IN VIVO ANTI-MALARIAL ACTIVITY OF SOME MALAYSIAN TRADITIONAL PLANTS: COMMUNITY'S AWARENESS AND ETHNOBOTANICAL SURVEY

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ABSTRACT

In Malaysia, located in the hot, humid equatorial region, malaria stills an important public health issue among rural and aboriginal people. In parallel with the implementation of Malaysia's malaria elimination programme, this cross-sectional was carried out among 223 households from forest-aboriginal and rural areas, Lipis district, Pahang state, to investigate the awareness of the communities regarding malaria: knowledge, attitudes and practices (KAP) and to establish a preliminary ethnobotanical database for the plants traditionally used to treat malaria. The *in vivo* anti-malarial activity of the methanol extracts of *Cocos nucifera*, *Labisia pumila*, *Languas galanga* and *Piper betle* selected based on the ethnobotanical survey were evaluated against the laboratory malaria model *Plasmodium berghei.* The acute oral toxicity (LD_{50}) was determined to ascertain the safety of the extracts. The phytochemical and antioxidant potentials of the crude extracts were also investigated to elucidate the possibilities of its anti-malarial effects. The rural population has shown a higher knowledge about the transmission of the disease (aboriginal 50%, rural 70.7%, $P<0.01$). However, the aboriginal participants showed higher practices in the use of medicinal plants and belief in witchcraft ($P < 0.05$, $P < 0.01$, respectively). On the other hand, the two communities did not show significant differences in their knowledge, attitudes and practices about the symptoms of malaria, severity of the disease and the use of mosquito bed nets, respectively. Nineteen species belonging to 17 families were identified. Twelve plant species have not previously been documented for the treatment of malaria in Peninsular Malaysia. The results of anti-malarial activity of *C. nucifera, L. pumila, L. galanga* and *P. betle* showed significant ($P < 0.05$) results during the early, established and residual *P. berghei* infections in mice. Overall results of the *in vivo* anti-malarial activity of the methanol extract of the plants during early, established and residual malaria infections with evidence of increasing mean survival time of the infected mice showed that *P.*

betle and *L. galanga* were the most effective followed by *C. nucifera* and *L. pumila*, respectively. The present study showed that the test extracts are toxicologically safe by oral administration. Phytochemical screening of the methanol extracts of the plants revealed the presence of alkaloids, terpenoids, anthraquinones, flavonoids, tannins, saponins, glycosides and steroids. At a concentration of 25 µg/mL *P. betle* showed potent DPPH scavenging antioxidant activity % (80.68%) followed by *L. pumila* (40.66%) and *L. galanga* (37.88%) where as *C. nucifera* exhibited a weak DPPH scavenging activity (5.52%). In conclusion, the aboriginal and rural communities showed awareness regarding malaria as a disease; however their KAP were inadequate, in particular, among the aboriginal population. Providing efficient health education to people residing in malaria endemic areas would improve their understanding about malaria prevention in order to bring about the elimination of malaria from the country. The ethnobotanical findings can be used as an ethnopharmacological basis for selecting plants for further anti-malarial phytochemical and pharmaceutical studies. The anti-malarial activity observed in this study also confirms that the Malaysian folkloric medicinal application of these plants has a pharmacological basis.

ABSTRAK

Di Malaysia, yang terletak di kawasan Khatulistiwa yang panas dan lembab, dimana penularan malaria adalah reseptif dan mudah dialami, malaria masih menjadi isu kesihatan awam yang penting di kawasan luar bandar dan pendalaman. Selari dengan implimentasi program pembasmian malaria di Malaysia, kajian 'cross-sectional' dengan soal selidik separuh struktur telah dijalankan dikalangan 223 isi rumah dari kalangan Orang Asli di hutan dan dari penduduk kawasan luar bandar, daerah Lipis, Negeri Pahang, untuk menyiasat kesedaran komuniti mengenai malaria (pengetahuan, sikap dan amalan) dan untuk membentuk satu databes etnobotanikal priliminari bagi pokok-pokok yang digunakan secara tradisional untuk merawat malaria. Aktiviti anti-malaria *in vivo* bagi ekstrak methanol *Cocos nucifera*, *Labisia pumila*, *Languas galanga* dan *Piper betle* telah dipilih berasaskan tinjauan etnobotanikal dan dinilai menentang model malaria makmal *Plasmodium berghei*. Toksisiti oral akut (LD_{50}) telah ditentukan bagi mencapai tahap selamat ekstrak tersebut. Fitokimia dan potensi antioksidan bagi ekstrak mentah juga telah disiasat untuk mempastikan kemungkinan terdapat kesan anti-malaria. Penduduk luar bandar telah menunjukkan pengetahuan lebih tinggi tentang penularan penyakit ini (Orang Asli 50%, luar bandar 70.7%, $P < 0.01$). Bagaimanapun, penggunaan pokok berubat dan kepercayaan pada ilmu sihir dan sihir dalam merawat penyakit demam adalah lebih tinggi signifikantnya di kalangan penduduk Orang Asli ($P< 0.05$, $P < 0.01$, masing-masing). Sebaliknya, tidak ada perbezaan yang signifikant diantara kedua komuniti dari segi pengetahuan tentang simptom malaria, sikap terhadap penyakit yang teruk dan amalan pencegahan malaria menggunakan kelambu. Sembilan belas spesies pokok yang tergolong dalam 17 famili telah dikenalpasti. Dua belas spesies pokok belum pernah didokumenkan sebelum ini untuk rawatan malaria di Semenanjung Malaysia. Keputusan aktiviti anti-malaria bagi *C. nucifera*, *L. pumila*, *L. galanga* dan *P. betle* menunjukkan yang setiap dari empat ekstrak pokok tersebut

mempamerkan aktiviti malaria supresi aktif lebih dari 30% terhadap jangkitan *P. berghei* dalam tikus semasa ujian supresif 4 hari. Keputusan keseluruhan dengan bukti peningkatan masa min survival menunjukkan yang *P. betle* dan *L. galanga* mengurangkan parasitemia secara signifikant ($P < 0.05$) dan meningkatkan masa survival tikus yang terjangkit. *C. nucifera*, *L. galanga* dan *P. betle* juga menunjukkan aktiviti anti-malaria yang signifikant ($P < 0.05$) semasa jangkitan malaria residual. Kajian ini menunjukkan yang ekstrak ujian adalah secara toksikologinya selamat diberi melalui oral. Ujian fitokimia ekstrak methanol bagi pokok-pokok ini menunjukkan wujudnya alkoloid, terpenoid, antraquinone, flavonoid, tanin, saponin, glikosid dan steroid. Pada kepekatan 25 µg/mL, *P. betle* menunjukkan aktiviti poten DPPH 'scavenging' antioksidan (80.68%) diikuti oleh *L. pumila* (40.66%) dan *L. galanga* (37.88%) dimana *C. nucifera* menunjukkan aktiviti DPPH 'scavenging' yang lemah (5.52%). Kedua-dua komuniti sedar akan malaria sebagai penyakit, tetapi pengetahuan, sikap dan amalan mereka tidak memadai, khususnya, di komuniti Orang Asli. Dengan membekalkan pelajaran kesihatan yang efisien kepada orang-orang yang tinggal di kawasan endemik malaria akan meningkatkan kefahaman mereka tentang pencegahan malaria untuk menuju kearah pembasmian malaria dari negara ini. Penemuan etnobotanikal boleh digunakan sebagai asas etnofarmakologi untuk memilih pokok-pokok bagi kajian fitokimia dan farmaseutikal selanjutnya. Aktiviti anti-malaria yang dilihat dalam kajian ini juga mengesahkan bahawa aplikasi berubat dari cerita rakyat Malaysia turun-temurun bagi pokok-pokok ini mempunyai asas farmakologi.

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LIST OF SYMBOLS AND ABBREVIATIONS

DPPH	1,1-diphenyl-2-picrylhydrazyl
$\chi 2$	Chi-square
D	Day
^0C	Degree centigrade
LI, LII, LIII	Degrees of resistance
DMSO	Dimethyl sulphoxide
DDT	Dichlorodiphenyltrichloroethane
$\geq$	Equals or larger than
$\leq$	Equals or smaller than
GHS	Globally Harmonized System of Classification and Labelling of Chemicals
g	Gram
IC_{50}	Half maximal inhibitory concentration
h	Hour
ICR	Imprinting Control Region mouse
IRS	Indoor residual spraying
ITNs	Insecticide-treated bed nets
i.p.	Intraperitoneal administration
LD_{50}	Median lethal dose or lethal dose, 50%
µg/mL	Microgram per milliliter
µL	Microliter
µL/kg	Microliter per kilogram
mg/kg	Milligram per kilogram
mg/mL	Milligram per milliliter
mL	milliliter
NO	Nitric oxide
n	Number of subjects
OECD	Organisation for Economic Co-operation and Development
RBCs	Red blood cells
RIDL	Release of insect carrying a dominant lethal gene
%	Percent
PBMC	Peripheral blood mononuclear cells
P	Significance level
<	Smaller than
km^2	Square kilometer
S.D.	Standard deviation
S.E.M	Standard error of the mean
SPSS	Statistical Package for the Social Sciences for Windows
H_2S0_4	Sulphuric acid
S.P.	Suppression percentage
UNICEF	the United Nations Children's Fund
UNDP	the United Nations Development Programme
VBDCP	Vector- Borne Diseases Control Program
w/w	Weight per weight
WHO	World Health Organization