UNIVERSITI PUTRA MALAYSIA

IN VITRO AND IN VIVO EFFECTS OF SELECTED MALAYSIAN MEDICINAL PLANT EXTRACTS ON Toxoplasma gondii

KUMARESWARAN A/L DEVANTHRAN

FPSK(M) 2016 78

IN VITRO AND *IN VIVO* EFFECTS OF SELECTED MALAYSIAN MEDICINAL PLANT EXTRACTS ON *Toxoplasma gondii*

By

KUMARESWARAN A/L DEVANTHRAN

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

April 2016

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

IN VITRO AND _IN VIVO_ EFFECTS OF SELECTED MALAYSIAN MEDICINAL PLANT EXTRACTS ON _Toxoplasma gondii_

By

KUMARESWARAN A/L DEVANTHRAN

April 2016

Chairman: **Professor Hj Wan Omar Abdullah, PhD**
Faculty : **Medicine and Health Sciences**

The sporozoan parasite *Toxoplasma gondii* is tissue parasite commonly causing infection to humans and animals, particularly in the immunocompromised hosts. Latest report stated that approximately one third of the global population has been infected with *T. gondii*. The World Health Organisation considers toxoplasmosis as one of the major parasitic diseases infecting human in the developing countries. General therapeutic drug for toxoplasmosis is pyrimethamine. Since it presents several adverse side effects, the need to develop new drugs for this condition is critical.

The aim of this study is to investigate anti-*toxoplasma* effect of some selected medicinal plants. Assays were developed to determine the anti-*toxoplasma* effects *in vitro* and *in vivo*. Four medicinal plants have been used, which are *Tinospora crispa, Piper sarmentosum*, *Andrographis paniculata* and *Curcuma longa*. The plants are reputed in traditional medicine for many treatments of diseases.

These medicinal plants reported in literature as antimalarial agent, were evaluated for their *in vitro* cytotoxicity activity against mammalian cell lines, Vero cell line, which was used as host cell in this anti-*toxoplasma* activity study. Methyl thiazolyl diphenyl tetrazolium (MTT) assay was used to measure *in vitro* cytotoxicity activity. The cell culture-based assays were performed in this study to evaluate the plant extract and determine their effectiveness in inhibiting the growth of *T. gondii in vitro*.

In *in vivo* experiment, survival analysis was conducted to estimate the survival time of *T. gondii* infected mice treated with plant extract. *T. gondii* brain cysts were inoculated orally in mice. In each study, three groups of mice were assigned to treatment with plant extract prior to *T. gondii* infection (prophylactic), after infection (therapeutic), or left untreated (infected untreated control). The plant extract effect on toxoplasmosis was evaluated by the assessment of survival rate and brain cyst burden.

All four plant extracts were found non toxic towards Vero cells. The IC_{50} value for all plant ethanolic extracts were above 100µg/ml. The least toxicity was the extract of *C. longa* followed by *T. crispa, P. sarmentosum* and *A. paniculata*. *T. gondii* tachyzoites was inhibited by plant extracts in Vero cells by concentration-dependent manner. Even at low concentration as at 25µg/ml, *T. crispa* and *P. sarmentosum* extract dramatically inhibited *T. gondii* tachyzoites in Vero cells. *T. crispa* extract showed the greatest inhibition on *T. gondii* tachyzoites growth in Vero cells followed by *P. sarmentosum*, *A. paniculata* and *C. longa*. *T. crispa* and *P. sarmentosum* extracts were comparable with positive control, clindamycin.

T. crispa extract was found as a potent anti-*toxoplasma* agent followed by *P. sarmentosum*, *A. paniculata* and *C. longa* for both *in vitro* and *in vivo* studies. In infection induced by inoculation of cysts of *T. gondii*, plant extract in prophylactic or therapeutic regimens significantly enhanced protection of infected mice against death. Delayed deaths of treated mice compare to untreated mice were observed throughout the 60 days observation period. The brains of infected mice treated with plant extract prophylactic or therapeutic groups showed low brain cyst burden compared to the infected untreated control. *T. crispa* and *P. sarmentosum* extracts reduced the brain cyst count almost to 25% compared to untreated infected mice.

These results suggest that all four plant extracts have potent anti-*toxoplasma* effect against *T. gondii* especially *T. crispa* and *P. sarmentosum* extracts. These plant extracts can be exploited for development of alternative medicine to treat *T. gondii* infections.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KESAN *IN VITRO* DAN *IN VIVO* EKSTRAK TUMBUHAN HERBA MALAYSIA TERHADAP PARASIT *Toksoplasma gondii*

Oleh

KUMARESWARAN A/L DEVANTHRAN

April 2016

Pengerusi: **Profesor Hj Wan Omar Abdullah, PhD**
Fakutli : **Perubatan dan Sains Kesihatan**

Parasit *Toksoplasma gondii* adalah parasit tisu yang menyebabkan jangkitan kepada manusia dan haiwan, terutamanya dalam perumah yang mempunyai sistem imun yang lemah. Satu pertiga daripada jumlah penduduk dunia kini telah dijangkiti parasit ini. Pertubuhan Kesihatan Dunia mempertimbangkan toksoplasmosis sebagai salah satu penyakit parasit utama menjangkiti manusia di kalangan negara membangun. Ubat umum terapeutik untuk merawat toksoplasmosis adalah 'pyrimethamine'. Memandangkan ubat tersebut menyebabkan beberapa kesan sampingan yang buruk, keperluan untuk menghasilkan dan menilai ubat-ubatan baru adalah penting.

Tujuan kajian ini adalah untuk mengkaji kesan anti-toksoplasma daripada herba. Kajian telah direka untuk menentukan kesan anti-toksoplasmal *in vitro* dan *in vivo*. Empat herba telah digunakan dalam kajian ini. Herba tersebut ialah *Tinospora crispa*, *Piper sarmentosum*, *Andrographis paniculata* dan *Curcuma longa*. Herba tersebut biasa digunakan dalam perubatan tradisional untuk merawat pelbagai penyakit.

Herba tersebut telah dilaporkan mempunyai kesan terhadap parasit malaria. Oleh yang demikian, herba tersebut telah dinilai aktiviti ketoksikan secara *in vitro* terhadap sel mamalia; sel Vero yang digunakan sebagai sel perumah untuk kajian aktiviti anti-toksoplasma. Aktiviti ketoksikan *in vitro* dikenalpasti melalui kajian 'methyl thiazolyl diphenyl tetrazolium'. Kajian berasaskan kultur sel telah dijalankan untuk menilai ekstrak herba yang berkesan untuk merencat aktiviti *T. gondii* dalam sel Vero melalui kaedah *in vitro*.

Dalam eksperimen *in vivo*, analisis jangka hayat hidup telah dijalankan untuk menganggarkan jangka hayat tikus yang dijangkiti *T. gondii* yang dirawat dengan ekstrak herba. Sista otak yang mengandungi *T. gondii* disuntik melalui mulut pada tikus. Dalam setiap kajian, tiga kumpulan rawatan tikus telah direka. Kumpulan rawatan tersebut adalah rawatan ekstrak herba sebelum jangkitan (pencegahan),

selepas jangkitan (terapeutik), dan kumpulan tanpa rawatan herba. Kesan ekstrak herba terhadap toksoplasmosis telah dinilai melalui pemerhatian jangka hayat hidup tikus dan beban sista otak tikus.

Kesemua ekstrak herba terbukti tidak toksik terhadap sel Vero. Nilai IC_{50} toksik untuk kesemua ekstrak etanol herba melebihi nilai 100µg/ml. Nilai IC_{50} toksik terendah adalah ekstrak *C. longa* diikuti oleh *T. crispa*, *P. sarmentosum* dan *A. paniculata*. Ekstrak herba menghalang pertumbuhan *T. gondii* dalam sel Vero dengan kepekatan yang berbeza. Walaupun pada kepekatan yang rendah, iaitu pada 25µg/ml, ekstrak *T. crispa* dan *P. sarmentosum* menghalang pertumbuhan *T. gondii* dalam sel Vero. Ekstrak *T. crispa* menunjukkan aktiviti perencatan yang ketara dalam pertumbuhan *T. gondii* dalam sel Vero diikuti oleh *P. sarmentosum*, *A. paniculata* dan *C. longa*. Ekstrak *T. crispa* dan *P. sarmentosum* menunjukkan potensi yang tinggi setanding dengan kawalan positif, ubat clindamycin.

Ekstrak *T. crispa* menghasilkan kesan anti-toksoplasma terbaik diikuti oleh *P. sarmentosum*, *A. paniculata* dan *C. longa* dalam kedua-dua kajian *in vitro* dan *in vivo*. Dalam jangkitan yang disebabkan oleh sista otak *T. gondii*, ekstrak herba dalam kumpulan pencegahan dan terapeutik telah meningkatkan perlindungan tikus yang dijangkiti daripada kematian. Pengurangan jangka hayat hidup tikus tanpa rawatan berbanding tikus yang dirawat telah diperhatikan sepanjang tempoh pemerhatian 60 hari. Tikus yang dijangkiti dan dirawat dengan ekstrak herba dalam kumpulan pencegahan dan terapeutik menunjukkan beban sista otak yang rendah berbanding dengan kumpulan tikus yang dijangkiti tanpa rawatan. Ekstrak *T. crispa* dan *P. sarmentosum* telah mengurangkan sista otak hampir 25% berbanding dengan tikus yang dijangkiti tanpa rawatan.

Kajian ini menunjukkan bahawa kesemua ekstrak herba mempunyai kesan anti-*toksoplasma* terhadap *T. gondii* terutamanya ekstrak *T. crispa* dan *P. sarmentosum*. Ekstrak herba ini boleh dieksploitasi untuk pembangunan perubatan alternatif bagi merawat jangkitan *T. gondii.*

ACKNOWLEDGEMENTS

Firstly, I would like to express my appreciation to my respected supervisor Professor. Dr. Hj. Wan Omar Abdullah and my utmost gratitude to him for his supervision throughout my study. Professor Wan was my research mentor in providing me with guidance, expertise and encouragement without which I could have never completed this study. His enthusiasm and support were the great factors contributing to all of my physical, intellectual, moral and spiritual upliftments. I can never thank him enough for all the time he has invested in me, and he will be an unforgettable figure in my life.

I am delighted to express my gratitude to my co supervisor Dr Ngah Zasmy Unyah for his patience, progressive advices and guidance throughout my project, without him I won't be able to complete this research. Special appreciation for all the people in my lab; Medical Parasitology lab especially Cik Norhanim and Pn. Siti Farah for helping me with my research. Special thanks also to my dear lab mates who were part of my research life in laboratory as well as outside campus life.

I extend my sincere thanks to Animal Experimental Unit, Virology Lab, Faculty of Medicine and Health Scienecs, Universiti Putra Malaysia, Institute of Medical Research, Universiti Malaya and Forest Research Institute Malaysia for providing great assistance for my research. I would like to acknowledge UPM for providing Graduate Research Fellowship (UPM/SPS/GS37441), and Research University Grant Scheme, UPM (RUGS 04-02-12-1763RU) as well as Ministry of Higher Education (MOHE) Malaysia for providing MyMaster [KPM(B) 880730055563] scholarship. Without the funding and support this research would not become a reality.

My special thanks to my family for patiently putting up with me emotionally and financially throughout my studies. Also not forgetting their love and continuous support and encouragement throughout my study. Last but never the least, is God Almighty for allowing me to finish this thesis, overcoming many obstacles and preserve my sanity through my research period.

I certify that a Thesis Examination Committee has met on (date of viva voce) to conduct the final examination of Kumareswaran A/L Devanthran on his thesis entitled "*In vitro* and *in vivo* effects of extracts of selected Malaysian plants on *Toxoplasma gondii*" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science

Members of the Thesis Examination Committee were as follows:

Prof. Dr. Zamberi Sekawi, MPath
Professor
Department of Medical Microbiology and Parasitology
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Rusliza Basir, PhD
Associate Professor
Department of Anatomy
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

Nasuruddin Hj. Abdullah, MMed
Professor
Department of Pathology and Laboratory Medicine
Kulliyah of Medicine
International Islamic University Malaysia
(External Examiner)

(Prof. Dr. Zulkarnain Zainal)
Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia
Date:

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the supervisory committee are as follows:

Hj. Wan Omar Abdullah, PhD
Professor
Faculty of Mediciine and Health Sciences
University Putra Malaysia
(Chairman)

Ngah Zasmy A/L Unyah, PhD
Senior Lecturer
Faculty of Medicine and Health Sciences
University Putra Malaysia
(Member)

_____
BUJANG KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

Declaration by graduate student

I hereby confirm that:

- This thesis is my original work;
- Quotations, illustrations and citations have been duly referenced;
- This thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- Intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- Written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before the thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- There is no plagiarism or data falsification/ fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism software.

Signature: ---------- Date: ----------

Name and Matric No.: Kumareswaran A//L Devanthran, GS37441

Declaration by Members of Supervisory Committee

This is to confirm that:

- The research conducted and the writing of this thesis was under our supervision;
- Supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to:

Signature: _____

Name of Chairman of
SupervisoryCommittee: Prof. Dr. Hj. Wan Omar Abdullah

Signature: _____

Name of Member of
Supervisory Committee: Dr. Ngah Zasmy Unyah

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

ACUC	Animal Care and Use Committee
AIDS	Acquired Immune Deficiency Syndrome
CD4	Cluster of differentiation 4
CDC	Centers for Disease Control
CNS	Central Nervous System
CT	Computed Tomography
Da	Dalton
DHFR	Dihydrofolate Reductase
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme linked immno assay
FAS	Fatty Acid Synthesis
FBS	Fetal Bovine Serum
HBSS	Hanks' balanced salt solution
HIV	Human immunodeficiency virus
IC_{50}	Half maximal inhibitory concentration
IFA	Indirect fluorescent antibody
Ig	Immunoglobulin
LC_{50}	Half maximal lethal concentration
LD	Lethal Dose
mg	miligram
mg/ml	milligram/ mililitre
ml	mililitre
MRI	Magnetic Resonance Imaging
MRSA	Methicillin-resistant *Staphylococcus aureus*
MTT	(3-(4,5-Dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide)
PABA	Para-Aminobenzoic Acid
PAS	Periodic Acid Schiff
PBS	Phosphate Buffer Solution
PCR	Polymerase Chain Reaction
PI	post-infection
ppm	part per million
rpm	revolutions per minute
RPMI	Roswell Park Memorial Institute
T. gondii	*Toxoplasma gondii*
µg/ml	microgram/ mililitre

CHAPTER 1

INTRODUCTION

Toxoplasmosis caused by the ubiquitous obligatory intracellular coccidian protozoan, *Toxoplasma gondii* (Negash *et al.,* 2008) that has many forms. There are three infectious stages of *Toxoplasma gondii.* They are tachyzoites (rapidly multiplying form), bradyzoites (tissue cyst form), and sporozoites (in oocysts). Tachyzoites are found in the acute phase of the disease and are responsible for clinical manifestations. They are susceptible to the immunological response of the host and to drug action. Cysts are the resistant form of the parasite, persisting for the host's entire life. Cyst walls are resistant to both drugs and the immune system (Hill *et al.,* 2002).

Toxoplasma gondii has a wide range of hosts which includes humans, mammals and marine mammals. About one third of the world population has been exposed to this parasite. Humans or animals can acquire *Toxoplasma gondii* infection post-natal by ingestion of undercooked or raw meat from infected animals, or ingestion of food or water contaminated with oocysts excreted by infected cats (Dubey *et al.,* 2008). The population structure of *Toxoplasma gondii* consists of three main clonal lineages; Type I (including RH,a highly virulent strain), Type II (including avirulent strains like Me49), and Type III (including avirulent strains like NED) correlated with virulence expression in mice (Howe *et al.,* 1995).

The treatment of toxoplasmosis is essential as *Toxoplasma gondii* causes serious mortality and morbidity in pregnant women and in immunocompromised patients who are suffering from the Acquired Immune Deficiency Syndrome (AIDS) or those undergoing chemotherapy. It is therefore clear that anti-*Toxoplasmic* therapy need to be potent against all strains of *Toxoplasma gondii*, be capable of killing tachyzoites and have a high ocular and cerebral penetration. However, their side effects, lack of efficacy against the tissue cyst of parasite and the potential appearance of resistant strains are particular drawback of the available treatments. (Mui *et al.,* 2008)

Several4 treatment failures of toxoplasmic encephalitis, chorioretinitis, and congenital toxoplasmosis have been reported (Baatz *et al.,* 2006; Doliwa *et al.,* 2013; Petersen 2007; Torres *et al.,* 1997). In addition, long term use of these drugs may lead to hematologic and renal toxicity (Crespo *et al.,* 2000) and the condition which lead to clinical failure by selecting drug resistant parasite variants. The current use of pyrimethamine for treatment of *T. gondii* infection is associated with suppression of bone marrow and may lead to neutropenia condition. Moreover, the combination of pyrimethamine with sulfadiazine can give rise to further concern due to allergy, kidney stones, or hepatic or renal complications (Mui *et al.,* 2005).

Nowadays, there is an increase of awareness on therapeutic potential of natural products and medicinal plants. Natural products are usually considered to be less toxic

and free from side effects than synthetic drugs in treating various diseases. The importance of these plants as sources of natural product bioactive compounds to medicine lies not only in their pharmacological or chemotherapeutic effect, but also in their role as template compounds for the production of new drug compounds (Phillipson *et al.,* 1994).

Previous studies showed that, lack of vaccine availability, and with the rise of parasite resistance towards therapeutic drugs, natural products can be efficient alternative against intracellular parasites such as *Plasmodium falciparum* and *Trypanosoma cruzi*. (Cui *et al.,* 2007, Kolodziej *et al.,* 2005) Natural products play an important role in the process of developing new drugs in the field of cancer research and infectious diseases (Newman *et al.,* 2003). The medicinal value of these plants lies in some chemical compounds that induce a definite physiological activity on the human body (Balunas *et al.,* 2005). In recent years, people have tended to use traditional medicine for the treatment of diseases. Medicinal plants are assumed to possess numerous secondary metabolites as flavonoids, alkaloids, terpenoids, tannins and others that help to protect the body from a variety of diseases.

Problem Statement

Standard therapies for toxoplasmosis involving combinations of pyrimethamine with sulfadiazine, clindamycin, azithromycin, or atavaquone. Drug treatment is often associated with severe side effects such as bone marrow suppression, cutaneous rash, leukopenia and thrombocytopenia. Alternative drugs with lesser side effects are needed to combat the disease by utilising medicinal plants.

1.1 Objectives

The general objective of this study is to evaluate the effects of selected Malaysian medicinal plants on *Toxoplasma gondii* infection.

1.2 Specific objectives

1. To determine *in vitro* activity of selected Malaysian medicinal plants against *Toxoplasma gondii* infection on Vero cell and the inhibitory concentration values for each treatment condition.
2. To demonstrate *in vivo* anti-*toxoplasma* activity in animal experiments for effective control of infection using infusions of selected Malaysian medicinal plants.
3. To determine cytotoxicity levels of selected Malaysian medicinal plants on mammalian cell.

REFERENCES

Abu El-Ezz NM. (2005) Effect of *Nigella sativa* and *Allium cepa* oils on *Trichinella spiralis* in experimentally infected rats. *Journal of Egyptian Society of Parasitology*; 35(2):511-23.

Aguilar-Diaz, H., Carrero, J.C., Arguello-Garcia, R., Laclette, J.P., and Morales-Montor, J. (2011) Cyst and encystment in protozoan parasites: Optimal targets for new life-cycle interrupting strategies? *Trends in Parasitology 27*; 450-458.

Al-Zanbagi N A (2009) *In vivo* effect of some home species extracts on *Toxoplasma gondii* tachyzoites. *Journal of Family and Community Medicine*; 16: 59-65.

Aldini R, Budriesi R, Roda G, Micucci M, Ioan P (2012) *Curcuma longa* extract exerts a myorelaxant effect on the ileum and colon in a mouse experimental colitis model, independent of the anti-inflammatory effect. *PLoS ONE*; 7(9).

Allam G (2009) Immunomodulatory effects of curcumin treatment on murine schistosomiasis mansoni. *Immunobiology*; 214: 712-727.

Angela L, Stüdli A, Brun R, Stephens CE, Boykin DW, Hemphill A. (2008) Host cells participate in the *in vitro* effects of novel diamidine analogues against tachyzoites of the intracellular apicomplexan parasites *Neospora caninum* and *Toxoplasma gondii*. *Antimicrobial Agents Chemotherapy*; 52:1999–2008.

Araújo CAC, Alegrio LV, Castro D, Lima MEF, Leon LL. (1998) *Leishmania amazonensis*: *in vivo* experiments with diarylheptanoids from Leguminosae and Zingiberaceae plants. *Memorias do Instituto Oswaldo Cruz 93* (Suppl. II): 306.

Araújo CAC, Alegrio LV, Castro D, Lima MEF, Gomes- Cardoso L, Leon LL. (1999) Studies on the effectiveness of diarylheptanoids derivatives against *Leishmania amazonensis*. *Memorias do Instituto Oswaldo Cruz 94*: 791-794.

Araujo F, Slifer T. (2003) Different strains of *Toxoplasma gondii* induce different cytokine responses in CBA/Ca mice. *Infection and Immunology*; 71(7):4171-4174.

Ariffin SHZ, Haryani WH, Ariffin ZZ, Safian MF, Senafi S, Wahab RMA. (2009) Intrinsic anticarcinogenic effects of *Piper sarmentosum* ethanolic extract on a human hepatoma cell line. *Cancer Cell International*; 9:6.

Arora, D. R., & Arora, B. (2005). *Medical Parasitology* (2nd Edition ed.): CBS Publisher and Distributer.

Aspinall, T. V., Joynson, D. H. M., Guy, E. (2002). The molecular basis of sulfonamide resistance in *Toxoplasma gondii* and implications for the clinical management of toxoplasmosis. *Journal of Infecious Disease*; 185, 1637–1643.

Athanasiadou S., J. Githiori and I. Kyriazakis. (2007) Medicinal plants for helminth parasite control: *Facts and Fiction*; 1: 1392-1400.

Atiax E, Ahmad F, Sirat HM, Arbain D. (2011) Antibacterial activity and cytotoxicity screening of Sumatran Kaduk (*Piper sarmentosum* Roxb). *Iranian Journal Pharmacology Therapy*; 10:1-5.

Baatz H, Mirshahi A, Puchta J, Gumbel H, Hattenbach L. (2006) Reactivation of *Toxoplasma* retinochoroiditis under atovaquone therapy in an immunocompetent patient. *Ocular Immunology and Inflammation*; 14: 185-187.

Baker, J. T., Borris, R. P., Carté, B., Madulid, D. R., and Tyler, V. E. (1995) Natural product drug discovery and development: New perspectives on international collaboration., *Journal of Natural Product*; 58: 1325-1357.

Balunas MJ, Kinghorn AD (2005) Drug discovery from medicinal plants. *Life Science*; 78: 431-441.

Belloni A, Villena I, Gomez JE, Aubert D. (2003) Regulation of tumor necrosis factor alpha and its specific receptors during *Toxoplasma gondii* infection in human monocytic cells. *Parasitology Research*; 89: 207–13.

Bermudez-Almada, M. D., Miranda-Vasquez, L., Espinosa- Plascencia, A. (2001) Sulfonamide residues in muscle of pigs slaughtered in the Northwest of Mexico. *Revista Cientifica (Maracaibo)* 11, 127–132.

Biswas TK, Mukherjee B. (2003) Plant medicines of Indian origin for wound healing activity: A healing review. *International Journal of Lower Extremity Wounds*; 2(1): 25-39.

Bjurling, P., Baxter, G. A., Caselunghe, M. (2000) Biosensor assay of sulfadiazine and sulfamethazine residues in pork. *Analyst*; 125: 1771–1774.

Black, M. W & Boothroyd, J. C. (2000). Lytic cycle of *Toxoplasma gondii*. *Microbiology and molecular biology reviews, Microbiology and Molecular Biology Reviews*; 64(3): 607-623.

Blancquaert, D., Storozhenko, S., Van Der Straeten, D. (2010) Folates and folic acid: From fundamental research toward sustainable health. *Critical Reviews in Plant Sciences*; 29: 14–35

Burkhardt, D., Wiesner,J., Stoesser,N., Ramharter,M., Uhlemann,A.C., Issifou,S. (2007) Delayed parasite elimination in human infections treated with clindamycin parallels 'delayed death' of *Plasmodium falciparum in vitro. International Journal of Parasitology*; 37(7): 777–785.

Camps, M., Arrizabalaga, G. & Boothroyd, J. (2002). An rRNAv mutation identifies the apicoplast as the target for clindamycin in *Toxoplasma gondii. Molecular Microbiology*; 43: 1309–1318.

CDC (2008). Toxoplasmosis, from http://www.cdc.gov/toxoplasmosis/epi.html

Chaithong U, Choochote W, Pitasawat B. (2006) Larvicidal effect of pepper plants on *Aedes aegypti* (L.) (Diptera: Culicidae). *Journal of Vector Ecology*; 31:138-144.

Chang, C. F., Yeh, T. M., Chou, C. C. (2002) Antimicrobial susceptibility and plasmid analysis of *Actinobacillus pleuropneumoniae* isolated in Taiwan. *Veterinary Microbiology*.; 84: 169–177.

Chatterton JM, Evans R, Ashburn D, Joss AW, Ho-Yen DO. (2002) *Toxoplasma gondii in vitro* culture for experimentation. *Journal of Microbiology Methods*; 51: 331–335.

Chew WK, Wah MJ, Ambu S, Segarra I. (2012) *Toxoplasma gondii*: Determination of the onset of chronic infection in mice and the *in vitro* reactivation of brain cysts. *Experimental Parasitology*; 130: 22–25.

Choi KM, Gang J, Yun J. (2008) Anti-*Toxoplasma gondii* RH strain activity of herbal extracts used in traditional medicine. *International Journal Antimicrobiology Agents*; 32: 360–362.

Choochote W, Chaithong U, Pitasawat B. (2006) Adulticidal activity against *Stegomyia aegypti* (Diptera: Culicidae) of three *Piper* spp. *Journal of the Institute of Tropical Medicine of São Paulo*; 48: 33-37.

Choudhury B.R., Haque SJ., Poddar M.K. (1987) *In vivo* and *in vitro* effects of kalmegh (*Andrographis paniculata*) extract and andrographolide on hepatic microsomal drug metabolizing enzymes. *Planta Medica*; 53(2): 135-40.

Choudhury B.R., Poddar M.K. (1984). Andrographolide and kalmegh (*Andrographis paniculata*) extract: *In vivo* and *in vitro* effect on hepatic lipid peroxidation. *Methods and Findings in Experimental and Clinical Pharmacology Journal*; 6(9): 481-5.

Choudhary MI, Ismail M, Ali Z, Shaari K, Lajis NH, Attaur R. (2010) Alkaloidal constituents of *Tinospora crispa*. *Natural Product Communications*; 5(11): 1747-50.

Colombo, F. A., Vidal, J. E., Penalva de Oliveira, A. C., Hernandez, A. V., Bonasser-Filho, F., Nogueira, R. S. (2005) Diagnosis of cerebral toxoplasmosis in AIDS patients in Brazil: Importance of molecular and immunological methods using peripheral blood samples. *Journal of Clinical Microbiology*; 43(10): 5044-5047.

Coppens, I., Dunn, J.D., Romano, J.D. and Joiner, K.A. (2006) *Toxoplasma gondii* sequesters lysosomes from mammalian hosts in the vacuolar space. *Cell*; 125: 261-274.

Cos, P., Vlietinck, A. J., Berghe, D. V., and Maes, L. (2006) Anti-infective potential of natural products: How to develop a stronger *in vitro* "proof-of-concept", *Journal of Ethnopharmacology*; 106: 290-302.

Crespo M, Quereda C, Pascual J, Rivera M, Clemente L. (2000) Patterns of sulfadiazine acute nephrotoxicity. *Clinical Nephrology*; 54: 68 -72.

Cui L, Miao J, Cui L (2007) Cytotoxic effect of curcumin on malaria parasite *Plasmodium falciparum*: Inhibition of histone acetylation and generation of reactive oxygen species. *Antimicrobial Agents Chemotherapy*; 51: 488-494.

Darcy F, Santoro F (1994) Toxoplasmosis. In F. Kierszenbaum, ed. Parasitic infections and the immune system, Academic Press Inc., San Diego, Calif. Pp 163–201.

Díaz de la Garza, R. I., Gregory, J. F. and Hanson, A. D. (2007) Folate biofortification of tomato fruit. *Proceedings of the National Academy of Science*; 104: 4218–4222

Denkers EY, Gazzelini RT (1998) Regulation and function of T-cell-mediated immunity during *Toxoplasma gondii* infection. *Clinical Microbiology Review*; 11: 569–588.

Derouin F, Jacqz-Aigrain E, Thulliez P, Couvreur J, Leport C (2002) Cotrimoxazole for prenatal treatment of congenital toxoplasmosis? *Parasitology Today*; 16: 254-256.

Derouin, F., Almadany, R., Chau, F., Rouveix, B., & Pocidalo, J. J. (1992). Synergistic activity of azithromycin and pyrimethamine or sulfadiazine in acute experimental toxoplasmosis. *Antimicrobial Agents and Chemotherapy*.

Djurković-Djaković O, Milenkovic V, Nikolic A, Bobić B, Grujić J. (2002) Efficacy of atovaquone combined with clindamycin against murine infection with a cystogenic (Me49) strain of *Toxoplasma gondii*. *Journal of Antimicrobial and Chemotherapy*; 50:981-987.

Dobrowolski, J.M., and Sibley, L.D. (1996) *Toxoplasma* invasion of mammalian cells is powered by the actin cytoskeleton of the parasite. *Cell*; 84: 933-939.

Doliwa C, Xia D, Escotte-Binet S, Newsham EL, Sanderson SJ (2013) Identification of differentially expressed proteins in sulfadiazine resistant and sensitive strains of *Toxoplasma gondii* using difference-gel electrophoresis (DIGE). *International Journal of Parasitology Drugs and Drug Resistance*; 3: 35–44.

Dua VK, Ojha VP, Roy R. (2004) Antimalarial activity of some xanthones isolated from the roots of *Andrographis paniculata*. *Journal of Ethnopharmacology*; 95:247-251.

Dua VK, Verma G, Dash AP. (2009) *In vitro* antiprotozoal activity of some xanthones isolated from the roots of *Andrographis paniculata*. *Phytotherapy Research*; 23: 126-128.

Dubey J P. (2010) Toxoplasmosis of animals and humans, 2nd ed. Boca Raton: CRC Press; pp313.

Dubey, J.P. (1997) Bradyzoite-induced murine toxoplasmosis: Stage conversion, pathogenesis and tissue cyst formation in mice fed bradyzoites of different strains of *Toxoplasma gondii*. *Journal of Eukaryotic Microbiology*; 44: 592-602.

Dubey, J. P. (1998) Advances in the life cycle of *Toxoplasma gondii*. *International Journal for Parasitology*; 28(7): 1019-1024.

Dutta A, Sukul NC. (1982) Filaricidal properties of a wild herb, *Andrographis paniculata*. *Journal of Helminthology*; 56: 81-84.

Dweck AC and Cavin J. (2006) Andawali *(Tinospora crispa)-A Review. Personal Care Magazine*; 7: 1-3.

Ee GCL, Lim CM, Lim CK, Rahmani M, Shaari K, Bong CFJ. (2009) Alkaloids from *Piper sarmentosum* and *Piper nigrum*. *National Product Research*; 23: 1416-1423.

El-Sayed N M, Aly E M (2014) *Toxoplasma gondii* infection can induce retinal DNA damage: An experimental study. *International Journal of Ophthalmology*; 7: 431-436.

Esklid P. (2007) Toxoplasmosis. *Seminars in Fetal and Neonatal Medicine*; 12(3): 214-223.

Fabricant, D. S., and Farnsworth, N. R. (2001) The value of plants used in traditional medicine for drug discovery. *Environmental Health Perspectives*. National Institute of Environmental Health Science; 109: 69-75.

Farkaad A. Kadir, Faizah Othman and Pouya Hassandarvish. (2011) Effect of *Tinospora crispa* on thioacetamide-induced liver cirrhosis in rats. *Indian Journalof Pharmacology*; 43(1): 64–68.

Faucher B, Moreau J, Zaegel O, Frank J, Piarroux P (2011) Failure of conventional treatment with pyrimethamine and sulfadiazine for secondary prophylaxis of cerebral toxoplasmosis in a patient with AIDS. *Journal of Antimicrobial Chemotherapy*; 66:1654 –1656.

Ferguson D, Mark J, Araujo F, Remington J. (1994) A morphological study of chronic cerebral toxoplasmosis in mice: Comparison of four different strains of *Toxoplasma gondii*. *Parasitology Research*; 80: 493-501.

Ferguson, D.J., Hutchison, W.M., and Pettersen, E. (1989) Tissue cyst rupture in mice chronically infected with *Toxoplasma gondii*: An immunocytochemical and ultrastructural study. *Parasitology Research*; 75: 599-603.

Ferreira R, Oliveira AB, Ribeiro MF, Tafuri WL, Vitor RW. (2006) *Toxoplasma gondii*: *In vitro* and *in vivo* activities of the hydroxynaphthoquinone 2-hydroxy-3-(1`-propen- 3-phenyl)-1, 4-naphthoquinone alone or combined with sulphadiazine. *Experimental Parasitology*; 113:125-129.

Fichera, M.E., Bhopale,M.K., Roos,D.S. (1995) *In vitro* assays elucidate peculiar kinetics of clindamycin action against *Toxoplasma gondii*. *Antimicrobial Agents Chemotherapy*; 39(7): 1530–1537.

Fichera, M.E., Roos,D.S. (1997) Aplastid organelle as a drug target in apicomplexan parasites. *Nature*; 390 (6658): 407–409.

Grag SR, Menon KV. (2003) Spices and herbs- promising antimicrobial agents and natural food preservatives. *Haryana Agricultural University Journal of Research*; 3(1): 1-6.

Gescher A, Sharma R, Steward W. (2005) Curcumin: the story so far. *European Journal Cancer*; 41(13): 1955-1968.

Goodman, C.D., McFadden, G.I. (2007) Fatty acid biosynthesis as a drug target in apicomplexan parasites. *Current Drug Targets*; 8 (1): 15–30.

Grujic J, Djurkovic-Djakovic O, Nikilic A, Klun I, Bobic D. (2005) Effectiveness of spiramycin in murine models of acute and chronic toxoplasmosis. *Journal of Antimicrobial Agents*; 25: 226 –230.

Hanisa H, MohdAzmi ML, Suhaila M, Somchit MN. (2014) Effects of *Centella asiatica* L., *Curcuma longa* L. and *Strobilanthes crispus* L.extracts on 3 kidney cell lines: *In vitro* cytotoxicity analysis. *International Journal of Pharmacy and Pharmaceutical Sciences*; 6(2): 388-392.

Haverkos HW. (1987) The TE study group: Assessment of therapy for toxoplasma encephalitis. *The American Journal of Medicine*; 82: 907-914.

Hermes G, Ajioka J, Kelly K (2008) Neurological and behavioral abnormalities, ventricular dilatation, altered cellular functions, inflammation, and neuronal injury in brains of mice due to common persistent parasitic infection. *Journal of Neuroinflammation*; 5:48.

Hill D & Dubey JP (2002) *Toxoplasma gondii*: Transmission, diagnosis and prevention. *Clinical Microbiology Infection*; 8: 634-640.

Huskinson-Mark, J., Araujo, F. G., & Remington, J. S. (1991). Evaluation of the effect of drugs on the cyst form of *Toxoplasma gondii*. *The Journal of Infectious Diseases*; 164(1): 170-171.

Hussain K, Ismail Z, Sadikun A, Ibrahim P, Malik A. (2009) Cytotoxicity evaluation and characterization of chloroform extract of leaf of *Piper sarmentosum* possessing antiangiogenic activity. *Pharmacology online*; 2: 379-391.

Hoff, E.F., and Carruthers, V.B. (2002) Is *Toxoplasma egress* the first step in invasion? *Trends in Parasitology*; 18: 251-255.

Howe DK and Sibley LD (1995) *Toxoplasma gondii* comprises three clonal lineages: Correlation of parasite genotype with human disease. *Journal of Infectious Disease*; 172: 1561–1566.

Hu, K., Mann, T., Striepen, B., Beckers, C.J., Roos, D.S., and Murray, J.M. (2002) Daughter cell assembly in the protozoan parasite *Toxoplasma gondii*. *Journal of Molecular Cell Biology*; 13: 593-606.

Ibrahim M.J., Wan M.Z., Narimah A.H., Nurul A.Z. (2011) Anti-proliferative and antioxidant effects of *Tinospora crispa*. *Biomedical Research*; 22(1): 57-62

Ishita C, Kaushik B, Uday B, Ranajit KB (2004) Turmeric and curcumin: Biological actions and medicinal applications. *Current Science*; 87: 44-53.

Israelski DM, Remington JS (1993) *Toxoplasma gondii* is an intracellular protozoan parasite: Toxoplasmosis in the non- AIDS immunocompromised host. *Current Clinical Topics in Infectious Diseases*; 13: 322–356.

Joynson, D. H. M. & Wreghitt, T. G. (2001) Toxoplasmosis: A comprehensive clinical guide. Cambridge University Press, Cambridge, UK.

Kapil A, IB Koul, SK Banerjee, and BD Gupta. (1993). Antihepatotic effects of major diterpenoid constituents of *A. paniculata*. *Biochemical Pharmacology*; 48(1): 182-185.

Keas, B. E. (1993). ZOL 316 Parasitology Online Retrieved July 30, 2015.

Kiuchi F, Goto Y, Sugimoto N, Akao N, Kondo K, Tsuda Y 1993. Nematocidal activity of Turmeric: Synergistic action of curcuminoids. *Chemical and Pharmaceutical Bulletin*; 41: 1640-1643.

Kodym P, Blazˇek K, Maly M, Hrda S (2002) Pathogenesis of experimental toxoplasmosis in mice with strains differing in virulence. *Acta Parasitology*; 47: 239–248.

Kolodziej H, Kiderlen A F (2005) Antileishmanial activity and immune modulatory effects of tannins and related compounds on *Leishmania* parasitized RAW 264.7 cells. *Phytochemistry*; 66: 2056-2071.

Kongkathip, N.; Dhumma-upakorn, P.; Kongkathip, B.; Chawananoraset, K.; Sangchomkaeo, P.; Hatthakitpanichakul, S. (2002) Study on cardiac

contractility of cycloeucalenol and cycloeucalenone isolated from *Tinospora crispa*. *Journal of Ethnopharmacology*; 12(83): p.95-99

Kongsaktrakoon B, Temsiririrkkul R, Suvitayavat W, Nakornchai S and Wongkrajang Y (1994) The Antipyretic effect of *Tinospora crispa* Mier ex. Hock F. & Thoms, Mahidol University. *Journal of Pharmaceutical Sciences*; 21(1): 1–6.

Krouwer, H. G. J. & Wijdicks, E. F. M. (2003). Neurologic complications of bone marrow transplantation. *Neurology Clinical Practice*; 21: 319–352.

Lambert, H., Hitziger, N., Dellacasa, I., Svensson, M., and Barragan, A. (2006) Induction of dendritic cell migration upon *Toxoplasma gondii* infection potentiates parasite dissemination. *Cell Microbiology*; 8: 1611-1623.

Lanz, R., Kuhnert, P. & Boerlin, P. (2003) Antimicrobial resistance and resistance gene determinants in clinical *Escherichia coli* from different animal species in Switzerland. *Veterinary Microbiology*; 91: 73–84.

Lee YH, Channon JY, Matsuura T, Schwartzman JD, Shin DW (1999) Functional and quantitative analysis of splenic T cell immune responses following oral *Toxoplasma gondii* infection in mice. *Experimental Parasitology*; 91: 212–221.

Leblebiciouglu, H., & Hökelek, M. (2006) Toxoplasmosis. Retrieved from http://emedicine.medscape.com/article/1000028-overview

Lee C.W., Roziahanim M., Rahmah N., Suthagar P.P., Shanmugapriya P., Sabariah I. (2012) Alkaloids content, cytotoxicity and anti-*toxoplasma gondii* activity of *Psidium guajava* L. and *Tinospora crispa*. *Bangladesh Journal of Pharmacology*; 7; 272-276

Lithen, W., Rafeal, G.A., (2006). Toxoplasmosis.

Luft, B.J., Hafner, R., Korzun,., Jacobson, J. (1993) Toxoplasmic encephalitis in patients with the acquired immunodeficiency syndrome. *New England Journal of Medicine*; 329: 995-1000.

Luder CG, Bohne W, Soldati D (2001) Toxoplasmosis: A persisting challenge. *Trends in Parasitology*; 17: 460–463.

Madav, H. C. Tripathi, and S. K. Mishra (1995) "Analgesic, antipyretic and antiulcerogenic effects of andrographolide," *Indian Journal of Pharmaceutical Sciences*; 57 (3): 121–125.

Mahmoud MR, El-Abhar HS, Saleh S. (2002) The effect of *Nigella sativa* oil against the liver damage induced by *Schistosoma mansoni* infection in mice. *Journal of Ethnopharmacology*; 79: 1-11.

Mandell, G.L., Bennett, J.E. and Dolin, R. (2005) Emerging and re-emerging infectious disease threats. *Principles and practice of infectious diseases*; Ch. 13: 173-192.

Mastuda, T. Kuroyanagi, M. Sugiyama, S. Umchara, K. Ueno, A and Nishi, K. (1994) *Chemical and Pharmaceutical Bulletin*; 42: 1216-1225.

McFadden, G.I., Roos, D.S. (1999) Apicomplexan plastids as drug targets. *Trends in Microbiology*; 7 (8): 328–333.

Melissa Johnson. (2012) Clindamycin: An overview. *UpToDate, Wolters Kluwer Health*

Micucci M, Aldini R, Cevenini M, Colliva C, Spinozzi S (2013) *Curcuma longa* L. as a therapeutic agent in intestinal motility disorders. 2: safety profile in mouse. *PLoS ONE*; 8 (11).

Mishra K, Dash AP, Swain BK, Dey N. (2009) Anti-malarial activities of *Andrographis paniculata* and *Hedyotis corymbosa* extracts and their combination with curcumin. *Malaria Journal*; 8: 26.

Mital, J. (2006) Investigating the role of Apical Membrane Antigen 1 (TgAMA1) during *Toxoplasma gondii* invasion. In *Microbiology and Molecular Genetics*; Ph.D thesis Burlington, VT: University of Vermont.

Mital, J., Meissner, M., Soldati, D., and Ward, G.E. (2005) Conditional expression of *Toxoplasma gondii* apical membrane antigen-1 (TgAMA1) demonstrates that TgAMA1 plays a critical role in host cell invasion. *Molecular Biology of the Cell*; 16: 4341-4349.

Monsef, H.R., Ghobadi, A., Iranshahi, M., Abdollahi, M. (2004) Antinociceptive effects of *Peganum harmala* L. alkaloid extract on mouse formalin test. *Journal of Pharmacy and Pharmaceutical Sciences*; 19: 65–69.

Montoya JG, Liesenfeld O (2004) Toxoplasmosis. *Lancet*; 363: 1965–1976.

Morrissette, N.S., Murray, J.M., and Roos, D.S. (1997) Subpellicular microtubules associate with an intramembranous particle lattice in the protozoan parasite *Toxoplasma gondii*. *Journal of Cell Science*; 110 (Pt 1): 35-42.

Mosmann T. (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assay. *Journal of Immunological Methods*; 65: 55-63.

Mou, Z., He, Y., Dai, Y., Liu, X., Li, J. (2000) Deficiency in fatty acid synthase leads to premature cell death and dramatic alterations in plant morphology. *Plant Cell*; 12 (3): 405 418.

Moudy, R., Manning, T.J., and Beckers, C.J. (2001) The loss of cytoplasmic potassium upon host cell breakdown triggers egress of *Toxoplasma gondii*. *Journal of Biological Chemistry*; 276: 41492-41501.

Mui E J, Schiehser G A, Milhous W K, Hsu H, Roberts CW (2008) Novel Triazine JPC-2067-B inhibits *Toxoplasma gondii in vitro* and *in vivo*. *PLoS Neglected Tropical Disease*; 2: 190.

Mui E J, Jacobus D, Milhous W K, Schiehser G, Hsu H (2005) Triazine inhibits *Toxoplasma gondii* tachyzoites *in vitro* and *in vivo*. *Antimicrobial Agents Chemotherapy*; 49: 3463 –3467.

Najib Nik A, Rahman N, Furuta T (1999) Antimalarial activity of extracts of Malaysian medicinal plants. *Journal of Ethnopharmacology*; 64: 249-254.

Negash T, Tilahun G, Medhin G (2008) Seroprevalence of *Toxoplasma gondii* in Nazareth town Ethiopia. *East Africa Journal Public Health*; 5: 211-214.

Newman D J, Cragg G M, Snader K M (2003) Natural products as sources of new drugs over the period 1981- 2002. *Journal of Natural Product*; 66: 1022-1037.

Newman, D. J., and Cragg, G. M. (2007) Natural products as sources of new drugs over the last 25 years. *Journal of Natural Products American Chemical Society*; 70: 461-477.

Ng P, McCluskey PJ (2002) Treatment of ocular toxoplasmosis. *Australian Prescriber*; 25: 88-90.

Nguyen, T.D., Bigaignon, G., Van Snick, J., and Coutelier, J.P. (2003) Virulent *Toxoplasma gondii* strain RH promotes T-cell independent overproduction of proinflammatory cytokines IL-12 and gamma interferon. *Journal of Medical Microbiology*; 52: 869-876.

Nissapatorn,V., Lee CKC and Khairul Anuar A. (2003) Seroprevalance of toxoplasmosis among AIDS patients in Hospital Kuala Lumpur, 2001. *Singapore Medical Journal*; 44(4): 194–196.

Nissapatorn,V., NoorAzmi,M.A., Cho,S.M., Fong,M.Y., Init,I., Rohela,M. (2003) Toxoplasmosis: Prevalence and risk factors. *Journal of Obstetrics and Gynecology*; 23: 618–624.

Nissapatorn,V., Suwanrath,C., Sawangjaroen,N., Ling,L.Y., and Chandeying,V. (2011) Toxoplasmosis-serological evidence and associated risk factors among pregnant women in southern Thailand. *Am Journal Tropical Medicine and Hygiene*; 85: 243–247.

Noor H, Ashcroft SJ (1989) Antidiabetic effects of *Tinospora crispa* in rats. *Journal of Ethnopharmacology*; 27(1-2): 149-61.

Padma, C. L. Narasimhudu, S. Devi, N. M. B. Natha, R. B. Naga, and G. H. Philip (2011) "*In vitro* antihelmintic activity of *Andrographis paniculata* (burm.f.) nees." *International Journal of Pharmaceutical Research and Development*; 3(3): 202–205.

Okeke, I. N., Laxminarayan and Klugman, K. P. (2005) Antimicrobial resistance in developing countries. Part I: Recent trends and current status, *Lancet Infectious Disease*; 5: 481-493.

Pappas, G., Roussos, N., and Falagas, M.E. (2009) Toxoplasmosis snapshots: global status of *Toxoplasma gondii* seroprevalence and implications for pregnancy and congenital toxoplasmosis. *International Journal of Parasitology*; 39: 1385-1394.

Park H, Kim MS, Jeon BH, Kim TK, Wataya Y, Kimm HS (2003) Antimalarial activity of herbal extracts used in traditional medicine in Korea. *Biological and Pharmaceutical Bulletin*; 26: 1623–1624.

Pathak SK, Jain DC and Sharma RP (1995) Chemistry and biological activities of the Genera *Tinospora*, a review. *International Journal of Pharmaceutical Sciences*; 33(4): 277–87.

Perreten, V. & Boerlin, P. (2003) A new sulfonamide resistance gene (sul3) in *Escherichia coli* is widespread in the pig population of Switzerland. *Antimicrobial Agents and Chemotherapy*; 47: 1169–1172.

Perry LM. (1981) Medicinal plants of East and Southeast Asia: Attributed properties and uses. (London, England: *MIT Press*), p. 620.

Petersen E (2007a) Toxoplasmosis. *Semin Fetal Neonatal Medicine*;12: 214-223.

Petersen E (2007b) Prevention and treatment of congenital toxoplasmosis. *Expert Review of Anti-Infective Therapy*; 5: 285–293.

Peungvicha P, S Thirawarapan S, Temsiririrkkul R, Watanabe H, Kumar Prasain J, Kadota S. (1998) Hypoglycemic effect of the water extract of *Piper sarmentosum* in rats. *Journal of Ethnopharmacology*; 60: 27-32.

Phillipson JD (1994) Natural products as drugs. *Transactions Royal Society Tropical Medicine Hygiene*; 88: S17–S19.

Prakash ELS, Kadar Ali SH, Nagireddy Divya, Reeta Vijaya Rani K, Manavalan R (2011) Evaluation of *In vitro* antioxidant activity of leaf extract of *Andrographis paniculata*. *Research Journal Pharmaceutical Biological and Chemical Sciences*; 2: 891-895.

Pulford A. (2003) Turmeric root. Health and Fitness: *In Homeopathic material medica of graphical drug pictures*: p 149-150.

Puri A, Saxena R, Saxena RP, Saxena KC, Srivastava V, Tandon JS. (1993) *Immunostimulant agents from Andrographis paniculata. Journal Natural Products*; 56: 995-999.

Ralph, S.A.,Van Dooren, G. G. (2004) Tropical infectious diseases: Metabolic maps and functions of the *Plasmodium falciparum* apicoplast. *Nature Reviews Microbiology*; 2: 203–216.

Ramya, T.N., Mishra,S., Karmodiya,K., Surolia,N., Surolia,A. (2007) Inhibitors of non housekeeping functions of the apicoplast defy delayed death in *Plasmodium falciparum*. *Antimicrobial Agents and Chemotherapy*; 51(1): 307–316.

Raskin, I., and Ripoll, C. (2004) Can an apple a day keep the doctor away?, *Current Pharmaceutical Design*; 10: 3419-3429.

Rasmussen HB, Christensen SB, Kvist LP, Karazmi A (2000) A simple and efficient separation of the curcumins, the antiprotozoal constituents of *Curcuma longa*. *Planta Medica*; 66: 396-398.

Ridtitid W, Rattanaprom W, Thaina P, Chittrakarn, S, Sunbhanich M (1998) Neuromuscular blocking activity of methanolic extract of *Piper sarmentosum* leaves in the rat phrenic nerve hemidiaphragm preparation. *Journal of Ethnopharmacology*; 61: 135-142.

Robert-Gangneux, F. and M. L. Darde (2012) Epidemiology of and diagnostic strategies for toxoplasmosis. *Clinical Microbiology Reviews*; 25(2): 264-296.

Rodrigues, R.V., Lanznaster, D., Longhi Balbinot, D.T., Gadotti Vde, M., Facundo, V.A., Santos, A.R. (2009) Antinociceptive effect of crude extract, fractions and three alkaloids obtained from fruits of *Piper tuberculatum*. *Biological and Pharmaceutical Bulletin*; 32: 1809–1812.

Rukachaisirikul T, Siriwattanakit P, Sukcharoenphol K, Wongvein C, Ruttanaweang P, Wongwattanavuch P, Suksamrarn A (2004) Chemical constituents and bioactivity of *Piper sarmentosum*. *Journal of Ethnopharmacology*; 93: 173-176.

Rungruang T, Boonmars T (2009) *In vivo* antiparasitic activity of the Thai traditional medicine plant *Tinospora crispa* against *Plasmodium yoelii*. *The Southeast Asian Journal of Tropical Medicine and Public Health*; 40(5): 898-900.

Saadatnia G, Haj Ghani H, Khoo BY, Maimunah A, Rahmah N (2010) Optimization of *Toxoplasma gondii* cultivation in VERO cell line. *Tropical Biomedicine*; 27: 125-30.

Sabu KK, Padmesh P, Seeni S. (2001). Intraspecific variation in active principle content and isozymes of *Andrographis paniculata* Nees (Kalmegh): A traditional hepatoprotective medicinal herbs of India. *Journal of Medicinal and Aromatic Plant Sciences*; 23: 637-647.

Salibay,C.C., Dungca,J.Z., and Claveria,F.G. (2008) Serological survey of *Toxoplasma gondii* infection among Urban (Manila) and Suburban (Dasmariñas, Cavite) Residents, Philippines. *Journal of Protozool Research*; 18: 26–33.

Sawangjaroen N, Sawangjaroen K, Poonpanang P (2004) Effects of *Piper longum* fruit, *Piper sarmentosum* root and *Quercus infectoria* nut gall on caecal amoebiasis in mice. *Journal of Ethnopharmacology*; 91: 357-360.

Sayed N M, Ismail K A, Ahmed S AG, Hetta M H (2012) *In vitro* amoebicidal activity of ethanol extracts of *Arachis hypogaea L*., *Curcuma longa L*. and *Pancratium maritimum L*. on *Acanthamoeba castellanii* cysts. *Parasitology Research*; 110: 1985–1992.

Sagdic O, Ozcan M. (2003). Antibacterial activity of Turkish spice hydrosols. *Food Control*; 14: 141-143.

Scholtyseck, E. and Piekarski, G. (1965). Elektronen mikroskopische Untersuchungen über die Merozoiten von *Eimeria stiedae* und *E. perforans* und *Toxoplasma gondii*. Zur systematischen Stellung von *T. gondii*. *Zeitschrift fur Parasitenkunde*; 26: 91-115.

Schwab, J.C., Beckers, C.J., and Joiner, K.A. (1994) The parasitophorous vacuole membrane surrounding intracellular *Toxoplasma gondii* functions as a molecular sieve. *Proceedings of the National Academy of Sciences*; 91: 509-513.

Schwarz, S. & Chaslus-Dancla, E. (2001) Use of antimicrobials in veterinary medicine and mechanisms of resistance. *Veterinary Research*; 32: 201–225.

Sheng-Xia C, Liang W, Xu-Gan J, Yao-Yu F, Jian-Ping C (2008) Anti-*Toxoplasma gondii* activity of GAS *in vitro*. *Journal of Ethnopharmacology*; 118: 503–507.

Sinai, A. P., Webster, P. and Joiner, K. A. (1997) Association of host cell mitochondria and endoplasmic reticulum with the *Toxoplasma gondii* parasitophorous vacuole membrane - a high affinity interaction. *Journal of Cell Science*; 110: 2117-2128.

Skallova´ A, Kodym P, Frynta D, Flegr J (2006) The role of dopamine in *Toxoplasma*-induced behavioural alterations in mice: an ethological and ethopharmacological study. *Parasitology*; 133: 525–535.

Strobl JS, Cassell M, Mitchell SM, Reilly CM, Lindsay DS (2007) Scriptaid and suberoylanilide hydroxamic acid are histone deacetylase inhibitors with potent anti–*Toxoplasma gondii* activity *in vitro*. *Journal of Parasitology*; 93: 694–700.

Suzuki Y, Orellana M, Wong S, Conley F, Remington J. (1993) Susceptibility to chronic infection with *Toxoplasma gondii* does not correlate with susceptibility to acute infection in mice. *Infection and Immunity*; 61(6): 2284-2288.

Suzuki Y, Joh K (1994) Effect of the strain of *Toxoplasma gondii* on the development of toxoplasmic encephalitis in mice treated with antibody to interferon-gamma. *Parasitology Research*; 80(2): 125-30.

Suzuki, Y., Wang, X., Jortner, B.S., Payne, L., Ni, Y., Michie, S.A., Xu, B., Kudo, T., and Perkins, S. (2010). Removal of *Toxoplasma gondii* cysts from the brain by perforin mediated activity of CD8+ T cells. *American Journal of Pathology*; 176: 1607-1613.

Tamma, P. (2007) Toxoplasmosis. *Pediatric Review*; 28: 470-471.

Torres R A, Weinberg W, Stansell J, Leoung G, Kovacs J (1997) Atovaquone for salvage treatment and suppression of toxoplasmic encephalitis in patients with AIDS. *Clinical Infectious Disease*; 24: 422-429.

Trikha I, Wig N (2001) Management of toxoplasmosis in AIDS. *Indian Journal of Medical Sciences*; 55: 87-98.

Villena I, Aubert D, Leroux B, Dupouy D, Pinon JM (1998) Pyrimethamine-sulfadoxine treatment of congenital toxoplasmosis: follow-up of 78 cases between 1980 and 1997. Reims Toxoplasmosis Group. *Scandinavian Journal of Infectious Diseases*; 30: 295–300.

Vimala, I.Mohd, R. Abdull, and S. Rohana. (2003) Natural Antioxidants: *Piper sarmentosum* (Kadok) and *Morinda elliptica*. *Malaysian Journal of Nutrition*; 9(1): 41–51.

VukovicM, D., DjurkoviM-DjakoviM, O., KovaQeviM, S., BobiM, B., NikoliM, A., TodoroviM, V. (1997) Effect of clindamycin in a murine model of acute toxoplasmosis. *Clinical Microbiology and Infection*; 3: 89–94.

Waller, R.F., Ralph,S.A., Reed,M.B., Su,V., Douglas,J.D., Minnikin,D.E. (2003)A type II pathway for fatty acid biosynthesis presents drug targets in *Plasmodium falciparum*. *Antimicrobial Agents and Chemotherapy*; 47(1): 297–301.

Wan Omar A., Ngah,Z.U, Zaridah, MZ, Noor Rain,A. (2007) *In vitro* and *in vivo* antiplasmodial properties of some Malaysian plants used in traditional medicine. *Infectious Disease Journal of Pakistan*; 16(4): 97-101

Wang, P., Wang, Q., Aspinall, T. V., Sims, P. F., and Hyde, J. E. (2004) Transfection studies to explore essential folate metabolism and antifolate drug synergy in the human malaria parasite *Plasmodium falciparum*. *Molecular Microbiology*; 51: 1425–1438.

Waree P, Ferguson D, Pongponratn E, Chaisri U, Sukthana Y (2007) Immunohistochemical study of acute and chronic toxoplasmosis in experimentally infected mice. *Southeast Asian Journal of Tropical Medicine and Public Health*; 38(2): 223-31.

Weiss, L.M., Kim, K (2007) *Toxoplasma gondii. The model apicomplexan: Perspectives and methods.* London, UK: Academic Press.

World Health Organisation (2003) World health report. Geneva. 41

Xavier G A, Cademartori B G, Cunha Filho N A, Farias NA R (2013) Evaluation of seroepidemiological toxoplasmosis in HIV/AIDS patients in the south of Brazil. *Journal of the Institute of Tropical Medicine of São Paulo*; 55: 25-30.

Zaidan MRS, Noor Rain A, Badrul AR, Adlin A, Norazah A, Zakiah I (2005) *In vitro* screening of five local medicinal plants for antibacterial activity using disc diffusion method. *Tropical Biomedicine*; 22: 165-170.

Zakaria ZA, Mat Jais AM, Somchit MN (2006) The *in vitro* antibacterial activity of *Tinospora crispa* extracts. *Journal of Biological Sciences*; 6: 398-401.

Zaridah, S. Z. Idid, A. Wan Omar, and S. Khozirah (2001) *In vitro* antifilarial effects of three plant species against adult worms of subperiodic *Brugia malayi*," *Journal of Ethnopharmacology*; 78(1): 79–84.

Zirihi GN, Mambu L, Guede-Guina F, Bodo B, Grellier P (2005) *In vitro* antiplasmodial activity and cytotoxicity of 33 West African plants used for treatment of malaria. *Journal of Ethnopharmacology*; 98: 281-85.

Zulkhairi A, Jr., Abdah MA, NH MK, Nursakinah I, Moklas MA, Hasnah B (2008) Biological properties of *Tinospora crispa* (akar patawali) and its antiproliferative activities on selected human cancer cell lines. *Malaysian Journal of Nutrition*; 14(2): 173-87.