UNIVERSITI PUTRA MALAYSIA

DECIPHERING THE PHARMACOLOGICAL EFFECTS OF ANDROGRAPHOLIDE ON ERYTHROCYTES AND *Plasmodium falciparum* 3D7 VIA METABOLIC CHANGES BY THE ^{1}H NMR-BASED METABOLOMICS APPROACH

ASHRAF AHMAD ISSA ALAPID

FPSK(p) 2021 27

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By

ASHRAF AHMAD ISSA ALAPID

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

March 2021

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DEDICATION

This thesis is dedicated to my dear mother, my dear father, my beloved wife, my children, my brothers and sisters.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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March 2021

Chairman : Associate Professor Rusliza Binti Basir, PhD
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Malaria is a serious health problem associated with high morbidity and mortality rates, affecting millions of people across the world. The evolution of drug resistance among various strains of *Plasmodium falciparum* has thwarted the control efforts, thereby prompting scientists to seek for new and effective alternative therapeutic agents in order to forestall the menace caused by the parasite. This study was undertaken to evaluate and elucidate the pharmacological effects of andrographolide (AG) on *P. falciparum* 3D7 and erythrocytes using ^{1}H-NMR-based metabolomics approach. The first part of the study was aimed at investigating the anti-plasmodium effect of AG against *P. falciparum* 3D7, its time-dependent effect as well as its impact on the cellular morphology of various stages of plasmodium intra-erythrocytic cycle as compared to the conventional drug chloroquine (CQ). The malaria drug sensitivity assay was carried out using pLDH and Giemsa-stained thin blood smears to determine the differences and the morphological changes at different time intervals during the growth stages of the parasite.

In the second part of this study, the IC_{50} and time-dependent of AG and CQ were used to determine the pharmacological effects of AG and CQ on the metabolic change of uninfected erythrocytes (uRBCs), infected erythrocytes (iRBCs) and the *P. falciparum* 3D7 parasite *in vitro*. The ^{1}H NMR-based metabolomics approach using Principal Component Analysis (PCA) and Orthogonal Partial Least Square discriminant analysis (OPLS-DA) were used. Overall, the results reveal that AG showed a good growth inhibitory effect (IC_{50} =4.14µM) that was substantially lower than that of CQ (IC_{50} =20.19 nM). Unlike CQ, which showed its utmost activity within the first 12 hours of the cycle, the AG effect was more prominent during the second 12 hours interval of the cycle (early trophozoites stage). Although AG failed to produce any effect on the morphology of the ring stage, it produced a noticeable change in the morphological

appearance and the sizes of the mature trophozoites after 12 hours. In contrast, the rings and trophozoites stage of the parasites were fairly affected in the chloroquine-treated flasks within the first 12 hours and 24 hours of the cycle, respectively.

Based on unsupervised data analysis PCA, the effects of AG and CQ on the metabolic changes of uRBCs showed a clear separation between all uRBCs samples with a total variance of 89.10%. A total of 28 and 32 metabolites were identified as biomarkers in uRBCs-AG and uRBCs-CQ, respectively. In uRBCs-AG, ten metabolic pathways were determined as disturbed metabolic pathways, including riboflavin metabolic pathway, D-Glutamine and D-glutamate metabolism, phenylalanine metabolism, arginine and proline metabolism, glutathione metabolism, arginine biosynthesis, citrate cycle, pyruvate metabolism, alanine, aspartate and glutamate metabolism and glycolysis/gluconeogenesis. In contrast, in uRBCs-CQ, nine metabolic pathways have been determined as disturbed metabolic pathways similar to uRBCs-AG except for glutathione metabolism. These findings suggest an evident relationship between AG and CQ associated with metabolic perturbations in uRBCs.

The effects of AG and CQ on the metabolic changes of iRBCs, the PCA and OPLS-DA showed ideal differentiation between iRBCs samples treated and untreated. Thirty-five blood metabolites were identified from the ^{1}H-NMR spectra analysis of iRBCs samples. The outcome of PCA showed clear discrimination between AG and CQ. Both PC1 and PC2 show a total variance of 77.10%. A total of 23 and 24 metabolites were identified as biomarkers in iRBCs-AG and iRBCs-CQ, respectively. The metabolic pathways analysis revealed ten metabolic pathways were identified as disturbed in all groups. The iRBCs untreated group had a high number of disturbed metabolic pathways, including alanine, aspartate and glutamate metabolism, glutathione metabolism, arginine and proline metabolism, and riboflavin metabolism. In the group of iRBCs-CQ, the disturbed metabolic pathways identified as alanine, aspartate and glutamate metabolism, arginine and proline metabolism, and glutathione metabolism. Whereas in the iRBCs-AG, the disturbed metabolic pathways identified include glyoxylate and dicarboxylate metabolism, glycine, serine and threonine metabolism, and histidine metabolism.

The effects of AG and CQ on the metabolic changes of *P. falciparum* 3D7 *in-vitro* were identified. The results of multivariate data analysis show a clear discriminant between *P. falciparum* 3D7 samples treated and untreated. The model showed a total variance of 89.9% described by the PC1 and PC2. A total of 19 and 21 metabolites were identified as biomarkers in groups of *P. falciparum* 3D7-AG and *P. falciparum* 3D7- CQ, respectively. In *P. falciparum* 3D7-AG, very few metabolites biomarkers were observed, including threonine, ornithine, riboflavin, lactate and glutathione, compared to the group treated with CQ, which showed a high number of biomarkers. Analysis of the metabolic pathways reveals two metabolic pathways were significantly disturbed in *P. falciparum* 3D7-AG group; arginine and proline metabolism, and glutathione metabolism. In *P. falciparum* 3D7-CQ group, six disturbed metabolic pathways were identified: glyoxylate and dicarboxylate metabolism, glutathione metabolism, alanine, aspartate and glutamate metabolism, arginine biosynthesis, purine metabolism and citrate cycle.

In conclusion, the present study is the first to report on the antimalarial activity of AG utilizing the ^{1}H NMR-based metabolomics approach. Results from this study suggest that the disturbed metabolic pathways identified could well serve as drug targets for future development of andrographolide-based therapeutic agents against *P. falciparum* 3D7.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

MENGHURAIKAN KESAN FARMAKOLOGI ANDROGRAPHOLIDE KE ATAS ERITROSIT DAN *Plasmodium falciparum* 3D7 MELALUI PERUBAHAN METABOLIK MENGGUNAKAN PENDEKATAN METABOLOMIK BERASASKAN ^{1}H-NMR

Oleh

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Mac 2021

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Malaria adalah masalah kesihatan yang serius yang dikaitkan dengan kadar mortaliti dan morbiditi yang tinggi melibatkan jutaan manusia di seluruh dunia. Evolusi kerintangan drug di kalangan pelbagai jenis *Plasmodium falciparum* telah membantutkan usaha mengawal, justeru mendesak para saintis mencari agen terapeutik baru dan berkesan bagi mengekang ancaman parasit ini. Kajian ini dijalankan bagi menilai dan menghuraikan kesan farmakologi andrographolide ke atas *P. falciparum* 3D7 dan eritrosit menggunakan pendekatan metabolomik berasaskan ^{1}H-NMR. Bahagian pertama kajian ini bertujuan menyelidiki kesan anti-plasmodium AG terhadap *P. falciparum* 3D7, kesan kebergantungan masa dan juga impaknya ke atas morfologi sel pelbagai peringkat kitaran intra-eritrositik plasmodium berbanding drug chloroquine (CQ) konvensional. Asai sensitiviti drug malaria dilakukan menggunakan pLDH dan calitan darah nipis berlumur Giemsa bagi menentukan perbezaan dan perubahan morfologi pada selang masa berbeza semasa peringkat pertumbuhan parasit.

Dalam bahagian kedua kajian ini, IC_{50} dan kebergantungan masa AG dan CQ digunakan bagi menentukan kesan farmakologi AG dan CQ ke atas perubahan metabolik eritrosit yang tidak terjangkit (uRBCs), eritrosit terjangkit (IRBCs) dan parasit *P. falciparum* 3D7 *in vitro*. Pendekatan metabolomik berasaskan ^{1}H-NMR mengguna pakai *"Principal Component Analysis (PCA)"* dan *"Orthogonal Partial Least Square discriminant analysis (OPLS-DA)"* telah digunakan. Secara keseluruhannya, keputusan menunjukkan bahawa AG memberikan kesan perencatan pertumbuhan yang baik (IC_{50} =4.14µM), yang lebih rendah daripada CQ (IC_{50} =20.19 nM). Tidak seperti CQ yang menunjukkan aktiviti paling tinggi dalam masa 12 jam pertama kitaran, kesan AG lebih ketara semasa selang 12 jam kedua kitaran (peringkat trofozoit awal). Walau pun AG gagal menghasilkan sebarang kesan ke atas morfologi peringkat gegelang semasa 12 jam pertama, ia menghasilkan perubahan yang nyata

dalam rupa bentuk morfologi dan saiz trofozoit matang selepas 12 jam. Sebaliknya, peringkat gegelang dan trofozoit parasit masing-masing hanya sedikit terkesan di dalam kelalang terawat CQ dalam masa 12 dan 24 jam pertama kitaran.

Berdasarkan data analisis PCA tanpa pengawasan, kesan AG dan CQ ke atas perubahan metabolik uRBCs menunjukkan pemisahan yang ketara di antara kesemua sampel uRBCs dengan varians keseluruhan 89.10%. Sebanyak 28 dan 32 metabolit masing masing dikenalpasti sebagai biopenanda dalam uRBCs-AG dan uRBCs-CQ. Dalam uRBCs-AG, sepuluh laluan metabolik telah ditentukan sebagai laluan metabolik terganggu, termasuk laluan metabolik riboflavin, metabolisme D-Glutamine and D-glutamate, metabolisme phenylalanine, metabolisme arginine and proline, metabolisme glutathione, biosintesis arginine, kitaran citrate, metabolisme pyruvate, metabolisme alanine, aspartate and glutamate, and glycolysis/gluconeogenesis. Sebaliknya, dalam uRBCs-CQ, sembilan laluan metabolik telah ditentukan sebagai laluan metabolik terganggu menyamai uRBCs-AQ kecuali metabolisme gluthathione. Dapatan ini mencadangkan hubungan jelas antara AG dan CQ yang berkait dengan gangguan metabolik dalam uRBCs.

Kesan AG dan CQ ke atas perubahan metabolik iRBCs, PCA dan OPLS-DA menunjukkan perbezaan yang ideal antara sample iRBCs terawat dan tidak terawat. Tiga puluh lima metabolit darah dikenal pasti dari analisis spektra ^{1}H-NMR sampel iRBCs. Keputusan PCA menunjukkan diskriminasi yang jelas antara AG dan CQ. Kedua dua PC1 dan PC2 menunjukkan varians keseluruhan 77.10%. Sejumlah 23 dan 24 metabolit masing masing dikenal pasti sebagai biopenanda dalam iRBCs-AG dan iRBCs-CQ. Analisis laluan metabolik mendedahkan sepuluh laluan metabolik dikenal pasti sebagai terganggu dalam kesemua kumpulan. Kumpulan iRBCs tidak terawat mempunyai bilangan laluan metabolik yang tinggi, termasuk metabolisme alanine, aspartate, glutamate, metabolisme glutathione, metabolisme arginine and proline, and metabolisme riboflavin. Dalam kumpulan iRBCs-CQ, laluan metabolik terganggu yang dikenal pasti termasuk metabolisme alanine, aspartate and glutamate, metabolisme arginine and proline, and metabolisme glutathione. Sementara dalam iRBCs-AG, laluan metabolik terganggu yang dikenal pasti termasuk metabolisme glyoxylate and dicarboxylate, metabolisme glycine, serine, threonine, and metabolisme histidine.

Kesan AG dan CQ ke atas perubahan metabolik *P. falciparum in vitro* telah dikenalpasti. Keputusan analisa data multivariat menunjukkan perbezaan jelas antara sampel *P. falciparum* 3D7 terawat dan tidak terawat. Model menunjukkan varians keseluruhan 89.9% diterangkan oleh PC1 dan PC2. Sejumlah 19 dan 21 metabolit masing masing dikenal pasti sebagai biopenanda dalam kumpulan *P. falciparum* 3D7-AG dan *P. falciparum* 3D7-CQ. Dalam *P. falciparum* 3D7-AG, hanya sedikit biopenanda metabolit diperhatikan termasuk threonine, ornithine, riboflavin, lactate dan glutathione, berbanding kumpulan yang dirawat dengan CQ, yang menunjukkan jumlah biopenanda yang tinggi. Analisa laluan metabolik mendedahkan dua laluan metabolik telah terganggu secara signifikan dalam kumpulan *P. falciparum* 3D7-AG; metabolisme arginine dan proline dan metabolisme glutathione. Dalam kumpulan *P. falciparum* 3D7-CQ, enam laluan metabolik terganggu dikenal pasti sebagai metabolisme glycoxylate and dicarboxylate, metabolisme glutathione, metabolisme

alanine, aspartate dan glutamate, biosintesis arginine, metabolisme purine and kitaran citrate.

Kesimpulannya, kajian ini adalah yang pertama melaporkan tentang aktiviti antimalaria AG menggunakan pendekatan metabolomik berasaskan ^{1}H NMR. Keputusan dari kajian ini mencadangkan bahawa laluan metabolik terganggu ini boleh menjadi sasaran drug bagi pengembangan agen terapeutik berasaskan andrographolide terhadap *P. falciparum* 3D7 di masa hadapan.

ACKNOWLEDGEMENTS

In the beginning, all praise is to ALLAH (SWT), the creator of the world.–May the peace and blessings be upon his messenger, and noble prophet Mohammad (PBUH). The PhD journey, It really has been an arduous task. However, hardworking, resilience more effort and perseverance made it possible to achieve this great opportunity. Only by ALLAH's grace and mercy, this work has been completed and Insha Allah, it was a huge success.

For the entire period of my research, there is a lot of people that deserve appreciation. Firstly, special gratitude to my able supervisor, Dr Roslaini Abd Majid for giving me the beneficial advice, for his constant support during the PhD journey as well as reviewing the experimental design step by step which had influenced positively on my scientific study. I learnt a lot from his wealth of experience not only in academic life, but also in social life. Once again, thank you for everything.

In the same vein, I would like to express my sincere appreciation to my co-supervisor Assoc. Prof. Dr. Rusliza Binti Basir for her kind help, great support, and beneficial advice. I also highly appreciate her understanding, patience and words of encouragement throughout my research study. Finally, my appreciation goes to Assoc. Prof. Dr. Rusliza Binti Basir for her acceptance to be the chairman of the new supervisory committee. May Almighty Allah reward her abundantly.

Special appreciation to my co-supervisor Assoc. Prof. Dr. Intan Safinar Ismail, I truly appreciate your contributions to the design of the metabolic part and also to your valuable explanation in my study. Assoc. Prof. Dr. Intan Safinar Ismail, being an expert in utilizing the metabolomics tool, she helped me from how to prepare the samples, extraction of the metabolites, identification of the metabolites, data analyses and drawing metabolic pathway. This has guided me through to the easy take-off steps that It would have taken a long time to optimize, May Almighty Allah reward her abundantly.

This thesis is a product of relentless effort that was achieved by consistently working hard day and night, with prayers, support and advice of my parents. Also, I appreciate the patience of my wife and children (Mohamed, Mahmoud and Abd-Almuhaymin) their encouragements and moral support help me to finish this thesis.

I appreciate the assistance of my co-students and friends Muhammad Nasiru Wana, Fatma Ibrahem, Azi Simon Onyeama, who had help me in one way or the other to achieve this great milestone. Besides, my appreciation goes to my colleagues, Dr Sharif Alhassan Abdullahi (Sharifosis), Dr Ahmed Mediani, Dr Amalina Ahmad Azam, Dr Aliyu Mahmuda and Mr Tijjani Mustapha for their assistance during my PhD journey. To our Lab support staff; Kak Farah Musa and Kak Hanim, I appreciate your wonderful cooperation.

Lastly, I thank the Ministry of Higher Education Malaysian who supported us with the research grant through the Fundamental Research Grant Scheme [grant number FRGS/1/2015/SKK12/UPM/03/01]. I also appreciate the support received from the Ministry of Higher Education Libya- University of Gharyan Libya for providing the scholarship system for my PhD study.

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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LIST OF SYMBOLS AND ABBREVIATIONS

%PI	Percentage of parasite inhibition
~	Approximately
<	Less than
=	Equals
>	Greater than
±	Plus-minus sign
1D	One-Dimensional
^{1}H-NMR	Proton Nuclear Magnetic Resonance Spectroscopy
2D	Two-Dimensional
A.A	Amine acids
Abs	Absorbance
AG	Andrographolide
ANOVA	Analysis Of Variance
APAD	3-acetylpyridine adenine dinucleotide
cMCM	Complete Malaria Culture Medium
CO_2	Carbon dioxide
CPD	Citrate Phosphate Dextrose
CQ	Chloroquine
d	Doublet
D_2O	Deuterium Oxide
dd	Doublet of doublet
DModx	distance to the model in X-space
DMSO	Di-methylsulphoxide

et al.,	alia: and others
g	Gram
g.m.wt	Gram Molecular Weight
H_2O_2	Hydrogen peroxide
Hb	Haemoglobin
HCA	Hierarchical Cluster Analysis Hierarchical Cluster Analysis
HCL	Hydrochloric acid
Hct	Hemeatocrit
HEPES	(4-(2-hydroxyethyl)-1-piperazine-ethan-sulphonic acid)
HMDB	Human metabolome database
Hz	Hertz
i.e,	That is
IC_{20}	inhibitory concentration at 20% of maximal growth
IC_{50}	inhibitory concentration at 50% of maximal growth
IC_{90}	inhibitory concentration at 90% of maximal growth
iMCM	incomplete Malaria Culture Medium
iRBCs	infected red blood cells
J	Coupling constant in Hz
KCL	Potassium chloride
KH_2PO_4	Dihydrogen phosphate
L	Liter
m	Multiplet
MHz	MegaHertz
Min	Minute

mL	Milliliter
Mm	Milli Molar
MVDA	multivariate data analysis
Na_2HPO_4	Disodium Hydrogen Phosphate
NaCl	Sodium Chloride
NADPH	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide
NaOD	Sodium Deuterium Oxide
NaOH	Sodium hydroxide
NBT	Nitroblue tetrazolium chloride
nM	Nano Molar
ºC	Degree in Celsius
P	Statistical significance level
P%	Parasitaemia percentage
PBS	Phosphate buffer saline
pLDH	Plasmodial lactic dehydrogenase enzyme
PC	Principal component
PCA	Principal component analysis
PLS	Partial Least Squares
OPLS-DA	Orthogonal Partial Least Squares–Discriminant Analysis
ppm	Part Per Million
pRBCs	Parasitized red blood cells
P. falciparum	*Plasmodium falciparum*
q	quartet

R^2	Coefficient of determination of linear regression
RBCs	Red Blood Cells
RMSEE	Root Mean Square Error of Estimation
RMSEP	Root Mean Square Error of Prediction
rpm	Revolution per minute
s	Singlet
SEM	standard error of the mean
SIMCA	Soft Independent Modeling of Class Analogy
TSP	trimethylsilyl-2,2,3,3-tetradeuteropropionate acid sodium salt
uRBCs	Uninfected red blood cells
uRBCs-AG	Uninfected red blood cells expose to Andrographolide
uRBCs-CQ	Uninfected red blood cells expose to chloroquine
VIP	variable Importance in the Projection
VP	Viability of parasite
Vs	Versus
WHO	World Health Organization
X	Times
δ	Chemical Shift in ppm
µL	Microliter

CHAPTER 1

INTRODUCTION

1.1 Background

For centuries, malaria has been one of the major devastating health issues to humanity. According to the World Health Organization (WHO) report in 2018, 228 million people were estimated to be suffering from the disease globally, causing about 405,000 deaths per annum (WHO, 2019). The majority of the recorded cases were from the African region with 93%, South East Asia with 3.4% and Eastern Mediterranean region had about 2.1% of the cases (WHO, 2019).

The partial success achieved by the eradication programs resulted in a sharp spatial concentration of malaria in the tropical areas. However, the number of malaria cases globally fell from an estimated 400 million in 2010 to 260 million in 2018. This translates to a decline of 18% globally, with the largest decline observed in the South-East Asia region, which had 50%, while the least decline was recorded in the African region (20%). Nevertheless, malaria remains a major killer of the world population, particularly in Sub-Saharan Africa (WHO, 2019).

Malaria is a life-threatening disease caused by one or a combination of five species of *Plasmodium* genus of the protozoan parasites. The *Plasmodium* parasite is transmitted to humans by infected female Anopheles mosquitoes, while taking a blood meal. The genus *Plasmodium* comprises of five species; *Plasmodium falciparum, Plasmodium ovale, Plasmodium vivax, Plasmodium malariae* and *Plasmodium knowlesi* (Cox-singh & Singh, 2008; Garnham 1966). *P. falciparum* is responsible for 99.7% of disease related deaths, especially in Sub-Saharan Africa (WHO, 2018).

1.2 Problem statement

Malaria is still one of the major public health concerns and persistent diseases worldwide. It is responsible for life-threatening infections in most endemic areas of the world. The Sub - Saharan Africa region carries a disproportionately more significant proportion of the global malaria burden (WHO, 2020). Ironically, nearly half of the world's population lives in endemic malaria areas (WHO, 2019). One of the significant problems of malaria infection is the development of drug resistance in malaria parasites that have become widespread, coupled with the limited number of effective antimalarial drugs available. Hence, disease management faces increasing difficulties to control or eradicate the disease (Mishra et al., 2016).

Resistance to the commonly used drugs such as chloroquine (CQ) against the *P. falciparum* strains has been reported in the past (Wellems & Plowe, 2001). Recently, there is an increasing report of the development of resistance against the recommended first line antimalarial treatment including the artemisinin-based combination therapies (ACTs) at the Thai-Cambodian border (Dondorp et al., 2009; Ashley et al., 2014) and Thai-Myanmar border with Vietnam (Miotto et al., 2013). Similarly, in recent years, there has been an increasing interest in efforts to eradicate the disease through vaccine development, avoidance measures and drug therapies.

Undoubtedly, these efforts led to the fractional success recorded by the eradication programs, which has also resulted in the spatial concentration of malaria cases in the resource poor tropical areas (WHO, 2017). Nevertheless, malaria remains a major killer of the world population and the widespread drug-resistant strains of *Plasmodium* are continuously evolving (WHO, 2019). It has, therefore, become necessary to understand, identify and develop new antimalarial drugs with a novel mechanism of actions against these strains.

1.3 Significance of the study

In most of the Asian, African and South American countries, approximately 90% of the people rely on traditional or alternative medicines that are based largely on different plants as a source of medicines (Fabricant & Farnsworth, 2001; Verpoorte et al., 2006; Appendino et al., 2010). One of the most commonly used medicinal plant is *Andrographis paniculata*. The plant is very popular among traditional medicine practitioners in China and other South East Asian countries (Kuroyanagi et al., 1987). The phytochemical analysis of *A. paniculata* reveals more than 55 ent-labdane diterpenoids, 30 flavonoids, 8 quinic acids, 4 xanthones, and 5 rare noriridoids (Hossain et al., 2014). The extracts from the aerial parts of the *A. paniculata* plant equally has many diterpenoids such as Andrographolide (AG), neoAndrographolide and dehydroAndrographolide.

Among these, the AG is considered one of the most essential bioactive compounds. It is a labdane diterpenoid derivative and it possesses plenty of medicinal and pharmaceutical properties, including antimicrobial, anti-inflammatory and antioxidant effects (Wasman et al., 2011). In addition, AG also possesses cardio-protective, hepato-protective, anti-HIV, anti-carcinogenic, anti-diabetic and anti-trypanosomal as well as antimalarial activity (Banerjee et al., 2017; Mishra et al., 2011; Hossain et al., 2014; Kandanur et al., 2019; Mishra et al., 2009; Misra et al., 1992; Niranjan et al., 2010; Sheeja & Kuttan, 2007; Sheeja et al., 2006; Widyawaruyanti et al., 2017; Yoopan et al., 2007; Zaid et al., 2015; Zaridah et al., 2001). Despite the extensive properties of AG, studies exploring the potentials and mechanisms of action of the AG as an anti-malaria are still scarce. Hence, their activity on uninfected red blood cells (uninfected RBCs), infected red blood cells (infected RBCs) and *P. falciparum* 3D7 as antimalarial agent has not been investigated to a great extent. Therefore, more studies need to be conducted considering the determination of the mechanism of action of this

compound will certainly highlight a pathway for the development of a new and improved antimalarial drug with a novel mechanism of actions.

1.4 Research hypothesis

The chemotherapeutic impact of plenty of drugs depends on their ability to compromise parasite growth without having affected the host cells selectively. Besides, it might alter the ability of the parasite to resist the conventional antimalarials. Andrographolide is considered one of the essential phytochemical compounds in the *A. paniculata* plant. It is a labdane diterpenoid derivative and possesses plenty of medicinal and pharmaceutical properties including an antimalarial activity. Nonetheless, studies exploring the potentials and mechanisms of action of the AG and their activity on uninfected RBCs, infected RBCs and *P. falciparum* 3D7 as an antimalarial agent has not been extensively investigated. Hence, this research is based on the hypothesis that AG might have significant biological activity on the metabolic pathways of uninfected RBCs, infected RBCs with *Plasmodium* parasite and the *P. falciparum* 3D7.

1.5 Objective

1.5.1 General objective

The main objective of the study was to identify the effect of Andrographolide compound (AG) on the metabolic pathway of the uninfected RBCs, infected RBCs and the *P. falciparum* 3D7 by ^{1}H-NMR metabolomics approach.

1.5.2 Specific objectives

1. To determine the efficacy of AG and CQ (IC_{50}) and the time dependency of antimalarial action on the intraerythrocytic cycle progression and morphology changes of *P. falciparum* 3D7 at a different time interval.
2. The determination of the expected metabolic derangement on uninfected RBCs following exposure to (AG) and (CQ) by ^{1}H-NMR based metabolomics approach.
3. The discovery of the expected metabolic derangement on infected RBCs following exposure to (AG) and (CQ) by ^{1}H-NMR based metabolomics approach.
4. To identify the biomarker(s) of the antimalarial activity of AG and CQ on *P. falciparum* 3D7 by ^{1}H-NMR based metabolomics approach.

1.6 Outline of the thesis

The purpose of this study was to evaluate the pharmacological effects of AG on infected RBCs, uninfected RBCs and *P. falciparum* 3D7 using the ^{1}H NMR-based metabolomics approach. The research is presented in a thesis report, which comprises of several chapters that provide and elaborate the details of the current study. Figure 1.1 exhibits the outline of the thesis chapters. Henceforth, a brief explanation of the specific objectives of the study is presented.

At the initial stage of the study, the efficacy IC_{50} (Inhibitory concentration to reduce the growth by 50%) of AG and CQ were determined. In addition, the time-dependency of antimalarial action on the intraerythrocytic cycle progression and morphological changes of *P. falciparum* 3D7 at the different time interval 12, 24 and 48 hours were determined (objective one). Then, the IC_{50} of AG and its time-action as well as IC_{50} of CQ and its time-action were used as a standard to achieving the other objectives. Subsequently, the metabolic variations on uninfected RBCs were determined using ^{1}H NMR-based metabolomics approach after exposure to (AG) and (CQ) (objective two). Next, the metabolic variations on infected RBCs were determined using ^{1}H-NMR after the exposure to (AG) and (CQ) (objective three). Finally, using the ^{1}H NMR-based metabolomics approach a several biomarker(s) and disturbed metabolic pathways were identified on *P. falciparum* 3D7 after exposure to AG and CQ (objective four).

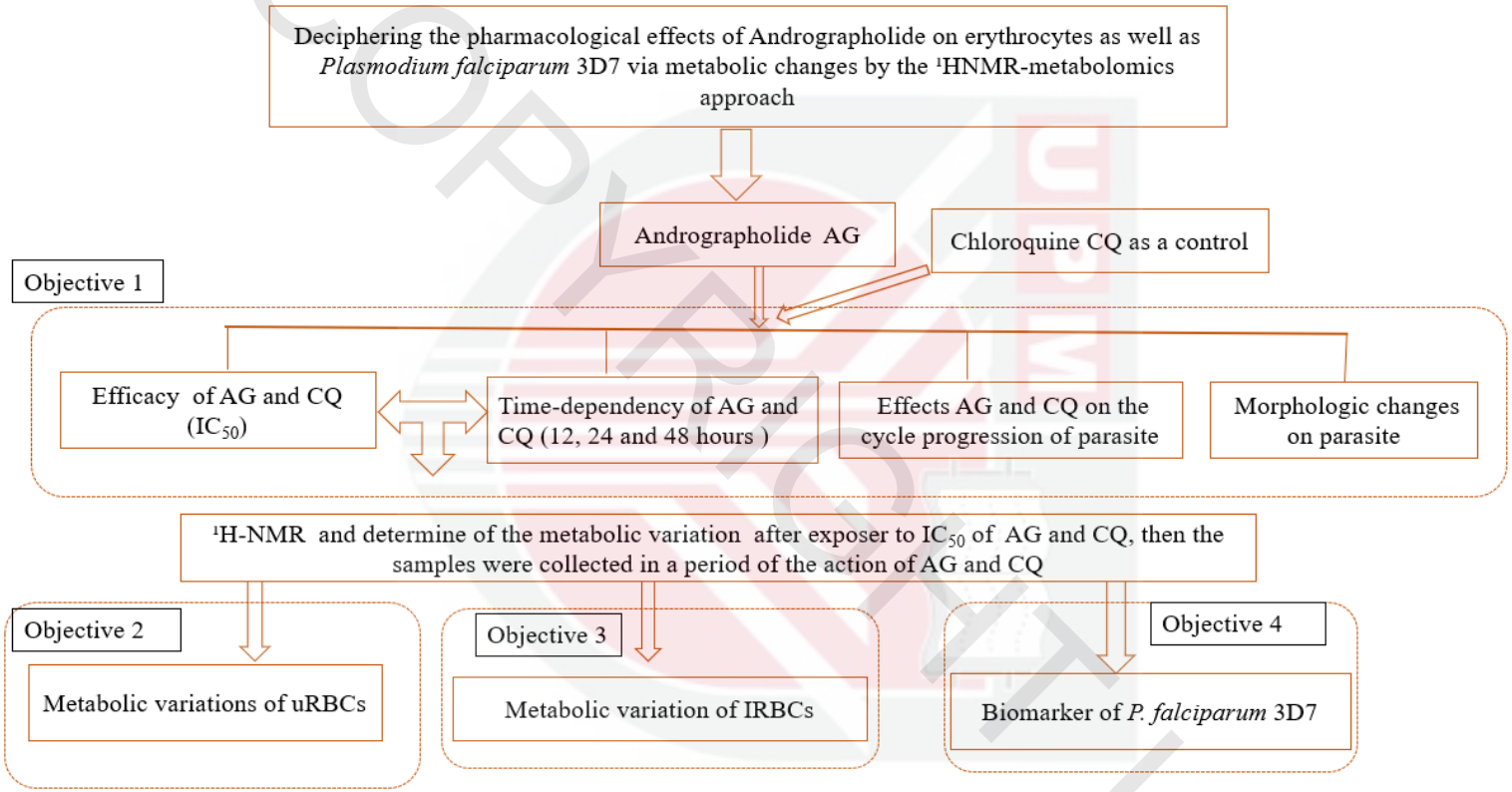

Figure 1.1 : Outline of the thesis objectives

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BIODATA OF STUDENT

The student, Ashraf Ahmed Issa Alapid, is a Libyan, he was born on 23rd January 1982 at Gharyan Libya. He studied the primary, intermediate and secondary schools in his hometown during the period from 1987 to 1998. he did bachelor's degree at university of Al Jabal Gharbi, department of biology science under Faculty of science in 1999. He graduated in 2003 and obtained a Degree in biology science. In 2004, he started his master study in the Libyan Academy, school basic science, department biological science. He graduated in 2008 and obtained M.Sc. Degree in medical parasitology. After that, he was worked as Assistant lecturer in Al Jabal Gharbi University as well as Gharyan University from 2009 to 2014. Through this job, he gained a thorough teaching experience that may help him to continue in his future academic life. In Jaan 2015, he decided to quit teaching, and he obtained admission in Universiti Putra Malaysia to pursue his PhD program under the supervision of the esteemed Dr Roslaini Bin Abd Majid. Then he came to Malaysia and studied English in ELS UPM school, for six months before joining a PhD program in Medical parasitology Unit/department of medical microbiology and parasitology at Faculty of Medicine and Health Sciences/ Universiti Putra Malaysia (UPM). From 2016 to 2020. On personal life, he is married, and they are blessed with three sons only.

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Awards

1. The best poster award at the Global health and Infectious Diseases. Infections 2017, 24th-25th October 2017. Faculty of Medicine and Health Sciences, Universiti Putra Malaysia.

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