_{21}NS$	295.14162	0.508
Nicotinic acid	1.242	$C_6H_5NO_2$	123.03196	0.195
N-({(2R,4S,5R)-5-[3-(4-Fluorophenyl)-1-methyl-1H-pyrazol-5-yl]-1- azabicyclo[2.2.2]oct-2-yl} methyl)acetamide	4.342	$C_{20}H_{25}FN_4O$	378.18611	0.180
3-(5-phenyl-1,3-oxazol-2-yl)-4- (trifluoromethyl) pyridine	0.876	$C_{15}H_9F_3N_2O$	290.06081	0.172
N-Cyclohexyl-N-methylcyclohexanamine	7.309	$C_{13}H_{25}N$	195.19846	0.168
Nicotinamide	1.244	$C_6H_6N_2O$	122.04794	0.131
2-oxa-4-azatetracyclo[6.3.1.1~6,10~.0~1,5~] tridecan-3-one	1.245	$C_{11}H_{15}NO_2$	193.11	0.114
α-Pyrrolidinopropiophenone	16.621	$C_{13}H_{17}NO$	203.13083	0.109

Compounds	Rt (Retention time)	Formula	Calculated Molecular weight	Area percentage (%)
N1-[4-(6-Methyl-1,3-benzothiazol-2- yl)phenyl]-2-cyclopentyl-2-phenylacetamide	5.327	$C_{27}H_{26}N_2OS$	426.17861	0.100
2-[(5-methyl-3-phenyl-4-isoxazolyl) methyl]-1H-1,2-benzisothiazole-1,1,3(2H)-trione	0.875	$C_{18}H_{14}N_2OS$	376.04021	0.082
Total				39.934
Phenolic				
6-Gingerol	13.036	$C_{17}H_{26}O_4$	276.17204	0.808
(1r,3R,4s,5S)-4-{[(2E)-3-(3,4- dihydroxyphenyl) prop-2-enoyl]oxy}-1,3,5- trihydroxycyclohexane-1-carboxylic acid	2.844	$C_{16}H_{18}O_9$	354.09453	5.572
2,3-Dihydroxybenzoic acid	2.365	$C_7H_6O_4$	154.02632	1.673
4-oxo-4,5,6,7-tetrahydrobenzo[b]furan-3-carboxylic acid	2.721	$C_9H_8O_4$	180.04195	1.806
4-methoxy-6-(prop-2-en-1-yl)-2H-1,3- benzodioxole	7.716	$C_{11}H_{12}O_3$	192.07833	1.703
3,4-Dihydroxyphenylpropionic acid	13.453	$C_9H_{10}O_4$	164.04711	0.735
3,5-di-tert-Butyl-4-hydroxybenzaldehyde	16.963	$C_{15}H_{22}O_2$	234.16168	0.691
Vanillin	6.163	$C_8H_8O_3$	152.04714	0.389
Diethyl phthalate	13.151	$C_{12}H_{14}O_4$	222.08889	0.386
4-Methoxybenzaldehyde	4.703	$C_8H_8O_2$	136.0522	1.348
4-Hydroxy-6-methyl-2-pyrone	5.603	$C_6H_6O_3$	126.03156	0.329
4-hydroxy-6-[2-(2-methyl-1,2,4a,5,6,7,8,8a-octahydronaphthalen-1-yl)ethyl]oxan-2-one	15.002	$C_{18}H_{28}O_3$	314.18777	0.619
Diacetoxyscirpenol	6.914	$C_{19}H_{26}O_7$	388.14525	0.243
7-Hydroxycoumarine	14.839	$C_9H_6O_3$	162.0314	0.374
methyl 3,4,5-trihydroxycyclohex-1-ene-1-carboxylate	1.922	$C_8H_{12}O_5$	170.05773	0.197
Caffeic acid	4.78	$C_9H_8O_4$	180.04195	0.170
(1S,4S,5R,9R,13S)-5,9-dimethyl-14- methylidenetetracyclo[11.2.1.0Âa,Âaâ•°.0â•´, âa•]hexadec-10-ene-5-carboxylic acid	12.897	$C_{20}H_{28}O_2$	300.20848	0.170
Apocynin	1.691	$C_9H_{10}O_3$	166.06278	0.368
3,5-di-tert-Butyl-4-hydroxybenzoic acid	14.936	$C_{15}H_{22}O_3$	250.15651	0.134
Pyrogallol	0.866	$C_6H_6O_3$	126.03156	0.074
4-Hydroxybenzaldehyde	6.164	$C_7H_6O_2$	122.0367	0.067
4-(4-hydroxy-2,6,6-trimethyl-3-{[(2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6- (hydroxymethyl)oxan-2-yl]oxy} cyclohex-1- en-1-yl)butan-2-one	6.096	$C_{19}H_{32}O_8$	410.18354	2.091
(2S,4R,5S,6S,7R)-5,6,12,14-tetrahydroxy-4-(hydroxymethyl)-13-methoxy-3,8-dioxatricyclo[8.4.0.0Â?,â·]tetradeca-1(14),10,12-trien-9-one	0.975	$C_{14}H_{16}O_9$	350.06099	0.383
Sulcatol	14.22	$C_8H_{16}O$	128.12004	0.249

Compounds	Rt (Retention time)	Formula	Calculated Molecular weight	Area percentage (%)
(1R,8S,9S)-3,4-dihydroxy-8-methoxy-11,11-dimethyl-5-(propan-2-yl)-16- oxatetracyclo[7.5.2.0Âa, Âaâ•°.0Â?,â·]hexade ca-2(7),3,5-trien-15-one	14.307	$C_{21}H_{28}O_5$	360.19322	0.217
methyl (1S)-6-hydroxy-7-methyl-1-{[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6- (hydroxymethyl)oxan-2-yl] oxy}-1H,4aH,5H,6H,7H,7aH-cyclopenta[c] pyran-4- carboxylate	5.522	$C_{17}H_{26}O_{10}$	412.13381	0.215
(1R,2S,3R,4R)-3-(Isobutylamino)-4-(3- pyridinyl)-1,2-cyclopentanediol	12.43	$C_{14}H_{22}N_2O_2$	250.17185	0.165
2-(Cyclohexylmethylidene)-1,2,3,4-tetrahydronaphthalen-1-one	1.258	$C_{17}H_{20}O$	240.14693	0.157
(1aR,2E,4aR,6S,7S,7aR,8R,11aS)-1,1,3,6- Tetramethyl-9-methylene-4-oxo-1,1a,4,5,6,7,7a,8,9,10,1 1,11a-dodecahydro-4aH- cyclopenta[a]cyclopropa[f] [11]annulene-4a,7,8-triyl triacetate	7.828	$C_{26}H_{36}O_7$	460.24538	0.152
(7R,8S)-7,8-Dihydroxy-3,7-dimethyl-6-oxo-7,8-dihydro-6H-isochromene-5- carbaldehyde	1.621	$C_{12}H_{12}O_5$	236.06816	0.148
Bis(4-ethylbenzylidene)sorbitol	14.616	$C_{24}H_{30}O_6$	414.20366	0.129
4-oxo-5-phenylpentanoic acid	8.51	$C_{11}H_{12}O_3$	192.07833	0.311
2-[3-ethenyl-5-(methoxycarbonyl)-2-{[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl] oxy}-3,4-dihydro-2H-pyran-4-yl]acetic acid	6.164	$C_{17}H_{24}O_{11}$	426.11327	0.071
Total				22.143
Terpenoid				
Crotonic acid	1.246	$C_4H_6O_2$	86.03695	0.429
(-)-Caryophyllene oxide	7.494	$C_{15}H_{24}O$	220.18235	0.425
Limonin	6.769	$C_{26}H_{30}O_8$	470.18907	0.227
Loganin	5.523	$C_{17}H_{26}O_{10}$	407.17848	0.158
(9cis)-Retinal	16.888	$C_{20}H_{28}O$	284.21358	0.150
1,4a-dimethyl-9-oxo-7-(propan-2-yl)-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxylic acid				0.164
Total				1.554
Fatty acid				
Eicosatetraynoic acid	12.608	$C_{20}H_{24}O_2$	296.17699	2.433
Tetranor-12(S)-HETE	16.975	$C_{16}H_{26}O_3$	248.17731	0.199
Total				2.632
Miscellaneous compounds (phthalate, sulphate, glucose, amino acid derivates)				
Diisobutylphthalate	17.925	$C_{16}H_{22}O_4$	278.15105	5.949
n-Pentyl isopentyl phthalate	17.934	$C_{18}H_{26}O_4$	323.20897	0.597

Compounds	Rt (Retention time)	Formula	Calculated Molecular weight	Area percentage (%)
Triisobutyl phosphate	16.507	$C_{12}H_{27}O_4P$	266.16423	0.433
Tributyl phosphate	16.716	$C_{12}H_{27}O_4P$	266.16426	0.159
D-(+)-Maltose	0.877	$C_{12}H_{22}O_{11}$	364.09755	0.419
Glucose 1-phosphate	0.861	$C_6H_{13}O_9P$	260.02924	0.336
D-(+)-Proline	0.951	$C_5H_9NO_2$	115.06329	7.227
Prolylleucine	1.251	$C^{11}H_{20}N_2O_3$	228.14704	0.166
Total				15.285

Table 5: Secondary metabolite profiles of ethyl acetate extract of *S. lucida* R. Br.

Compounds	Rt (Retention time)	Formula	Calculated Molecular weight	Area percentage (%)
Alkaloid				
EPK	6.139	$C_{16}H_{28}N_4O_6$	394.18804	16.076
2,2,6,6-Tetramethyl-1-piperidinol (TEMPO)	12.329	$C_9H_{19}NO$	157.14621	2.010
Dibenzylamine	6.995	$C_{14}H_{15}N$	197.11987	1.694
ethyl 4-(3,5-dimethylpiperidino)-2-(trifluoromethyl) quinoline-6- carboxylate	6.496	$C_{20}H_2F_3N_2O_2$	380.17256	1.580
11-piperidino-2,3-dihydro-1H-cyclopenta[4,5] pyrido[1,2- a]benzimidazole-4-carbonitrile	13.017	$C_{20}H_{20}N_4$	316.16432	0.818
SPK	4.668	$C_{14}H_{26}N_4O_5$	352.17793	0.584
Caprolactam	0.895	$C_6H_{11}NO$	113.08395	0.379
N-{4-[(2R,3R)-3-(Hydroxymethyl)-4- methyl-5-oxo-2-morpholinyl]phenyl}-2,2-dimethylpropanamide	15.79	$C_{17}H_{24}N_2O_4$	320.17449	0.341
N1-[4-(6-Methyl-1,3-benzothiazol-2-yl) phenyl]-2-cyclopentyl-2- phenylacetamide	6.226	$C_{27}H_{26}N_2OS$	426.17809	0.334
Methyl 2-[(3S)-1-(2-methylbenzyl)-3-pyrrolidinyl]-1,3-benzoxazole-7- carboxylate	6.043	$C_{21}H_{22}N_2O_3$	350.16219	0.278
(15E)-15-ethylidene-18-(methoxycarbonyl)-17-methyl -12-oxo-10,17- diazatetracyclo[12.3.1.0Âl,ÂaÂa.0â´,â• a]octadeca-3(11),4,6,8-tetraen-17-ium-17-olate	5.479	$C_{21}H_{24}N_2O_4$	368.17284	0.224
Levalbuterol	13.427	$C_{13}H_{21}NO_3$	261.13585	0.200
(-)-Strychnine	5.997	$C_{21}H_{22}N_2O_2$	334.16731	0.188
β-Hydroxythiofentanyl	15.514	$C_{20}H_{26}N_2O_2$	358.17476	0.170
6-Methyl-2-pyridinemethanol	1.01	C_7H_9NO	123.06831	0.141
Nicotinamide	0.933	$C_6H_6N_2O$	122.04795	0.127
Choline	0.964	$C_5H_{13}NO$	103.0998	0.124
1-[(1,2-dimethyl-1H-imidazol-4-yl)sulfonyl]-4-(9H-fluoren-9- yl)piperazine	0.954	$C_{22}H_{24}N_4O_2S$	446.12343	0.111
N-(2,4-Dimethylphenyl)formamide	1.002	$C_9H_{11}NO$	149.08383	0.102

Compounds	Rt (Retention time)	Formula	Calculated Molecular weight	Area percentage (%)
Indan-1-one 1-(4,5-dihydro-1H-imidazol-2-yl) hydrazone	0.938	$C_{12}H_{14}N_4$	214.11771	0.099
N-[4-(benzyloxy)phenyl]-4-[2-(4-pyridinyl)ethyl] tetrahydro-1(2H)- pyrazinecarboxamide	17.905	$C_{25}H_{28}N_4O_2$	416.21633	0.096
6-(4-methoxyphenyl)pyrimidine-2,4-diamine	0.932	$C_{11}H_{12}N_4O$	216.09706	0.056
Reserpine	21.123	$C_{33}H_{40}N_2O_9$	608.26189	0.116
Total				25.847
Phenolic				
7-Hydroxycoumarine	0.864	$C_9H_6O_3$	180.0418	1.354
(1S,3R,4S,5R)-3,5-bis({[(2E)-3-(3,4- dihydroxyphenyl) prop-2-enoyl]oxy})- 1,4-dihydroxycyclohexane-1- carboxylic acid	8.15	$C_{25}H_{24}O_{12}$	516.12584	0.854
(1r,3R,4s,5S)-4-{[(2E)-3-(3,4- dihydroxyphenyl) prop-2-enoyl]oxy}- 1,3,5-trihydroxycyclohexane-1- carboxylic acid	0.86	$C_{16}H_{18}O_9$	372.10479	0.335
4-[(1S,3aR,4S,6aR)-4-(4-hydroxy-3- methoxyphenyl)-hexahydrofuro[3,4- c]furan-1-yl]-2-methoxyphenol	0.873	$C_{20}H_{22}O_6$	340.13017	0.234
Vanillin	6.102	$C_8H_8O_3$	152.04697	0.163
1-Naphthol	13.018	$C_{10}H_8O$	144.05706	1.299
4-Methoxybenzaldehyde	0.885	$C_8H_8O_2$	136.05203	4.157
4-methoxy-6-(prop-2-en-1-yl)-2H-1,3-benzodioxole	0.877	$C_{11}H_{12}O_3$	192.07822	1.117
Bis(3,5,5-trimethylhexyl) phthalate	21.26	$C_{26}H_{42}O_4$	418.30724	0.180
3,5-di-tert-Butyl-4-hydroxybenzaldehyde	16.939	$C_{15}H_{22}O_2$	234.16141	0.119
DEET	11.709	$C_{12}H_{17}NO$	191.13056	0.091
6-(3-hydroxybutan-2-yl)-5-(hydroxymethyl)-4-met hoxy-2H-pyran-2-one	0.955	$C_{11}H_{16}O_5$	266.05525	0.506
(2R)-5-hydroxy-7-methoxy-2-phenyl- 3,4-dihydro-2H-1-benzopyran-4-one	16.015	$C_{16}H_{14}O_4$	270.08852	0.114
6-Gingerol	13.02	$C_{17}H_{26}O_4$	276.17175	3.571
Shogaol	15.997	$C_{17}H_{24}O_3$	276.17174	1.060
2-hydroxy-6-[(8Z,11Z)-pentadeca-8,11,14-trien-1-yl] benzoic acid	16.886	$C_{22}H_{30}O_3$	324.20555	0.300
Zearalenone	18.047	$C_{18}H_{22}O_5$	300.13289	0.188
1-(3-ethyl-2,4-dihydroxy-6-methoxyphenyl) butan-1-one	18.144	$C_{13}H_{18}O_4$	238.12007	0.061
(2E)-5-[(8aS)-2,5,5,8a-tetramethyl-3- oxo-3,4,4a,5,6,7,8,8a- octahydronaphthalen-1-yl]-3- methylpent-2-enoic acid	16.674	$C_{20}H_{30}O_3$	300.20799	2.101
Citreoviridin	16.819	$C_{23}H_{30}O_6$	402.20082	0.220
Nor-9-carboxy-δ9-THC	15.47	$C_{21}H_{28}O_4$	344.19545	0.141
Acetophenone	20.556	C_8H_8O	120.05737	0.159
Total				18.324
Terpenoid				

Compounds	Rt (Retention time)	Formula	Calculated Molecular weight	Area percentage (%)
All trans retinal	16.875	$C_{20}H_{28}O$	284.21318	1.666
4-(4-hydroxy-2,6,6-trimethyl-3-{[(2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6- (hydroxymethyl)oxan-2- yl]oxy} cyclohex-1-en-1-yl)butan-2- one	5.72	$C_{19}H_{32}O_8$	410.18303	10.770
Kahweol	14.976	$C_{20}H_{26}O_3$	314.18736	1.843
(1S,4S,5R,9R,13S)-5,9-dimethyl-14-methylidenetetracyclo[11.2.1.0Âa,Âa â•°.0â´,â•a]hexadec-10-ene-5-carboxylic acid	14.651	$C_{20}H_{28}O_2$	300.20815	0.338
Carbaprostacyclin	17.64	$C_{21}H_{34}O_4$	372.22655	0.139
6-[(2E,6E)-7-(5-hydroxy-3-methyl-2-oxocyclopent-3-en-1-yl)-6- methylhepta-2,6-dien-2-yl]-3-methyl-5,6-dihydro-2H-pyran-2-one	12.736	$C_{20}H_{26}O_4$	312.17168	0.118
4-decyl-3-hydroxy-5-oxooxolane-2,3-dicarboxylic acid	0.957	$C_{16}H_{26}O_7$	352.14351	0.099
1,4a-dimethyl-9-oxo-7-(propan-2-yl)-1,2,3,4,4a,9,10,10a- octahydrophenanthrene-1-carboxylic acid	0.939	$C_{20}H_{26}O_3$	336.1696	0.096
Isobutyraldehyde	2.839	C_4H_8O	72.05778	0.551
Total				22.859
Fatty acid				
Palmitic Acid	14.436	$C_{16}H_{32}O_2$	273.26586	1.982
Oleanolic acid	20.685	$C_{30}H_{48}O_3$	438.34899	0.163
9-Oxo-10(E),12(E)-octadecadienoic acid	17.62	$C_{18}H_{30}O_3$	294.21871	2.308
Oleamide	21.566	$C_{18}H_{35}NO$	281.27095	1.531
Arachidonic acid	20.496	$C_{20}H_{32}O_2$	286.22875	3.457
4-hydroxy-6-[2-(2-methyl-1,2,4a,5,6,7,8,8a-octahydronaphthalen-1-yl)ethyl]oxan-2-one	18.337	$C_{18}H_{28}O_3$	314.18736	1.376
Hexadecanamide	21.708	$C_{16}H_{33}NO$	255.25551	2.646
(±)13-HpODE	17.426	$C_{18}H_{32}O_4$	294.21871	1.103
Stearamide	23.81	$C_{18}H_{37}NO$	283.28646	1.932
α-Eleostearic acid	16.926	$C_{18}H_{30}O_2$	278.22392	0.683
Oleoyl ethanolamide	21.941	$C_{20}H_{39}NO_2$	307.28676	0.757
Eicosapentaenoic acid	20.613	$C_{20}H_{30}O_2$	308.2343	1.318
Stearoyl Ethanolamide	23.079	$C_{20}H_{41}NO_2$	309.3022	0.325
Sphingosine (d18:1)	20.011	$C_{18}H_{37}NO_2$	321.26604	0.161
Linoleoyl ethanolamide	18.932	$C_{20}H_{37}NO_2$	323.28163	0.117
4-oxo-5-phenylpentanoic acid	8.492	$C_{11}H_{12}O_3$	192.07814	0.093
2-Amino-1,3,4-octadecanetriol	17.261	$C_{18}H_{39}NO_3$	317.29225	0.653
Tetranor-12(S)-HETE	17.795	$C_{16}H_{26}O_3$	288.17171	0.552
Ethyl palmitoleate	18.535	$C_{18}H_{34}O_2$	282.25524	0.201
Erucamide	25.527	$C_{22}H_{43}NO$	337.33382	0.079
Total				21.436

Compounds	Rt (Retention time)	Formula	Calculated Molecular weight	Area percentage (%)
Steroid				
Galaxolidone	20.638	$C_{18}H_{24}O_2$	272.17665	3.280
17 beta-Trenbolone	16.342	$C_{18}H_{22}O_2$	270.16121	0.174
Total				3.453
Miscellaneous (Phthalate derivates)				
Diisobutylphthalate	17.911	$C_{16}H_{22}O_4$	278.15115	2.771
n-Pentyl isopentyl phthalate	0.892	$C_{18}H_{26}O_4$	328.16661	0.243
Bis(2-ethylhexyl) phthalate	23.026	$C_{24}H_{38}O_4$	390.27553	0.471
Sedanolide	20.55	$C_{12}H_{18}O_2$	176.11972	0.135
Total				3.620

Table 6: Secondary metabolite profiles of n-hexane extract of *S. lucida* R. Br.

Compounds	Rt (Retention time)	Formula	Calculated Molecular weight	Area percentage (%)
Alkaloid				
2,2,6,6-Tetramethyl-1-piperidinol (TEMPO)	12.355	$C_9H_{19}NO$	157.1464	3.609
Dibenzylamine	7.089	$C_{14}H_{15}N$	197.12	3.278
2-[(2-chlorobenzyl) sulfanyl]-4,6-dimethylnicotinonitrile	14.889	$C_{15}H_{13}ClN_2S$	326.0001	1.760
EPK	6.178	$C_{16}H_{28}N_4O_6$	394.1883	1.776
11-piperidino-2,3-dihydro-1H-cyclopenta[4,5] pyrido[1,2- a]benzimidazole-4-carbonitrile	13.064	$C_{20}H_{20}N_4$	316.1645	1.264
2-[(2S,3R,4S,5R)-5-(Aminomethyl)-3,4-dihydroxytetra hydro-2-furanyl]-N-(4- methoxybenzyl)acetamide	0.965	$C_{15}H_{22}N_2O_5$	332.1386	0.952
2-Amino-1,3,4-octadecanetriol	14.647	$C_{18}H_{39}NO_3$	317.2923	0.472
Caprolactam	0.876	$C_6H_{11}NO$	113.0841	0.418
Dextrorphan	12.946	$C_{17}H_{23}NO$	514.355	0.414
Levalbuterol	13.465	$C_{13}H_{21}NO_3$	261.1359	0.343
N-Butylbenzenesulfonamide	27.821	$C_{10}H_{15}NO_2S$	213.0819	0.449
N-[4-(benzyloxy)phenyl]-4-[2-(4-pyridinyl)ethyl] tetrahydro-1(2H)- pyrazinecarboxamide	17.947	$C_{25}H_{28}N_4O_2$	416.2166	0.217
LSD-d3 (Lysergic acid diethylamide-D3)	18.838	$C_{20}H_{22[2]}H_3N_3O$	326.2216	0.208
Flecainide	0.967	$C_{17}H_{20}F_6N_2O_3$	414.1439	0.160
Betaine	0.96	$C_5H_{11}NO_2$	117.0789	0.138
DEET (diethyltoluamide)	11.733	$C_{12}H_{17}NO$	191.1306	0.114
Choline	0.966	$C_5H_{13}NO$	103.0998	0.080
Total				15.652
Phenolic				
6-Gingerol	13.054	$C_{17}H_{26}O_4$	276.1718	5.751
Shogaol	16.033	$C_{17}H_{24}O_3$	276.1718	1.246

Compounds	Rt (Retention time)	Formula	Calculated Molecular weight	Area percentage (%)
2-hydroxy-6-[(8Z,11Z)-pentadeca-8,11,14-trien-1-yl] benzoic acid	16.916	$C_{22}H_{30}O_3$	324.2054	0.226
1-Naphthol	13.054	$C_{10}H_8O$	144.0571	2.354
4-Methoxybenzaldehyde	16.033	$C_8H_8O_2$	136.0521	5.303
3,5-di-tert-Butyl-4-hydroxybenzaldehyde	16.985	$C_{15}H_{22}O_2$	234.1616	0.232
4-hydroxy-6-[2-(2-methyl-1,2,4a,5,6,7,8,8a-o ctahydronaphthalen-1-yl)ethyl]oxan-2-one	18.391	$C_{18}H_{28}O_3$	314.1874	2.347
(1R,4aS)-7-(2-Hydroxypropan-2-yl)-1,4a-dimethyl-9-oxo-3,4,10,10a-tetrahydro-2H-phen anthrene-1-carboxylic acid	17.105	$C_{20}H_{26}O_4$	330.1825	1.569
Zearalenone	16.976	$C_{18}H_{22}O_5$	300.1331	2.009
Citreoviridin	16.865	$C_{23}H_{30}O_6$	402.2011	0.538
Acetophenone	20.581	C_8H_8O	120.0574	0.251
Nor-9-carboxy-δ9-THC	15.514	$C_{21}H_{28}O_4$	344.1958	0.224
3-(4-hydroxy-3-methoxyphenyl)propanoic acid	13.054	$C_{10}H_{12}O_4$	178.0626	0.424
4-Methoxycinnamic acid	20.675	$C_{10}H_{10}O_3$	178.0626	0.118
(2R)-5-hydroxy-7-methoxy-2-phenyl-3,4- dihydro-2H-1-benzopyran-4-one	16.056	$C_{16}H_{14}O_4$	270.0887	0.217
Total				22.807
Terpenoid				
(2E)-5-[(8aS)-2,5,5,8a-tetramethyl-3-oxo-3,4,4a,5,6,7, 8,8a-octahydronaphthalen-1-yl]-3-methylpent-2-enoic acid	16.707	$C_{20}H_{30}O_3$	300.2081	3.531
All trans retinal	16.915	$C_{20}H_{28}O$	284.2134	2.555
Kahweol	15.015	$C_{20}H_{26}O_3$	314.1875	1.345
(9cis)-Retinal	17.939	$C_{20}H_{28}O$	284.2134	1.273
Isobutyraldehyde	2.848	C_4H_8O	72.05779	1.099
6-[(2E,6E)-7-(5-hydroxy-3-methyl-2-oxocyclopent-3-en-1-yl)-6-methylhepta-2,6-dien-2-yl]-3-methyl-5,6-dihydro-2H- pyran-2-one	0.959	$C_{20}H_{26}O_4$	312.172	0.542
4,7-dihydroxy-4-(hydroxymethyl)-3,4a,8,8-tetramethyl-1,4,4a,5,6,7,8,8a-octahydronaphthalen-1-one	17.447	$C_{15}H_{24}O_4$	290.1511	0.374
(1S,4S,5R,9R,13S)-5,9-dimethyl-14- methylidenetet racyclo[11.2.1.0Âa,Âaâ•°.0â´,â•a]hexadec-10-ene-5-carboxylic acid	14.686	$C_{20}H_{28}O_2$	300.2082	0.622
Carbaprostacyclin	17.681	$C_{21}H_{34}O_4$	372.2269	0.308
Limonin	0.839	$C_{26}H_{30}O_8$	470.1888	0.120
(2Z)-5-(1,2,4a,5-tetramethyl-7-oxo-1,2,3,4,4a,7,8,8a-oc tahydronaphthalen-1-yl)-3-methylpent-2-enoic acid	12.929	$C_{20}H_{30}O_3$	340.2008	0.116
4-decyl-3-hydroxy-5-oxooxolane-2,3-dicarboxylic acid	0.96	$C_{16}H_{26}O_7$	352.1435	0.112
Total				11.999
Fatty acid				

Compounds	Rt (Retention time)	Formula	Calculated Molecular weight	Area percentage (%)
Oleamide	21.599	$C_{18}H_{35}NO$	281.2709	4.146
Hexadecanamide	21.651	$C_{16}H_{33}NO$	255.2556	3.710
Palmitic Acid	14.493	$C_{16}H_{32}O_2$	273.266	2.077
Arachidonic acid	20.54	$C_{20}H_{32}O_2$	286.2289	2.909
Stearamide	23.849	$C_{18}H_{37}NO$	283.2866	3.248
Oleoyl ethanolamide	21.968	$C_{20}H_{39}NO_2$	307.2869	1.572
Eicosapentaenoic acid	20.363	$C_{20}H_{30}O_2$	308.2345	1.733
Bis(7-methyloctyl) adipate	14.894	$C_{24}H_{46}O_4$	398.3388	0.909
γ-Linolenic acid ethyl ester	20.885	$C_{20}H_{34}O_2$	312.2658	0.752
13-OxoODE	15.843	$C_{18}H_{30}O_3$	316.2033	0.644
Stearoyl Ethanolamide	23.089	$C_{20}H_{41}NO_2$	309.3025	0.857
Tetranor-12(S)-HETE	17.836	$C_{16}H_{26}O_3$	288.1719	0.802
Eicosatetraynoic acid	0.897	$C_{20}H_{24}O_2$	296.1772	0.394
Oleanolic acid	20.718	$C_{30}H_{48}O_3$	438.349	0.231
9-Oxo-10(E),12(E)-octadecadienoic acid	17.662	$C_{18}H_{30}O_3$	294.2189	0.170
Total				24.154
Steroid				
Galaxolidone	20.469	$C_{18}H_{24}O_2$	272.1768	5.748
17beta-Trenbolone	16.387	$C_{18}H_{22}O_2$	270.1614	0.150
Total				5.897
Miscellaneous (Phthalate derivates)				
Sedanolide	19.877	$C_{12}H_{18}O_2$	176.1198	0.326
Diisobutylphthalate	17.957	$C_{16}H_{22}O_4$	278.1512	7.398
Bis(3,5,5-trimethylhexyl) phthalate	14.909	$C_{26}H_{42}O_4$	418.3072	4.735
Diisodecyl phthalate	14.914	$C_{28}H_{46}O_4$	446.3386	0.191
Total				12.650

Terpenoid compounds that had a high percentage of ethyl acetate extract were kahweol (1.2%), and trans retinal (1.65%). Palmitic acid, hexadecanamide were fatty acid compounds from ethyl acetate extract (Table 5).

The most abundant phytoconstituents from n-hexane extract are fatty acid (24.154%), phenolic derivates (22.80%), and alkaloid (15.652%) (Figure 2). Phenolic derivates from n-heksana extract mainly identified as aromatic compounds such as 6-gingerol and shogaol.[14] Oleamide, palmitic acid, stearamide composed the fatty acid compound of n-hexana extract (Table 6).

DISCUSSION

The *in vivo* anti-malarial activity showed that the water extract could inhibit the parasite's growth in mice infected with *P. berghei* more potently than the ethyl acetate, ethanol, and n-hexane extracts. The water and ethanol extract exhibited the same secondary metabolite profile; mostly contained alkaloids, and phenolic derivates. Some alkaloid compounds in water and ethanol extracts were choline, betaine, pyrazoline alkaloid derivatives: nicotinamide, and nicotinic acid.

Choline-derived compounds show anti-malarial activity by inhibiting membrane phospholipid biosynthesis in *P. falciparum* and interacting with metabolites resulted from hemoglobin degradation in food vacuoles.[15,16] Betaine lipid derivative

compound, monoacylglyceryl trimethyl homoserine, isolated from *Heterospora chenopodii*, inhibited the growth of *Plasmodium falciparum in vitro* with an IC_{50} 7 µM.[17] *In vitro* anti-malarial assay showed that nicotinamide and nicotinic acid performed as competitive inhibitors of PfSir2.[18] *Plasmodium falciparum*, "silent information regulator 2" (PfSir2), is a class III member of histone deacetylases (HDACs) that play a role in the epigenetic regulation of virulent genes in the pathogenesis of malaria. Inhibition of PfSir2 activates the silence var gene through chromatin modification.[19] HDAC and Histone Acetyltransferases (HATs) play a role in modifying histones' covalent bonds. Histones affect chromatin-based events such as transcription, replication, and DNA repair. Inhibition of HDAC causes histone hyperacetylation, thereby changing the lysine composition and transcription of *Plasmodium falciparum* DNA.[20] In the culture of *Plasmodium falciparum* strains CS2 and 3G8, nicotinamide inhibited parasite growth with IC_{50} 6.9 mM and 2.2 mM, respectively. The combination of nicotinamide, artemisinin, chloroquine, and pyrimethamine could show a synergistic effect.[21]

Benzylamine was identified as an alkaloid from ethyl acetate extract. Previous study showed that naphthaquinone, dibenzylamine derivative compounds, inhibited the growth of *Plasmodium falciparum* KI (multidrug-resistant) with IC_{50} between 0.77 – 4.05µg/mL.[22] In addition, the ethyl acetate extract also contained indole strychnine alkaloids. Bisindol compounds from the genus Strychnos; isosungucine, hydroxyisosungucine, and strychnogucine B, showed anti-malarial activity against *Plasmodium falciparum* W2-chloroquine resistance with IC_{50} 168; 85; 85 nM, respectively.[23] Strychnogucine B inhibited the growth of *Plasmodium berghei* in murine models by 36% on day 5 and 60% on day 7.[24] The indole alkaloid reserpine, isolated from *Corynanthe pachyceras*, inhibited the growth of *Plasmodium falciparum*-chloroquine resistance with IC_{50} 8.1 µM.[25]

The phenolic compounds in the water and ethanol extracts that showed anti-malarial activity included: caffeic acid, cinnamic acid, pyrogallol, coumarin, and hydroxybenzoic acid. Caffeic acid has an IC_{50} of 80.5 µM in inhibiting the growth of *Plasmodium falciparum* 3D7-chloroquine sensitive.[26] Several compounds derived from cinnamic acid (myristicine) showed *in vitro* anti-malarial activity.[13] Gallic acid derivative compounds with phenol functional groups exhibited inhibition of Plasmodium growth with IC_{50} between 20 micromolar and selectivity index >5.[27] One of the gallic acid derivatives, methyl gallate, performed *in vitro* anti-malarial activity against *Plasmodium falciparum* 3D7 with IC_{50} 0.0128 µM.[28] Coumarin derivatives exhibited anti-malarial activity through inhibition of hemozoin formation, resulting in toxicity to vacuole cells.[29] Methyl 4-benzoxy-3,5-dihydroxybenzoate is a water-soluble derivative of hydroxybenzoic which inhibited *Plasmodium falciparum* growth with an IC_{50} 3.72 mM.[27]

Terpenoid compounds that showed anti-malarial activity are Caryophyllene and limonin. Limonin inhibited the growth of parasites in the ring phase with an IC_{50} 2.7 µM.[30] The nanoparticle delivery system of Caryophyllene inhibited the growth of *Plasmodium falciparum* 3D7 chloroquine sensitive with IC_{50} 2.34 µg/mL and showed cytotoxic activity in lung cancer cells.[31]

Several compounds belonging to the fatty acid derivates could also inhibit the growth of Plasmodium parasites, including oleamide, palmitic acid, and olealinic acid. Some studied showed that palmitic acid was ineffective in inhibiting the ring phase maturation process into scizont on Plasmodium (MIC > 50 µg/mL).[32] One of the plants that contain oleamide was *Blumea balsamifera. Blumea balsamifera* inhibited plasmodium's growth *in vitro* with IC_{50} 9.66 g/mL and SI >20.70.[33]

It is required to measure the selectivity index to decide whether the sample has the potential for further development. An extract can be observed further if the selectivity index is > 10.[34] According to the result, all extracts were selective as anti-malarial with SI value > 10. However, based on the *in vitro* and *in vivo* anti-malarial assays, water, ethanol, and ethyl extracts acetate exhibited more effective activity. Water and ethanol extracts were categorized as highly active as anti-malarials (IC_{50} < 5 µg/mL), meanwhile, ethyl acetate and n-hexane extracts were categorized as active (IC_{50} > 5 to 50 µg/mL).[35] The phytoconstituents of the ethyl acetate extract displayed more abundant fatty acids than the ethanol and water extracts. Previous studies showed that fatty acid derivatives contributed slightly to anti-malarial activity.[36-38] According to the LC-HRMS profile and prior anti-malarial investigations, the alkaloids and phenols in the ethanol and water extracts contributed more to the anti-malarial activity of *Strychnos lucida* R. Br.

CONCLUSION

Ethanol and water extract of the stem from *Strycnos lucida* R. Br. provide selective anti-malarial activity according to selectivity index. The alkaloid and phenol groups contribute to the anti-malarial activity of water and ethanol extract based on LC-HRMS profiles, *in vitro* and *in vivo* assay. This research proves the effectiveness of *Strychnos lucida* decoct as anti-malarial that has been used empirically by local people in Indonesia. However, further studies are required to isolate and identify active compounds from *Strcyhnos lucida* that exhibit anti-malarial activity.

ACKNOWLEDGEMENT

This research was supported by Institute of Research and Community Service Faculty of Medicine, Brawijaya University, Malang, Indonesia (Contract number 234/2021).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

CMC-Na: Sodium carboxymethyl cellulose; **DEET:** N,N-diethyl-meta-toluamide; **DMSO:** Dimethyl sulfoxide; **ELISA:** The enzyme-linked immunosorbent assay; **HATs:** Histone Acetyltransferases; **HDACs:** Histone deacetylases; **HPLC:** High Performance Liquid Chromatography; **IC:** inhibitory Concentration; **ITD:** Institute of Tropical Disease; **LC-HRMS:** Liquid Chromatography High Resolution Mass Spectrometry; **MIC:** Minimum Inhibitory Concentration; **TEMPO:** 2,2,6,6-Tetramethyl-1-piperidinol.

SUMMARY

The powder of *Strychnos lucida* was extracted using various organic solvents, and determined for *in vitro/in vivo* anti-malarial, *in vitro* cytotoxic activity, and secondary metabolites profile. *In vivo* anti-malarial was conducted in using Peter's four suppressive days test, and *in vitro* antiplasmodial was performed against *Plasmodium falciparum* chloroquine sensitive. The result showed that water and ethanol extract exhibited the most potential anti-malarial activity both *in vitro* and *in vivo*. In addition, the selectivity index showed that water and ethanol extract were highly selective as antiplasmodial agent. According to the LC-MS profile, the most abundant secondary metabolite contained in water and ethanol extract is alkaloid and phenolic compounds.

REFERENCES

1. Ma'ruf NA. Ida ayu made Rai Astuti, Fairuz Wardaty, ani Mardhiani, titin Delia, martin lastly Marbun, Adid Muqtadiroh Rencana Aksi Program, translator. Penelitian dan Pengembangan Kesehatan tahun 2020-2024.
2. Jakarta: Badan Penelitian dan Pengembangan Kesehatan Kementerian Kesehatan Republik Indonesia; 2020.
3. Taek MM. Ew BP, Agil M. Plants used in traditional medicine for treatment of malaria by Tetun ethnic people in west Timor, Indonesia. Asian Pac J Trop Med. 2018;11(0668):630-7.
4. Taek MM, Tukan GD, Prajogo BEW, Agil M. Antiplasmodial activity and phytochemical constituents of selected anti-malarial plants used by native people in west Timor Indonesia. Turk J Pharm Sci. 2021;18(1):80-90. doi: 10.4274/tjps.galenos.2019.29000, PMID 33634682.
5. Sarmento C, Worachartcheewan A, Pingaew R, Nacional H, Valadares G, Leste T. Toxicology (EHT). Department of Clinical Microbiology and Applied Technology, Faculty of Medical Technology, Mahidol University. Bangkok, Thailand: CHE, Ministry of Education, 6 Department of Clinical Laboratory and Pathology. 2015;12(4):122-7.
6. Liu F, Wang X, Han X, Tan X, Kang W. Cytotoxicity and DNA interaction of brucine and strychnine-two alkaloids of semen strychni. Int J Biol Macromol. 2015;77:92-8. doi: 10.1016/j.ijbiomac.2015.03.017, PMID 25796448.
7. Gao Q, Wang J jun, mei HF, Chen Y. Effect of strychnine hydrochloride on liver cytochrome P450 mRNA expression and monooxygenase activities in rat. Acta Pharmacol Sin B. 2011;1(2):121-8. doi: 10.1016/j.apsb.2011.06.009.
8. Song X, Yang D, Wang Y, Liu W, Wang Y, Zhu J. Pharmacokinetic, acute toxicity, and pharmacodynamic studies of semen strychni total alkaloid microcapsules. Trop J Pharm Res. 2019;18(9):1985-92. doi: 10.4314/tjpr.v18i9.29.
9. Maher A, Radwan R, Breitinger HG. *In vivo* protection against strychnine toxicity in mice by the glycine receptor agonist ivermectin. BioMed Res Int. 2014;2014:640790. doi: 10.1155/2014/640790, PMID 25317421.
10. Trager W, Jensen JB JJ. Human *Malaria parasites* in Continuous Culture. Science. 1976;193(4254):673-5. doi: 10.1126/science.781840.
11. Khasanah U, Shalas AF, Resti NN, Nurdiana M, Widyawaruyanti A. Bioassay-guided fractionation and *in vitro* antiplasmodial activity of ethyl acetate fractions from *Alectryon serratus* (Sapindaceae) leaf. Trop J Pharm Res. 2022;21(1):99-104.
12. Dwivedi MK, Shukla R, Sharma NK, Manhas A, Srivastava K, Kumar N, *et al*. Evaluation of ethnopharmacologically selected *Vitex negundo* L. for *in vitro* anti-malarial activity and secondary metabolite profiling. J Ethnopharmacol. 2021;275:114076. doi: 10.1016/j.jep.2021.114076, PMID 33789139.
13. Peters WW.peters. The four-day suppressive *in vivo* anti-malarial test. Ann Trop Med Parasitol. 1975;69(2):155-71. doi: 10.1080/00034983.1975.11686997, PMID 1098584.
14. De P, Bedos-Belval F, Vanucci-Bacque C, Baltas M. Cinnamic acid derivatives in tuberculosis, malaria and cardiovascular diseases - a review. Curr Org Chem. 2012;16(6):747-68. doi: 10.2174/138527212799958020.
15. Trease GE, Evans WC; 2009. Trease and Evans' pharmacognosy. 16th ed [internet]. Available from: https://www.abebooks.com/Trease-Evans-Pharmacognosy-13th-Edition-William/14174467122/bd.
16. Duan S, Wang R, Wang R, Tang J, Xiao X, Li N, *et al. In vivo* anti-malarial activity and pharmacokinetics of artelinic acid-choline derivative liposomes in rodents. Parasitology. 2020;147(1):58-64. doi: 10.1017/S0031182019001306, PMID 31556865.
17. Peyrottes S, Caldarelli S, Wein S, Périgaud C, Pellet A, Vial H. Choline analogues in malaria chemotherapy. Curr Pharm Des. 2012;18(24):3454-66. doi: 10.2174/138161212801327338, PMID 22607139.
18. Martín J, Crespo G, González-Menéndez V, Pérez-Moreno G, Sánchez-Carrasco P, Pérez-Victoria I, *et al*. MDN-0104, an antiplasmodial betaine lipid from heterospora chenopodii. J Nat Prod. 2014;77(9):2118-23. doi: 10.1021/np500577v, PMID 25215605.
19. Prusty D, Mehra P, Srivastava S, Shivange AV, Gupta A, Roy N, *et al*. Nicotinamide inhibits *Plasmodium falciparum* Sir2 activity *in vitro* and parasite growth. FEMS Microbiol Lett. 2008;282(2):266-72. doi: 10.1111/j.1574-6968.2008.01135.x, PMID 18397290.
20. Coetzee N, von Grüning H, Opperman D, van der Watt M, Reader J, Birkholtz LM. Epigenetic inhibitors target multiple stages of *Plasmodium falciparum* parasites. Sci Rep. 2020;10(1):2355. doi: 10.1038/s41598-020-59298-4, PMID 32047203.
21. Miao J, Lawrence M, Jeffers V, Zhao F, Parker D, Ge Y, *et al*. Extensive lysine acetylation occurs in evolutionarily conserved metabolic pathways and parasite-specific functions during *Plasmodium falciparum* intraerythrocytic development. Mol Microbiol. 2013;89(4):660-75. doi: 10.1111/mmi.12303, PMID 23796209.
22. Tcherniuk SO, Chesnokova O, Oleinikov IV, Oleinikov AV. Nicotinamide inhibits the growth of *P. falciparum* and enhances the anti-malarial effect of artemisinin, chloroquine and pyrimethamine. Mol Biochem Parasitol. 2017;216:14-20. doi: 10.1016/j.molbiopara.2017.06.004, PMID 28633997.
23. Paengsri W, Promsawan N, Baramee A. Synthesis and evaluation of 2-hydroxy-1,4-naphthoquinone derivatives as potent anti-malarial agents. Chem Pharm Bull (Tokyo). 2021;69(3):253-7. doi: 10.1248/cpb.c20-00770, PMID 33431728
24. .Frederich M, Tits M, Angenot L. Potential anti-malarial activity of indole alkaloids. Trans R Soc Trop Med Hyg. 2008;102(1):11-9. doi: 10.1016/j.trstmh.2007.10.002, PMID 18035385.
25. Beaufay C, Ledoux A, Jansen O, Bordignon A, Zhao S, Teijaro CN, *et al. In vivo* anti-malarial and antitrypanosomal Activity of Strychnogucine B, a Bisindole alkaloid from *Strychnos icaja*. Planta Med. 2018;84(12-13):881-5. doi: 10.1055/a-0644-2723, PMID 29929205.
26. Staerk D, Lemmich E, Christensen J, Kharazmi A, Olsen CE, Jaroszewski JW. Leishmanicidal, antiplasmodial and cytotoxic activity of indole alkaloids from Corynanthe Pachyceras. Planta Med. 2000;66(6):531-6. doi: 10.1055/s-2000-8661, PMID 10985079.
27. Alson SG, Jansen O, Cieckiewicz E, Rakotoarimanana H, Rafatro H, Degotte G, *et al. In vitro* and *in vivo* anti-malarial activity of caffeic acid and some of its derivatives. J Pharm Pharmacol. 2018;70(10):1349-56. doi: 10.1111/jphp.12982, PMID 30033538.
28. Degotte G, Pirotte B, Frédérich M, Francotte P. Polyhydroxybenzoic acid derivatives as potential new anti-malarial agents. Arch Pharm (Weinheim). 2021;354(11):e2100190. doi: 10.1002/ardp.202100190, PMID 34346088.
29. Khasanah U, Widyawaruyanti A, Hafid AF, Tanjung M. Antiplasmodial activity of isolated polyphenols from *Alectryon serratus* leaves against 3D7 *Plasmodium falciparum*. Pharmacogn Res. 2017;9(S1):57-60.
30. Mbaba M, Dingle LMK, Zulu AI, Laming D, Swart T, De la Mare JA, *et al*. Coumarin-annulated ferrocenyl 1,3-oxazine derivatives possessing *in vitro* anti-malarial and antitrypanosomal potency. Molecules. 2021;26(5):1–18.
31. Lee SE, Kim MR, Kim JH, Takeoka GR, Kim TW, Park BS. Anti-malarial activity of anthothecol derived from *Khaya anthotheca* (Meliaceae). Phytomedicine. 2008;15(6-7):533-5. doi: 10.1016/j.phymed.2007.08.001, PMID 17913482.
32. Kamaraj C, Balasubramani G, Siva C, Raja M, Balasubramanian V, Raja RK, *et al*. Ag Nanoparticles Synthesized Using β-caryophyllene Isolated from *Murraya koenigii*: anti-malarial (*Plasmodium falciparum* 3D7) and Anti-cancer Activity (A549 and HeLa Cell Lines). J Clust Sci. 2017;28(3):1667-84. doi: 10.1007/s10876-017-1180-6
33. Lakshmi V, Srivastava S, Mishra SK, Srivastava MN, Srivastava K, Puri SK. Anti-malarial activity in *Xylocarpus granatum* (Koen). Nat Prod Res. 2012;26(11):1012-5. doi: 10.1080/14786419.2010.535000, PMID 21787243.
34. Chaniad P, Phuwajaroanpong A, Techarang T, Viriyavejakul P, Chukaew A, Punsawad C. Antiplasmodial activity and cytotoxicity of plant extracts from the *Asteraceae* and *Rubiaceae* families. Heliyon. 2022;8(1):e08848. doi: 10.1016/j.heliyon.2022.e08848, PMID 35141436.
35. Peña-Morán OA, Villarreal ML, Álvarez-Berber L, Meneses-Acosta A, Rodríguez-López V. Cytotoxicity, post-treatment recovery, and selectivity analysis of naturally occurring

podophyllotoxins from *Bursera fagaroides* var. fagaroides on breast cancer cell lines. Molecules. 2016;21(8):1-15. doi: 10.3390/molecules21081013, PMID 27527135.

36. Chinchilla M, Valerio I, Sánchez R, Mora V, Bagnarello V, Martínez L, *et al. In vitro* anti-malarial activity of extracts of some plants from a biological reserve in Costa Rica. Rev Biol Trop. 2012;60(2):881-91. doi: 10.15517/rbt.v60i2.4024, PMID 23894953.
37. Batista R, Silva ADJ, de Oliveira AB. Plant-derived anti-malarial agents: new leads and efficient phytomedicines. Part II. Non-alkaloidal natural products. Molecules. 2009;14(8):3037-72. doi: 10.3390/molecules14083037, PMID 19701144.
38. Kaur K, Jain M, Kaur T, Jain R. Anti-malarials from nature. Bioorg Med Chem. 2009;17(9):3229-56. doi: 10.1016/j.bmc.2009.02.050, PMID 19299148.Mojab F. Anti-malarial natural products: a review. Avicenna J Phytomed. 2012;2(2):52-62. PMID 25050231.

Cite this article: Khasanah U, Shalas AF, Ihsan BRP, Wulandari LAA, Nurtyas NC, Waril, *et al*. Evaluation of Selectivity Index and Phytoconstituents Profile of Various Extracts from the Stem of *Strychnos lucida* R. Br. as Anti-malarial. Pharmacog Res. 2023;15(4):733-50.