UNIVERSITI PUTRA MALAYSIA

ANTIDEPRESSANT EFFECT OF Centella asiatica (L.) Urb. EXTRACT ON RESERPINE-INDUCED DEPRESSION-LIKE ZEBRAFISH [Danio rerio (F. Hamilton, 1822)] MODEL VIA 1H NMR METABOLOMICS APPROACH

FAUZIAHANIM BINTI ZAKARIA

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By

FAUZIAHANIM BINTI ZAKARIA

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

September 2018

DEDICATION

This thesis is dedicated to my family, especially my parents, Zakaria b. Hj Awang and Seti Rakiah bt Haron, and my husband, Shaiful Anuar b. Shafingi, for their unconditional love and support during my study. I also would like to thanks to my main supervisor, Prof Dr Khozirah Shaari and the committee for their kindness in helping me and guiding me through out this journey. Last but not least, I would like to express my appreciation to my three daughters, Fatimah az zahra, Fatihah al Husna and Fasehah Nur Arina who always being on my side in my ups and downs during my study.

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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FAUZIAHANIM BINTI ZAKARIA

September 2018

Chairman : Khozirah Shaari, PhD
Institute : Bioscience

The World Health Organization (WHO) recorded approximately 350 millions people around the world have sufferred or is sufferring from mental health disorders, such as depression, anxiety, schizophrenia and addictive behaviors. Current antidepressants have associated problems related to their use, such as prolonged delays in symptom resolution, low rates of full remissions, substantial residual symptoms post-treatment and high rates of relapse. Use of rodents as translational animal model have several limitations such as high costs, low throughput and time-consuming among others. The zebrafish is a new promising model in drug discovery research. Thus, objective of this study is to determine the behavioral effects and metabolites changes after exposure of *C. asiatica* extract (RECA) on the established zebrafish model of reserpine-induced depression. The antidepressant effect of marker triterpenoid saponins of *C. asiatica* were also evaluated via behavioural analysis. Depression was successfully induced in the zebrafish model by single intraperitoneal injection of 80 mg/kg BW which produced significant increase ($F_{(3,32)} = 46.71$, $p<0.0001$) and decrease {$t(9.25) = -5.772$, $p<0.0001$} in freezing duration and reduced total distance travelled, respectively. A significant increase {$t(11) = 0.0001$} in cortisol level (6 fold increment) further supported the results. Treating the depression model with 150 mg/L of RECA via immersion technique, gave positive improvement in that the model showed significant reduction in freezing durations {$t(16)= 3.302$, $p=0.005$) and increase in total distance travelled {$t(9)=1.646$, $p=0.046$}. Similarly, the social interaction test (SIT) significantly increase {$t(11)= 7.778$, $p=0.0001$} contact duration of the zebrafishes. ^{1}H NMR-based metabolomics analysis of the zebrafish brain extract showed twelve metabolites, i.e lactate, β-hydroxyisovaleric acid, glutamine, choline, histidine, glutamate, histamine, valine, histidine, L-fucose, betaine, and GABA were significantly reduced in the depression model. Treatment with RECA was able to significantly increase the levels of six metabolites i.e L-fucose, lactate, betaine, valine, β-hydroxyisovaleric acid, and ethanolamine, back towards normal levels, consistent with moderate of the depressed

condition. The current study revealed that *C. asiatica* has antidepressant properties and further investigation should be carried out towards obtaining a better insight into the pathology of depression and mechanism of *C. asiatica* as antidepressant.

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

KESAN ANTIDEPRESSAN EKSTRAK *Centella asiatica* (L.) Urb. DALAM MODEL KEMURUNGAN IKAN ZEBRA (*Danio rerio*) TERARUH RESERPINA MELALUI KAJIAN ^{1}H NMR METABOLOMIK

Oleh

FAUZIAHANIM BINTI ZAKARIA

September 2018

Pengerusi : Khozirah Shaari, PhD
Institut : Biosains

Pertubuhan Kesihatan Sedunia merekodkan lebih kurang 350 juta manusia di seluruh dunia telah menderita penyakit mental seperti kemurungan, keresahan, schizophrenia dan ketagihan. Ubat anti-kemurungan yang ada mempunyai masalah akibat penggunaan seperti resolusi gejala yang mengambil masa yang panjang, kadar penyembuhan yang rendah, gejala selepas perawatan dan kembali sakit selepas beberapa ketika. Penggunaan tikus sebagai haiwan model mempunyai kekurangannya seperti melibatkan kos yang tinggi, dapatan kajian yang rendah dan mengambil masa yang sangat panjang. Ikan zebra ialah suatu model baru yang memberikan harapan dalam penyelidikan ubatan. Oleh itu, objektif kajian ini ialah untuk menentukan kesan tingkahlaku dan perubahan metabolit selepas dedahan kepada ektrak pegaga (RECA) pada model kemurungan zebrafish teraruh reserpina. Kesan antikemurungan oleh saponin triterpenoid dari pegaga juga dinilai menggunakan analisis tingkahlaku. Kemurungan berjaya diinduksi kepada ikan zebra melalui satu suntikan intraperitonium 80 mg/kg berat badan yang menghasilkan pertambahan ($F_{(3,32)} = 46.71$, $p<0.0001$) dan pengurangan {$t(9.25) = -5.772$, $p<0.0001$} secara signifikan dalam jangkamasa tidak bergerak dan jumlah jarak pergerakan, secara respektif. Pertambahan secara signifikan {$t(11) = 0.0001$} dalam paras kortisol (enam kali ganda) menyokong keputusan ini. Rawatan model dengan 150 mg/L RECA melalui teknik penyerapan memberikan impak positif pada model di mana ia menunjukkan pengurangan secara signifikan {$t(16)= 3.302$, $p=0.005$) dalam jangkamasa tidak bergerak dan pertambahan {$t(9)=1.646$, $p=0.046$} dalam jumlah jarak pergerakan. Kesan yang sama ditunjukkan dalam ujian interaksi sosial apabila jangkamasa interaksi bertambah secara signifikan {$t(11)= 7.778$, $p=0.0001$} antara ikan zebra. Analisis metabolomik menggunakan ^{1}H NMR ekstrak otak ikan zebra menunujukkan 12 metabolit iaitu laktat, asid β-hydroksiisovalerik, glutamina, kolina, histidin, glutamat, histamin, valina, histidin, L-fukos, betain dan GABA menunjukkan pengurangan secara signifikan dalam model kemurungan. Rawatan dengan RECA telah meningkatkan secara signifikan enam daripada metabolit iaitu L-fukos, laktat, betaine, valina, asid β-

hydroxyisovalerik dan ethanolamina kembali kepada paras normal, konsisten dengan penyembuhan sederhana keadaan murung. Kajian ini menunjukkan bahawa *C. asiatica* berpotensi menjadi ubat antikemurungan dan kajian lanjut perlu dilakukan untuk mendapatkan gambaran yang lebih jelas tentang penyakit kemurungan dan mekanisma yang terlibat dalam tindakbalas *C.asiatica* sebagai ubat antikemurungan.

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This thesis was submitted to the Senate of the 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:

Khozirah Shaari, PhD
Professor
Institute of Bioscience
Universiti Putra Malaysia
(Chairman)

Intan Safinar Ismail, PhD
Associate Professor,
Institute of Bioscience
Universiti Putra Malaysia
(Member)

Hafandi Ahmad, PhD
Senior Lecturer
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Nurhuda Manshoor, PhD
Senior Lecturer
Faculty of Pharmacy
Universiti Teknologi MARA (Puncak Alam)
(Member)

ROBIAH BINTI YUNUS, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

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Signature:_____ Date: _____

Name and Matric No: Fauziahanim binti Zakaria GS38772

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Signature: _____
Name of Chairman of Supervisory Committee: Professor Dr. Khozirah Shaari

Signature: _____
Name of Member of Supervisory Committee: Associate Professor Dr. Intan Safinar Ismail

Signature: _____
Name of Member of Supervisory Committee: Dr. Hafandi Ahmad

Signature: _____
Name of Member of Supervisory Committee: Dr. Nurhuda Manshoor

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LIST OF ABBREVIATIONS

°C	Degree centigrade
%	Percentage
α	Alpha
β	Beta
δ	Delta
µm	Micro meter
µg	Microgram
µL	Micro litre
ANOVA	Analysis of variance
BCAA	Branched chain amino acids
COSY	Homonuclear correlation spectroscopy
CQA	Caffeoylquinic acid
DCQA	Dicaffeoylquinic acid
D_2O	Deuterium oxide
HPLC	High performance liquid chromatography
JRES NMR	J resolved NMR spectroscopy
MS	Mass spectrometry
NMR	Nuclear magnetic resonance spectroscopy
PC	Principle component
PCA	Principal Component Analysis
SD	Standard deviation
TSP	Trimethylsilyl propionic acid-d4 sodium salt

CHAPTER 1

INTRODUCTION

1.1 Background

The World Health Organization (WHO) recorded approximately 350 million people around the world have sufferred or is sufferring from mental health disorders, such as depression, anxiety, schizophrenia, eating disorders and addictive behaviors (Akimoto et al., 2017). Depression is a complex and disabling psychiatric disorder. It is expected to be the leading cause of disability by the year 2030. This disorder is a large burden to any country due to the consequencial outcome of the disorder, such as higher incidences of suicide, increase medical costs for treatment, and decrease in work productivity and quality. It was estimated that the world's total expenditure for mental, neurological and substance use disorders was up to USD 2.5 to 8.5 trilion in 2010. The figure is expected to double by the year 2030 if intensive actions to curb it are not initiated (Chisholm et al., 2016).

Patients who suffer from depression will usually exhibit psychomotor retardation, anhedonia, reduced appetite, weight loss, reduced cognitive performance, HPA axis dysregulation, increased cardiovascular morbidity, increased amygdala responsiveness, abnormal social behavior and altered pain perception (Neumann et al., 2011). Depressed patients have been known to show increased immobility. They even struggle to wake up from bed (Neumann et al., 2011).

The zebrafish is a new promising model in drug discovery research (Howe et al., 2013). This is due to several positive points about its use, including high physiological homology to human (70% of human gene have at least one obvious zebrafish orthologue), high throughput value, genetic tractability, low cost, sensitive to various drugs and pharmacological agents (such as ethanol) and have a short reproductive cycle (Stewart et al., 2013). The use of zebrafish model to investigate anti depression activity is still at its infancy. A previous study investigated neurochemical and behavioural responses of Unpredictable Chronic Stress (UCS) following developmental isolation using a zebrafish model (Fulcher, et al, 2016). The study found that UCS protocol only increased anxiety-like behavior, while developmental isolation after UCS caused depressive-like behavior and reduced serotonin and dopamine levels in zebrafish brain. Pharmacologically-induced depression in zebrafish has been reported by several reseachers. Kyzar and his co-workers reported that immersion in 40 mg/L reserpine for 20 minutes daily caused hypolocomotion in zebrafish after 7 days of chronic exposure (Kyzar et al., 2013). Meanwhile, Saroya et al., (2010) carried out intraperitoneal (i.p) injection of reserpine to zebrafish to study the bystander effect of reserpine-treated fishes. Bystander effect happens when irradiated cells transmit signals to non-irradiated cells. In normal cases, irradiated fishes will also transmit signal to non-irradiated partnering fishes. In this study, however, irradiated reserpine-injected zebrafish did not

transmit any signal to non-irradiated zebrafish due to disturbance of the serotonine function. The study reported that the reserpine-treated zebrafish have 73% less serotonine compared to non-treated fishes (Saroya et al., 2010).

The search for new drugs from nature have drawn on many biological resources and human practices related to them. Traditional medicine and the ethnopharmacological uses of plants have often been the origin of discovery of a useful drug to fight important diseases such as cancer and diabetes. *Centella asiatica* is a small perennial medicinal plant, popularly used as brain tonic based on Ayurvedic (Orhan, 2012) and Traditional Chinese Medicine (Zheng & Qin, 2007) systems. The antidepressant effect of the plant in rodents has been reported since 2003 when Chen et al discovered that total triterpenes from *C. asiatica* have antidepressant effect in an acute rodent model of depression. Nine years later, Kalshetty et al conducted a chronic model of depression using olfactory bulbectomy (OBX) rat model to evaluate the antidepressive effect of *C. asiatica*. From this study, they found that administration of *C. asiatica* extract reversed the physiological effects of OBX model of depression such as body weight, ambulation, body temperature and heart rate. They also found that *C. asiatica* standardized extract significantly reduced open arm entries by 80% compared to depressed OBX rats (Kalshetty et al., 2012).

Most of the studies on the anti depression property of *C. asiatic*a focused on behavioural evaluation. However, the full biochemical pathology and mechanism of action of *C. asiatica* in imparting the antidepressive effect is still unclear and requires deeper investigations. The present study is an attempt to further understand these aspects using ^{1}H NMR-based metabolomics approach.

Recent developments in systems biology have also impacted natural products research as a whole through the introduction of metabolomics for analysis of mixtures. The metabolomics approach facilitates the simultaneous qualification and quantification of the huge population of metabolites whithin an organism. It gives a clearer picture of the metabolome of the living organism under specific conditions (Kim et al., 2010) and allows measurement of the changes arising from pertubations to the system at or over specific time points (Mushtaq et a., 2014). The approach is very suitable for investigating changes in zebrafish metabolites (whole body, liver or brains) following a perturbation or intervention such as drug-induced depression and treatment with a plant extract, respectively.

Various instrumental platforms can be used for a metabolomics study. The major platforms include high resolution Nuclear Magnetic Resonance (NMR) spectroscopy, mass spectrometry (MS) and infra-red (IR) spectroscopy. The approach combines the use of these platforms with multivariate data analysis and proper data mining methods to identify and correlate plant metabolites to the causal factors. ^{1}H NMR-based metabolomics offers a simple, rapid and powerful platform to identify metabolites. In this study, the ^{1}H NMR-based approach was used to evaluate the antidepressant effect

of *C. asiatica* on zebrafish and help identify biomarkers in the pathways affected by the reserpine-induced depression in a zebrafish model.

1.2 Problem statement

Current antidepressants, such as tricyclic antidepressants (TCAs), monoamine oxidase inhibitors (MAOIs), selective serotonin reuptake inhibitors (SSRIs), serotonin and noradrenaline reuptake inhibitors (SNRIs), noradrenergic and specific serotonergic antidepressant (NaSSA) and norepinephrine reuptake inhibitors (NRIs) have associated problems related to their use, such as prolonged delays in symptom resolution, low rates of full remissions, substantial residual symptoms post-treatment and high rates of relapse (Si & Yu, 2016).

In general, drug discovery research is very cost intensive, even for discovery of new antidepressants (Nguyen, Stewart, & Kalueff, 2014b). Preclinical research have mainly used rodents as the translational animal model. However, use of rodents for disease models have several limitations such as high costs, low throughput and time-consuming among others (Nguyen et al., 2014a). A complementary model to the rodent model would certainly be a beneficial addition towards obtaining a better insight into the pathology of depression.

1.3 Objectives

The main aim of this study is to investigate the effect of *C. asiatica* on reserpine-induced depression zebrafish model, towards obtaining a better understanding of the plants use in mitigating depression. Some of the research questions in the the study are: will *C. asiatica* show anti-depressant activity in the zebrafish model? What are the signature biochemical changes in the metabolome of the depression-induced zebrafish upon treatment with *C. asiatica* that could be associated with the anti-depressant action of the plant extract? Do the marker triterpenoid saponins of *C. asiatica* show any antidepressant effect on the reserpine-induced zebrafish model?

Answers to the research questions of this study were, therefore, sought through the following set of research specific objectives:

1. To establish a zebrafish model of reserpine-induced depression
2. To determine the behavioral effects of *C. asiatica* extract on the zebrafish model of depression
3. To characterise the biochemical profile of reserpine-induced depression zebrafish model before and after exposure to *C. asiatica* extract

4. To identify signature biochemical or metabolic changes in the metabolome of reserpine-induced depressed zebrafish, resulting from exposure to *C. asiatica* extract.
5. To evaluate the antidepressant effect of several individual triterpenoid saponins of *C. asiatica*

REFERENCES

Abas, F., Khatib, A., Shaari, K., & Lajis, N. H. (2014). Chemical characterization and antioxidant activity of three medicinal Apiaceae species. *Industrial Crops and Products*, *55*, 238–247. http://doi.org/10.1016/j.indcrop.2014.02.013

Abelaira, H. M., Réus, G. Z., & Quevedo, J. (2013). Animal models as tools to study the pathophysiology of depression. *Revista Brasileira de Psiquiatria (São Paulo, Brazil : 1999)*, *35*, 112–120. http://doi.org/10.1590/1516-4446-2013-1098

Ahmed-Farid, O. A. H., Ahmed, R. F., & Saleh, D. O. (2016). Combination of resveratrol and fluoxetine in an acute model of depression in mice: Prevention of oxidative DNA fragmentation and monoamines degradation ARTICLE INFO ABSTRACT. *Journal of Applied Pharmaceutical Science*, *6*(06), 1–7. http://doi.org/10.7324/JAPS.2016.60601

Akimoto, H., Oshima, S., Ohara, K., Negishi, A., Hiroyama, H., Nemoto, T., & Kobayashi, D. (2017). Metabolic Profiling of Hippocampal Tissue in Rats with Depression-Like Symptoms. *Biol. Pharm. Bull.*, *40*(6), 789–796.

Albiñana, E., Luengo, J. G., Baraibar, A. M., Muñoz, M. D., & Gandía, L. (2017). Choline induces opposite changes in pyramidal neuron excitability and synaptic transmission through a nicotinic receptor-independent process in hippocampal slices. *Neuroscience*, *469*, 779–795. http://doi.org/10.1007/s00424-017-1939-5

Alqahtani, A., Tongkao-On, W., Li, K. M., Razmovski-Naumovski, V., Chan, K., & Li, G. Q. (2015). Seasonal Variation of Triterpenes and Phenolic Compounds in Australian Centella asiatica (L.) Urb. *Phytochemical Analysis*, *26*(6), 436–443. http://doi.org/10.1002/pca.2578

An, L., Li, J., Yu, S.-T., Xue, R., Yu, N.-J., Chen, H.-X., … Zhang, Y.-Z. (2015). Effects of the total flavonoid extract of Xiaobuxin-Tang on depression-like behavior induced by lipopolysaccharide and proinflammatory cytokine levels in mice. *Journal of Ethnopharmacology*, *163*, 83–87. http://doi.org/10.1016/j.jep.2015.01.022

Anderson, H. D., Pace, W. D., Libby, A. M., West, D. R., & Valuck, R. J. (2012). Rates of 5 Common Antidepressant Side Effects Among New Adult and Adolescent Cases of Depression: A Retrospective US Claims Study. *Clinical Therapeutics*, *34*(1), 113–123. http://doi.org/10.1016/j.clinthera.2011.11.024

Andrisic, L., Dudzik, D., Barbas, C., Milkovic, L., & Grune, T. (2018). Redox Biology Short overview on metabolomics approach to study pathophysiology of oxidative stress in cancer. *Redox Biology*, *14*(August 2017), 47–58. http://doi.org/10.1016/j.redox.2017.08.009

Anroop B. Nair, S. J. (2016). A simple practice guide for dose conversion between animals and human. *Journal of Basic and Clinical Pharmacy* |, *7*, 27–31. http://doi.org/10.4103/0976-0105.177703

Antkiewicz-Michaluk, L., Wąsik, A., Mozdzeń, E., Romańska, I., & Michaluk, J. (2014). Antidepressant-like effect of tetrahydroisoquinoline amines in the animal model of depressive disorder induced by repeated administration of a low dose of reserpine: Behavioral and neurochemical studies in the rat. *Neurotoxicity Research*, *26*, 85–98. http://doi.org/10.1007/s12640-013-9454-8

Antkiewicz-Michaluk, L., Wąsik, A., Możdżeń, E., Romańska, I., & Michaluk, J. (2015). Withdrawal from repeated administration of a low dose of reserpine induced opposing adaptive changes in the noradrenaline and serotonin system function: A behavioral and neurochemical ex vivo and in vivo studies in the rat. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *57*, 146–54. http://doi.org/10.1016/j.pnpbp.2014.10.009

Aragão, G. F., Carneiro, L. M. V, Junior, a P. F., Vieira, L. C., Bandeira, P. N., Lemos, T. L. G., & Viana, G. S. D. B. (2006). A possible mechanism for anxiolytic and antidepressant effects of alpha- and beta-amyrin from Protium heptaphyllum (Aubl.) March. *Pharmacology, Biochemistry, and Behavior*, *85*(4), 827–34. http://doi.org/10.1016/j.pbb.2006.11.019

Artigas, F. (2015). Developments in the field of antidepressants, where do we go now? *European Neuropsychopharmacology*, *25*(5), 657–670. http://doi.org/10.1016/j.euroneuro.2013.04.013

Azerad, R. (2016). Chemical structures, production and enzymatic transformations of sapogenins and saponins from Centella asiatica (L.) Urban. *Fitoterapia*, *114*, 168–187. http://doi.org/10.1016/j.fitote.2016.07.011

Azis, H. A., Taher, M., Ahmed, A. S., Sulaiman, W. M. A. W., Susanti, D., Chowdhury, S. R., & Zakaria, Z. A. (2017). In vitro and In vivo wound healing studies of methanolic fraction of Centella asiatica extract. *South African Journal of Botany*, *108*, 163–174. http://doi.org/10.1016/j.sajb.2016.10.022

B. Brinkhaus, M. Lindner, D. S. and E. G. H. (2000). Chemical, pharmacological and clinical profile of the East Asian medicinal plant Centella asiatica. *Phytomedicine*, *7*(5), 427–448.

Bailey, C. P. T. H., Criss, C. J., Linton, C. J., Fried, C. J., Taylor, C. A., Padron, G., & Johnson, A. D. (2015). Evaluation of the Anxiolytic and Antidepressant Effects of Asiatic Acid, a Compound from Gotu Kola or Centella asiatica, in the Male Sprague Dawley Rat. *AANA Journal*, *83*(2), 91–98.

Bandmann, O., & Burton, E. a. (2010). Genetic zebrafish models of neurodegenerative diseases. *Neurobiology of Disease*, *40*, 58–65. http://doi.org/10.1016/j.nbd.2010.05.017

Baranyi, A., Amouzadeh-ghadikolai, O., Lewinski, D. Von, Rothenhäusler, B., Theokas, S., Robier, C., & Mangge, H. (2016). Branched-Chain Amino Acids as New Biomarkers of Major Depression - A Novel Neurobiology of Mood Disorder, *11*(8), 1–10. http://doi.org/10.1371/journal.pone.0160542

Beckonert, O., Keun, H. C., Ebbels, T. M. D., Bundy, J., Holmes, E., Lindon, J. C., & Nicholson, J. K. (2007). Metabolic profiling, metabolomic and metabonomic procedures for NMR spectroscopy of urine, plasma, serum and tissue extracts. *Nature Protocols*, *2*, 2692–2703. http://doi.org/10.1038/nprot.2007.376

Belzung, C. (2014). Innovative drugs to treat depression: did animal models fail to be predictive or did clinical trials fail to detect effects? *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *39*(5), 1041–51. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/24345817

Belzung, C., & Lemoine, M. (2011). Criteria of validity for animal models of psychiatric disorders: focus on anxiety disorders and depression. *Biology of Mood & Anxiety Disorders*. http://doi.org/10.1186/2045-5380-1-9

Berton, O., Hahn, C.-G., & Thase, M. E. (2012). Are we getting closer to valid translational models for major depression? *Science (New York, N.Y.)*, *338*(6103), 75–9. http://doi.org/10.1126/science.1222940

Berton, O., & Nestler, E. J. (2006). New approaches to antidepressant drug discovery: Beyond monoamines. *Nature Reviews Neuroscience*, *7*(2), 137–151. http://doi.org/10.1038/nrn1846

Best, J., Nijhout, H. F., Samaranayake, S., Hashemi, P., & Reed, M. (2017). A mathematical model for histamine synthesis, release, and control in varicosities. *Theoretical Biology and Medical Modelling*, *14*(1), 1–19. http://doi.org/10.1186/s12976-017-0070-9

Bhattacharya, R. D., Parmar, K. M., Itankar, P. R., & Prasad, S. K. (2017). Phytochemical and pharmacological evaluation of organic and non-organic cultivated nutritional Centella asiatica collected after different time intervals of harvesting. *South African Journal of Botany*, *112*, 237–245. http://doi.org/10.1016/j.sajb.2017.06.003

Biney, R. P., Benneh, C. K., Ameyaw, E. O., Boakye-Gyasi, E., & Woode, E. (2016). Xylopia aethiopica fruit extract exhibits antidepressant-like effect via interaction with serotonergic neurotransmission in mice. *Journal of Ethnopharmacology*, *184*, 49–57. http://doi.org/10.1016/j.jep.2016.02.023

Bobade, V., Bodhankar, S. L., Aswar, U., Vishwaraman, M., & Thakurdesai, P. (2015). Prophylactic effects of asiaticoside-based standardized extract of Centella asiatica (L.) Urban leaves on experimental migraine: Involvement of 5HT1A/1B receptors. *Chinese Journal of Natural Medicines*, *13*(4), 274–282. http://doi.org/10.1016/S1875-5364(15)30014-5

Bouwknecht, J. A. (2015). Behavioral studies on anxiety and depression in a drug discovery environment: Keys to a successful future. *European Journal of Pharmacology*, *753*, 158–76. http://doi.org/10.1016/j.ejphar.2014.09.051

Bradley, K. A. L., Mao, X., Case, J. A. C., Kang, G., Shungu, D. C., & Gabbay, V. (2016). Increased ventricular cerebrospinal fluid lactate in depressed adolescents. *European Psychiatry*, *32*, 1–8. http://doi.org/10.1016/j.eurpsy.2015.08.009

Brenes, J. C., & Fornaguera, J. (2009). The effect of chronic fluoxetine on social isolation-induced changes on sucrose consumption, immobility behavior, and on serotonin and dopamine function in hippocampus and ventral striatum. *Behavioural Brain Research*, *198*(1), 199–205. http://doi.org/10.1016/j.bbr.2008.10.036

Brown, E. S., Varghese, F. P., & McEwen, B. S. (2004). Association of depression with medical illness: does cortisol play a role? *Biological Psychiatry*, *55*(1), 1–9. http://doi.org/10.1016/S0006-3223(03)00473-6

Bruno, D., Nierenberg, J., Cooper, T. B., Marmar, C. R., Zetterberg, H., Blennow, K., … Pomara, N. (2017). Neurobiology of Learning and Memory The recency ratio is associated with reduced CSF glutamate in late-life depression. *Neurobiology of Learning and Memory*, *141*, 14–18. http://doi.org/10.1016/j.nlm.2017.03.011

Cachat, J. M., Canavello, P. R., Elkhayat, S. I., Bartels, B. K., Hart, P. C., Elegante, M. F., … Kalueff, A. V. (2011). Measuring Endocrine (Cortisol) Responses of Zebrafish to Stress. In *Zebrafish Neurobehavioral Protocols* (Vol. 51, pp. 1–14). http://doi.org/10.1007/978-1-60761-953-6

Cachat, J., Stewart, A., Grossman, L., Gaikwad, S., Kadri, F., Chung, K. M., … Kalueff, A. V. (2010). Measuring behavioral and endocrine responses to novelty stress in adult zebrafish. *Nature Protocols*, *5*(11), 1786–99. http://doi.org/10.1038/nprot.2010.140

Carvalho, R. C., Patti, C. C., Takatsu-Coleman, A. L., Kameda, S. R., Souza, C. F., Garcez-do-Carmo, L., … Silva, R. H. (2006). Effects of reserpine on the plus-maze discriminative avoidance task: dissociation between memory and motor impairments. *Brain Research*, *1122*(1), 179–83. http://doi.org/10.1016/j.brainres.2006.09.008

Chauhan, P. K., & Singh, V. (2012). Acute and Subacute Toxicity study of the Acetone Leaf extract of Centella asiatica in Experimental Animal Models. *Asian Pacific Journal of Tropical Biomedicine*, 511–513. http://doi.org/10.1016/S2221-1691(12)60263-9

Chen, C.-N., Chang, K.-C., Lin, R.-F., Wang, M.-H., Shih, R.-L., Tseng, H.-C., … Tsai, C.-C. (2016). Nitric oxide pathway activity modulation alters the protective effects of (−)Epigallocatechin-3-gallate on reserpine-induced impairment in rats. *Behavioural Brain Research*, *305*(92), 198–211. http://doi.org/10.1016/j.bbr.2016.02.038

Chen, G., Yang, D., Yang, Y., Li, J., Cheng, K., Tang, G., … Xie, P. (2015). Amino acid metabolic dysfunction revealed in the prefrontal cortex of a rat model of depression. *Behavioural Brain Research*, *278*(1), 286–292. http://doi.org/10.1016/j.bbr.2014.05.027

Chen, J., Zhou, C., Zheng, P., Cheng, K., & Wang, H. (2017). Di ff erential urinary metabolites related with the severity of major depressive disorder. *Behavioural Brain Research*, *332*(April), 280–287. http://doi.org/10.1016/j.bbr.2017.06.012

Chisholm, D., Sweeny, K., Sheehan, P., Rasmussen, B., Smit, F., Cuijpers, P., & Saxena, S. (2016). Scaling-up treatment of depression and anxiety : a global. *The Lancet Psychiatry*, *3*(5), 415–424. http://doi.org/10.1016/S2215-0366(16)30024-4

Chivapat, S., Chavalittumrong, P., & Tantisira, M. H. (2011). Acute and sub-chronic toxicity studies of a standardized extract of Centella asiatica ECa 233. *Thai Journal of Pharmaceutical Sciences*, *35*(2), 55–64.

Choudhury, P. R., Choudhury, M. D., Ningthoujam, S. S., Mitra, A., Nath, D., & Talukdar, A. Das. (2015). Plant utilization against digestive system disorder in Southern Assam, India. *Journal of Ethnopharmacology*, *175*, 192–197. http://doi.org/10.1016/j.jep.2015.09.020

Cryan, J. F., Hoyer, D., & Markou, A. (2003). Withdrawal from chronic amphetamine induces Depressive-Like behavioral effects in rodents. *Biological Psychiatry*, *54*(1), 49–58. http://doi.org/10.1016/S0006-3223(02)01730-4

Cunha, A. S., Matheus, F. C., Moretti, M., Sampaio, T. B., Poli, A., Santos, D. B., … Prediger, R. D. (2016). Agmatine attenuates reserpine-induced oral dyskinesia in mice: Role of oxidative stress, nitric oxide and glutamate NMDA receptors. *Behavioural Brain Research*, *312*, 64–76. http://doi.org/10.1016/j.bbr.2016.06.014

De Freitas, C. M., Busanello, A., Schaffer, L. F., Peroza, L. R., Krum, B. N., Leal, C. Q., … Fachinetto, R. (2016). Behavioral and neurochemical effects induced by reserpine in mice. *Psychopharmacology*, *233*(3), 457–467. http://doi.org/10.1007/s00213-015-4118-4

Dhingra, D., & Sharma, A. (2006). Antidepressant-like activity of Glycyrrhiza glabra L. in mouse models of immobility tests. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *30*(3), 449–454. http://doi.org/10.1016/j.pnpbp.2005.11.019

Dhingra, D., Sharma, A., Linn, B., Linn, C., Linn, C., Nutt, L., … Linn, H. (2005). A review on antidepressant plants. *Natural Product Radiance*, *5*(2), 144–152.

Di Rosso, M. E., Palumbo, M. L., & Genaro, A. M. (2016). Immunomodulatory effects of fluoxetine: A new potential pharmacological action for a classic antidepressant drug? *Pharmacological Research*, *109*, 101–107. http://doi.org/10.1016/j.phrs.2015.11.021

Diego-adeliño, J. De, Portella, M. J., Gómez-ansón, B., López-moruelo, O., Serra-blasco, M., Vives, Y., … Álvarez, E. (2013). Hippocampal abnormalities of glutamate / glutamine , N -acetylaspartate and choline in patients with depression are related to past illness burden, *38*(2), 107–116. http://doi.org/10.1503/jpn.110185

Diémé, B., Lefèvre, A., Nadal-desbarats, L., Galineau, L., Madji, B., Montigny, F., … Mavel, S. (2017). Journal of Pharmaceutical and Biomedical Analysis Workflow methodology for rat brain metabolome exploration using NMR , LC – MS and GC – MS analytical platforms. *Journal of Pharmaceutical and Biomedical Analysis*, *142*, 270–278. http://doi.org/10.1016/j.jpba.2017.03.068

Dipankar Chandra Roy, Shital Kumar Barman, M. M. S. (2013). Current Updates on Centella asiatica: Phytochemistry, Pharmacology and Traditional Uses. *Medicinal Plant Research*, *3*(4), 20–36. http://doi.org/10.5376/mpr.2013.03.0004

Du, H., Liu, M., & Chen, A. (2017). Metabolic analysis of the antidepressive effects of Yangxinshi Tablet in a vascular depression model in mice. *Biomedical Chromatography*, (July), 1–9. http://doi.org/10.1002/bmc.4114

Du, H., Lou, Z., & Lin, Q. (2017). Metabolic profiles revealed synergistically antidepressant effects of lilies and Rhizoma Anemarrhenae in a rat model of depression. *Biomedical Chromatography*, 1–10. http://doi.org/10.1002/bmc.3923

Duggina, P., Kalla, C. M., Varikasuvu, S. R., Bukke, S., & Tartte, V. (2015). Protective effect of centella triterpene saponins against cyclophosphamide-induced immune and hepatic system dysfunction in rats: its possible mechanisms of action. *Journal of Physiology and Biochemistry*, *71*(3), 435–54. http://doi.org/10.1007/s13105-015-0423-y

Duman, C. H. (2010). Models of depression. *Vitamins and Hormones*, *82*(10), 1–21. http://doi.org/10.1016/S0083-6729(10)82001-1

Ebshiana, A. A., Snowden, S. G., Thambisetty, M., Parsons, R., Hye, A., & Legido-Quigley, C. (2015). Metabolomic method: UPLC-q-ToF Polar and non-polar metabolites in the healthy rat cerebellum using an in-vial dual extraction. *PLoS ONE*, *10*(4), 1–20. http://doi.org/10.1371/journal.pone.0122883

Egan, R. J., Bergner, C. L., Hart, P. C., Cachat, J. M., Canavello, P. R., Elegante, M. F., … Kalueff, A. V. (2009). Understanding behavioral and physiological phenotypes of stress and anxiety in zebrafish. *Behavioural Brain Research*, *205*, 38–44. http://doi.org/10.1016/j.bbr.2009.06.022

Eghbalnia, H. R., Romero, P. R., Westler, W. M., Baskaran, K., Ulrich, E. L., & Markley, J. L. (2017). Increasing rigor in NMR-based metabolomics through validated and open source tools. *Current Opinion in Biotechnology*, *43*, 56–61. http://doi.org/10.1016/j.copbio.2016.08.005

Esch, C. De, Slieker, R., Wolterbeek, A., Woutersen, R., & Groot, D. De. (2012). Neurotoxicology and Teratology Zebra fi sh as potential model for developmental neurotoxicity testing : A mini review. *Neurotoxicology and Teratology*, *34*(6), 545–553. http://doi.org/10.1016/j.ntt.2012.08.006

Feierstein, C. E., Portugues, R., & Orger, M. B. (2015). REVIEW SEEING THE WHOLE PICTURE : A COMPREHENSIVE IMAGING APPROACH TO FUNCTIONAL MAPPING OF CIRCUITS IN BEHAVING ZEBRAFISH. *Neuroscience*, *296*, 26–38. http://doi.org/10.1016/j.neuroscience.2014.11.046

Feng, X.-Z., Li, X., Liu, X.-D., Li, T., Li, X., Feng, D.-F., … Xu, J. (2016). SiO2 nanoparticles cause depression and anxiety-like behavior in adult zebrafish. *RSC Adv.*, 1–26. http://doi.org/10.1039/C6RA24215D

Ferguson, J. M. (2001). SSRI Antidepressant Medications: Adverse Effects and Tolerability. *Primary Care Companion to the Journal of Clinical Psychiatry*, *3*(1), 22–27. http://doi.org/10.4088/PCC.v03n0105

Finnin, B. C., & Reed, B. L. (1973). The action of reserpine on teleost melanofores. *European Jurnal of Pharmacology*, *22*, 239–248.

Fisar Zdenk. (2015). Drugs related to monoamine oxidase activity. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *69*, 112–124. http://doi.org/10.1016/j.pnpbp.2016.02.012

Francis, S. C., & Thomas. (2013). Essential oil profiling of Centella asiatica (L.) Urb. a medicinally important herb. *South Indian Journal of Biological Sciences*, *2*(1), 309.

Fulcher, N., Tran, S., Shams, S., Chatterjee, D., & Gerlai, R. (2016). Neurochemical and Behavioral Responses to Unpredictable Chronic Mild Stress Following Developmental Isolation: The Zebrafish as a Model for Major Depression. *Zebrafish*, *00*(00), 1–12. http://doi.org/10.1089/zeb.2016.1295

Gao, Z. Y., Yang, P., Huang, Q. J., & Xu, H. Y. (2016). The influence of dizocilpine on the reserpine-induced behavioral and neurobiological changes in rats. *Neuroscience Letters*, *614*, 89–94. http://doi.org/10.1016/j.neulet.2016.01.006

Gleeson, M., Connaughton, V., & Arneson, L. S. (2007). Induction of hyperglycaemia in zebrafish (Danio rerio) leads to morphological changes in the retina. *Acta Diabetologica*, *44*(3), 157–163. http://doi.org/10.1007/s00592-007-0257-3

Gold, P. W. (2014). The organization of the stress system and its dysregulation in depressive illness. *Molecular Psychiatry*, *20*(October 2014), 1–16. http://doi.org/10.1038/mp.2014.163

Gong, M. juan, Han, B., Wang, S. mei, Liang, S. wang, & Zou, Z. jie. (2016). Icariin reverses corticosterone-induced depression-like behavior, decrease in hippocampal brain-derived neurotrophic factor (BDNF) and metabolic network disturbances revealed by NMR-based metabonomics in rats. *Journal of Pharmaceutical and Biomedical Analysis*, *123*, 63–73. http://doi.org/10.1016/j.jpba.2016.02.001

Gonzalez-Riano, C., Garcia, A., & Barbas, C. (2016). Metabolomics studies in brain tissue: A review. *Journal of Pharmaceutical and Biomedical Analysis*, *130*, 141–168. http://doi.org/10.1016/j.jpba.2016.07.008

Govindaraju, V., Young, K., & Maudsley, A. A. (2000). Proton NMR chemical shifts and coupling constants for brain metabolites. *NMR Biomed.*, *13*, 129–153.

Gray, N. E., Alcazar Magana, A., Lak, P., Wright, K. M., Quinn, J., Stevens, J. F., … Soumyanath, A. (2018). Centella asiatica: phytochemistry and mechanisms of neuroprotection and cognitive enhancement. *Phytochemistry Reviews*, *17*(1), 161–194. http://doi.org/10.1007/s11101-017-9528-y

Green, J., Collins, C., Kyzar, E. J., Pham, M., Roth, A., Gaikwad, S., … Kalueff, A. V. (2012). Automated high-throughput neurophenotyping of zebrafish social behavior. *Journal of Neuroscience Methods*, *210*(2), 266–71. http://doi.org/10.1016/j.jneumeth.2012.07.017

Gunnarsdottir, T. J., & Jonsdottir, H. (2010). Complementary Therapies in Clinical Practice Healing crisis in reflexology : Becoming worse before becoming better. *Complementary Therapies in Clinical Practice*, *16*(4), 239–243. http://doi.org/10.1016/j.ctcp.2010.01.005

Günther, B., & Wagner, H. (1996). Quantitative determination of triterpenes in extracts and phytopreparations of Centella asiatica (L.) urban. *Phytomedicine*, *3*(1), 59–65. http://doi.org/10.1016/S0944-7113(96)80011-0

Gupta, A., Verma, S., Kushwaha, P., Srivastava, S., & Rawat, A. K. S. (2014). Quantitative estimation of asiatic acid, asiaticoside and madecassoside in two accessions of Centella asiatica (L) Urban for morpho-chemotypic variation. *Indian Journal of Pharmaceutical Education and Research*, *48*(3), 75–78. http://doi.org/10.5530/ijper.48.3.9

Gupta, D., Radhakrishnan, M., & Kurhe, Y. (2015). Effect of a novel 5-HT3 receptor antagonist 4i, in corticosterone-induced depression-like behavior and oxidative stress in mice. *Steroids*, *96*, 95–102. http://doi.org/10.1016/j.steroids.2015.01.021

Hashim. (2011). Centella asiatica in food and beverage applications and its potential antioxidant and neuroprotective effect. *International Food Research Journal*, *18*(4), 1215–1222.

Hashimoto, K., Bruno, D., Nierenberg, J., Marmar, C. R., Zetterberg, H., Blennow, K., & Pomara, N. (2016). Abnormality in glutamine – glutamate cycle in the cerebrospinal fl uid of cognitively intact elderly individuals with major depressive disorder : a 3-year follow-up study. *Nature*, *6*(3), 1–6. http://doi.org/10.1038/tp.2016.8

Hengjumrut, P., Anukunwithaya, T., Tantisira, M. H., Tantisira, B., & Khemawoot, P. (2016). Comparative Pharmacokinetics between Madecassoside and Asiaticoside Presented in a Standardised Extract of *Centella Asiatica* , ECa 233 and Their Respective Pure Compound Given Separately in Rats. *Xenobiotica*, *0*(0), 1–29. http://doi.org/10.1080/00498254.2016.1273562

Howe, K., Clark, M. D., Torroja, C. F., Torrance, J., Berthelot, C., Muffato, M., … Stemple, D. L. (2013). The zebrafish reference genome sequence and its relationship to the human genome. *Nature*, *496*(7446), 498–503. http://doi.org/10.1038/nature12111

Hsu, Y. M., Hung, Y. C., Hu, L., Lee, Y. J., & Yin, M. C. (2015). Anti-diabetic effects of madecassic acid and rotundic acid. *Nutrients*, *7*(12), 10065–10075. http://doi.org/10.3390/nu7125512

Huang, F., Li, J., Shi, H. L., Wang, T. T., Muhtar, W., Du, M., … Wu, X. J. (2014). Simultaneous quantification of seven hippocampal neurotransmitters in depression mice by LC-MS/MS. *Journal of Neuroscience Methods*, *229*, 8–14. http://doi.org/10.1016/j.jneumeth.2014.04.004

Jacobs, B. L., Praag, H. Van, & Gage, F. H. (2000). Adult brain neurogenesis and psychiatry : a novel theory of depression. *Molecular Psy*, *5*, 262–269.

Jiang, H., Zheng, G., Lv, J., Chen, H., Lin, J., Li, Y., … Ding, X. (2016). Identification of Centella asiatica ’s Effective Ingredients for Inducing the Neuronal Differentiation. *Evidence-Based Complementary and Alternative Medicine*, *2016*. http://doi.org/10.1155/2016/9634750

Johnson, C. H., Ivanisevic, J., & Siuzdak, G. (2016). Metabolomics: beyond biomarkers and towards mechanisms. *Nature Publishing Group*, 1–9. http://doi.org/10.1038/nrm.2016.25

Kalshetty, P., Aswar, U., Bodhankar, S., Sinnathambi, A., & Mohan, V. (2012). Antidepressant effects of standardized extract of Centella asiatica L in olfactory bulbectomy model. *Biomedicine & Aging Pathology*, *2*(2), 48–53. http://doi.org/10.1016/j.biomag.2012.03.005

Kalueff, A. V., Echevarria, D. J., & Stewart, A. M. (2014). Gaining translational momentum: More zebrafish models for neuroscience research. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *55*, 1–6. http://doi.org/10.1016/j.pnpbp.2014.01.022

Kalueff, A. V, Gebhardt, M., Stewart, A. M., Cachat, J. M., Brimmer, M., Chawla, J. S., … Schneider, H. (2013). Towards a comprehensive catalog of zebrafish behavior 1.0 and beyond. *Zebrafish*, *10*(1), 70–86. http://doi.org/10.1089/zeb.2012.0861

Kalueff, A. V, Stewart, A. M., & Gerlai, R. (2014). Zebrafish as an emerging model for studying complex brain disorders. *Trends in Pharmacological Sciences*, *35*(2), 63–75. http://doi.org/10.1016/j.tips.2013.12.002

Kalueff, A. V, & Tuohimaa, P. (2004). Experimental modeling of anxiety and depression. *Acta Neurobiologiae Experimentalis*, *64*, 439–448.

Kalueff, A. V, Wheaton, M., & Murphy, D. L. (2007). What ' s wrong with my mouse model ? Advances and strategies in animal modeling of anxiety and depression. *Behav Brain Reseach*, *179*, 1–18. http://doi.org/10.1016/j.bbr.2007.01.023

Kar, B., & Subbiah, S. (2013). Zebrafish : An in vivo model for the study of human diseases. *International Journal of Genetics and Genomics*, *1*(1), 6–11. http://doi.org/10.11648/j.ijgg.20130101.12

Karolewicz, B., MacIag, D., O'Dwyer, G., Stockmeier, C. A., Feyissa, A. M., & Rajkowska, G. (2010). Reduced level of glutamic acid decarboxylase-67 kDa in the prefrontal cortex in major depression. *International Journal of Neuropsychopharmacology*, *13*(4), 411–420. http://doi.org/10.1017/S1461145709990587

Kawase, T., Nagasawa, M., Ikeda, H., Yasuo, S., Koga, Y., & Furuse, M. (2017). Gut microbiota of mice putatively modifies amino acid metabolism in the host brain. *British Journal of Nutrition*, *117*, 775–783. http://doi.org/10.1017/S0007114517000678

Kazmi, I., Afzal, M., Ali, B., Damanhouri, Z. A., Ahmaol, A., & Anwar, F. (2013). Anxiolytic potential of ursolic acid derivative-a stearoyl glucoside isolated from Lantana camara L. (verbanaceae). *Asian Pacific Journal of Tropical Medicine*, *6*(6), 433–437. http://doi.org/10.1016/S1995-7645(13)60069-3

Keller, J., Gomez, R., Williams, G., Lembke, A., Lazzeroni, L., Murphy, G. M., & Schatzberg, A. F. (2016). HPA axis in major depression: cortisol, clinical symptomatology and genetic variation predict cognition. *Molecular Psychiatry*, *2*(July 2015), 1–10. http://doi.org/10.1038/mp.2016.120

Kennedy, S. H. (2006). A review of antidepressant treatments today. *European Neuropsychopharmacology*, *16*, 619–624.

Khan, H., Amin, S., & Patel, S. (2018). Targeting BDNF modulation by plant glycosides as a novel therapeutic strategy in the treatment of depression. *Life Sciences*, *196*(2017), 18–27. http://doi.org/10.1016/j.lfs.2018.01.013

Kim, H. K., Choi, Y. H., & Verpoorte, R. (2010). NMR-based metabolomic analysis of plants. *Nature Protocols*, *5*, 536–549. http://doi.org/10.1038/nprot.2009.237

KOHLERT, J. G., MANGAN, B. P., KODRA, C., DRAKO, L., LONG, E., & SIMPSON, H. (2012). DECREASED AGGRESSIVE AND LOCOMOTOR BEHAVIORS IN *BETTA SPLENDENS* AFTER EXPOSURE TO FLUOXETINE [1]. *Psychological Reports*, *110*(1), 51–62. http://doi.org/10.2466/02.13.PR0.110.1.51-62

Krishnaiah D, Devi T, Bono A, & Sarbatly R. (2009). Studies on phytochemical constituents of six Malaysian medicinal plants. *Journal of Medicinal Plants Research*, *3*(2), 67–72. Retrieved from http://www.academicjournals.org/JMPR

Krishnamurthy, R. G., Senut, M., Zemke, D., Min, J., Frenkel, B., Greenberg, E. J., … Majid, A. (2010). Asiatic acid, a pentacyclic triterpene from Centella asiatica, is neuroprotective in a mouse of focal cerebral ischemia. *NIH Public Access*, *87*(11), 2541–2550. http://doi.org/10.1002/jnr.22071.Asiatic

Krishnan, V., & Nestler, E. J. (2008). The molecular neurobiology of depression. *Nature*, *455*, 894–902. http://doi.org/10.1038/nature07455

Kumar, M. H. V., & Gupta, Y. K. (2002). Effect of different extracts of Centella asiatica on cognition and markers of oxidative stress in rats, *79*, 253–260.

Kwon, K. J., Bae, S., Kim, K., An, I. S., Ahn, K. J., An, S., & Cha, H. J. (2014). Asiaticoside, a component of Centella asiatica, inhibits melanogenesis in B16F10 mouse melanoma. *Molecular Medicine Reports*, *10*(1), 503–507. Retrieved from http://www.spandidos-publications.com/mmr/10/1/503/abstract

Kyzar, E., Michael, A., Landsman, S., Collins, C., Gebhardt, M., Robinson, K., & Kalueff, A. V. (2013). Behavioral effects of bidirectional modulators of brain monoamines reserpine and d-amphetamine in zebra fi sh. *Brain Research*, *1527*, 108–116.

Kyzar, E., Stewart, A. M., Landsman, S., Collins, C., Gebhardt, M., Robinson, K., & Kalueff, A. V. (2013). Behavioral effects of bidirectional modulators of brain monoamines reserpine and d-amphetamine in zebrafish. *Brain Research*, *1527*, 108–116. http://doi.org/10.1016/j.brainres.2013.06.033

Leao, A. H. F. F., Sarmento-Silva, A. J., Santos, J. R., Ribeiro, A. M., & Silva, R. H. (2015). Molecular, neurochemical, and behavioral hallmarks of reserpine as a model for parkinson's disease: New perspectives to a long-standing model. *Brain Pathology*, *25*, 377–390. http://doi.org/10.1111/bpa.12253

Leibowitz, A., Boyko, M., Shapira, Y., & Zlotnik, A. (2012). Blood glutamate scavenging: Insight into neuro protection. *International Journal of Molecular Sciences*, *13*(8), 10041–10066. http://doi.org/10.3390/ijms130810041

Leith, N. J., & Barrett, R. J. (1980). Psychopharmacology Effects of Chronic Amphetamine or Reserpine on Self-stimulation Responding : Animal Model of Depression ? *Psychopharmacology*, *15*, 9–15.

Levin, E. D., Bencan, Z., & Cerutti, D. T. (2007). Anxiolytic effects of nicotine in zebrafish. *Physiology & Behavior*, *90*(1), 54–58. http://doi.org/10.1016/j.physbeh.2006.08.026

Liang, X., Xu, N., Cui, S., Liu, X. H., Zhang, H., Liu, S., … Dong, Y. (2008). Antidepressant-like effect of asiaticoside in mice. *Pharmacology Biochemistry and Behavior*, *89*(3), 444–449. http://doi.org/10.1016/j.pbb.2008.01.020

Liang, Z., Chen, Z., Wang, W., Wang, H., Gui, S., & Li, P. (2018). Metabolite-related antidepressant action of diterpene ginkgolides in the prefrontal cortex. *Neuropsychiatric Disease and Treatment*, 999–1011. http://doi.org/10.2147/NDT.S161351

Lin, J., Jiang, H., & Ding, X. (2017). Synergistic combinations of five single drugs from Centella asiatica for neuronal differentiation. *NeuroReport*, *28*(1), 23–27. http://doi.org/10.1097/WNR.0000000000000698

Liu, C. C., Wu, Y. F., Feng, G. M., Gao, X. X., Zhou, Y. Z., Hou, W. J., … Tian, J. S. (2015). Plasma-metabolite-biomarkers for the therapeutic response in depressed patients by the traditional Chinese medicine formula Xiaoyaosan: A 1H NMR-based metabolomics approach. *Journal of Affective Disorders*, *185*, 156–163. http://doi.org/10.1016/j.jad.2015.05.005

Liu, L., Zhou, X., Zhang, Y., Liu, Y., Yang, L., Pu, J., … Xie, P. (2016). The identification of metabolic disturbances in the prefrontal cortex of the chronic restraint stress rat model of depression. *Behavioural Brain Research*, *305*, 148–156. http://doi.org/10.1016/j.bbr.2016.03.005

Liu, L., Zhou, X., Zhang, Y., Pu, J., Yang, L., Yuan, S., & Zhao, L. (2018). Hippocampal metabolic differences implicate distinctions between physical and psychological stress in four rat models of depression. *Transl Psychiatry*, *20188*(4), 1–11. http://doi.org/10.1038/s41398-017-0018-1

Liu, X., Zheng, P., Zhao, X., Zhang, Y., Hu, C., Li, J., … Xu, G. (2015). Discovery and validation of plasma biomarkers for major depressive disorder classification based on liquid chromatography-mass spectrometry. *Journal of Proteome Research*, *14*(5), 2322–2330. http://doi.org/10.1021/acs.jproteome.5b00144

Liu, Y., Zhou, X., Yang, L., & Wang, H. (2017). Social defeat stress causes depression-like behavior with metabolite changes in the prefrontal cortex of rats. *PLoS ONE*, *10.1371*, 1–16.

Long, H. S., Stander, M. A., & Van Wyk, B. E. (2012). Notes on the occurrence and significance of triterpenoids (asiaticoside and related compounds) and caffeoylquinic acids in Centella species. *South African Journal of Botany*, *82*, 53–59. http://doi.org/10.1016/j.sajb.2012.07.017

Lu, Z., Wang, J., Li, M., Liu, Q., Wei, D., Yang, M., & Kong, L. (2014). 1H NMR-based metabolomics study on a goldfish model of Parkinson's disease induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). *Chemico-Biological Interactions*, *223*, 18–26. http://doi.org/10.1016/j.cbi.2014.09.006

Luo, L., Liu, X. L., Mu, R. H., Wu, Y. J., Liu, B. Bin, Geng, D., … Yi, L. T. (2015). Hippocampal BDNF signaling restored with chronic asiaticoside treatment in depression-like mice. *Brain Research Bulletin*, *114*, 62–69. http://doi.org/10.1016/j.brainresbull.2015.03.006

Majeed, Z. R., Ritter, K., Robinson, J., Blümich, S. L. E., Brailoiu, E., & Cooper, R. L. (2015). New insights into the acute actions from a high dosage of fluoxetine on neuronal and cardiac function: Drosophila, crayfish and rodent models. *Comparative Biochemistry and Physiology Part - C: Toxicology and Pharmacology*, *176–177*, 52–61. http://doi.org/10.1016/j.cbpc.2015.07.010

Marcon, M., Herrmann, A. P., Mocelin, R., Rambo, C. L., Koakoski, G., Abreu, M. S., … Piato, A. L. (2016). Prevention of unpredictable chronic stress-related phenomena in zebrafish exposed to bromazepam, fluoxetine and nortriptyline. *Psychopharmacology*, 1–10. http://doi.org/10.1007/s00213-016-4408-5

Matsuzaki, H., Shimizu, Y., Iwata, N., Kamiuchi, S., Suzuki, F., Iizuka, H., … Okazaki, M. (2013). Antidepressant-like effects of a water-soluble extract from the culture medium of Ganoderma lucidum mycelia in rats. *BMC Complementary and Alternative Medicine*, *13*, 370. http://doi.org/10.1186/1472-6882-13-370

Maximino, C., Gemaque, J., Benzecry, R., Lima, M. G., Batista, E. D. J. O., Picanço-Diniz, D. W., … Herculano, A. M. (2015). Role of nitric oxide in the behavioral and neurochemical effects of IB-MECA in zebrafish. *Psychopharmacology*, *232*(10), 1671–1680. http://doi.org/10.1007/s00213-014-3799-4

Maximino, C., & Herculano, A. M. (2010). A review of monoaminergic neuropsychopharmacology in zebrafish. *Zebrafish*, *7*(4), 359–78. http://doi.org/10.1089/zeb.2010.0669

McGirr, A., Vhringer, P. A., Ghaemi, S. N., Lam, R. W., & Yatham, L. N. (2016). Safety and efficacy of adjunctive second-generation antidepressant therapy with a mood stabiliser or an atypical antipsychotic in acute bipolar depression: a systematic review and meta-analysis of randomised placebo-controlled trials. *The Lancet Psychiatry*, *3*(12), 1138–1146. http://doi.org/10.1016/S2215-0366(16)30264-4

Ménard, C., Hodes, G. E., & Russo, S. J. (2017). Pathogenesis of depression: insights from human and rodent studies. *Neuroscience*, *321*, 138–162. http://doi.org/10.1016/j.neuroscience.2015.05.053.Pathogenesis

Mice, M., Yoshikawa, T., Nakamura, T., Shibakusa, T., Sugita, M., Naganuma, F., … Yanai, K. (2014). Insufficient Intake of L -Histidine Reduces Brain Histamine and Causes Anxiety-Like Behaviors in, (3). http://doi.org/10.3945/jn.114.196105.release

Michels, N., Van De Wiele, T., & De Henauw, S. (2017). Chronic Psychosocial Stress and Gut Health in Children: Associations with Calprotectin and Fecal Short-Chain Fatty Acids. *Psychosomatic Medicine*, *79*(8), 927–935. http://doi.org/10.1097/PSY.0000000000000413

Miranda, A. M., & Oliveira, T. G. (2015). Lipids under stress - A lipidomic approach for the study of mood disorders. *BioEssays*, *37*(11), 1226–1235. http://doi.org/10.1002/bies.201500070

Munari, L., Provensi, G., Passani, M. B., Galeotti, N., Cassano, T., Benetti, F., … Blandina, P. (2015). Brain histamine is crucial for selective serotonin reuptake inhibitors' behavioral and neurochemical effects. *International Journal of Neuropsychopharmacology*, *18*(10), 1–10. http://doi.org/10.1093/ijnp/pyv045

Murray, F., Smith, D. W., & Hutson, P. H. (2008). Chronic low dose corticosterone exposure decreased hippocampal cell proliferation, volume and induced anxiety and depression like behaviours in mice. *European Journal of Pharmacology*, *583*(1), 115–27. http://doi.org/10.1016/j.ejphar.2008.01.014

Mushtaq, M. Y., Choi, Y. H., Verpoorte, R., & Wilson, E. G. (2014). Extraction for metabolomics: Access to the metabolome. *Phytochemical Analysis*, *25*(October 2013), 291–306. http://doi.org/10.1002/pca.2505

Mushtaq, M. Y., Marçal, R. M., Champagne, D. L., Van Der Kooy, F., Verpoorte, R., & Choi, Y. H. (2014). Effect of acute stresses on zebra fish (danio rerio) metabolome measured by nmr-based metabolomics. *Planta Medica*, *80*(14), 1227–1233. http://doi.org/10.1055/s-0034-1382878

Nasir, M. N., Abdullah, J., Habsah, M., Ghani, R. I., & Rammes, G. (2012). Inhibitory effect of asiatic acid on acetylcholinesterase, excitatory post synaptic potential and locomotor activity. *Phytomedicine : International Journal of Phytotherapy and Phytopharmacology*, *19*(3–4), 311–6. http://doi.org/10.1016/j.phymed.2011.10.004

Nataraj, J., Manivasagam, T., Justin Thenmozhi, A., & Essa, M. M. (2016). Neuroprotective effect of asiatic acid on rotenone-induced mitochondrial dysfunction and oxidative stress-mediated apoptosis in differentiated SH-SYS5Y cells. *Nutritional Neuroscience*, *8305*(February), 1–9. http://doi.org/10.1080/1028415X.2015.1135559

Neumann, I. D., Wegener, G., Homberg, J. R., Cohen, H., Slattery, D. a., Zohar, J., … Mathé, a. (2011). Animal models of depression and anxiety: What do they tell us about human condition? *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *35*(6), 1357–1375. http://doi.org/10.1016/j.pnpbp.2010.11.028

Ngo, L. T., Okogun, J. I., & Folk, W. R. (2014). 21st Century Natural Product Research and Drug Development and Traditional Medicines. *Natural Product Reports*, *30*(4), 584–592. http://doi.org/10.1039/c3np20120a.21

Nguyen, M., Stewart, A. M., & Kalueff, A. V. (2014a). Aquatic blues: Modeling depression and antidepressant action in zebrafish. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *55C*, 26–39. http://doi.org/10.1016/j.pnpbp.2014.03.003

Nguyen, M., Stewart, A. M., & Kalueff, A. V. (2014b). Aquatic blues: Modeling depression and antidepressant action in zebrafish. *Progress in Neuropsychopharmacology & Biological Psychiatry*. http://doi.org/10.1016/j.pnpbp.2014.03.003

Niciu, M. J., Ionescu, D. F., Richards, E. M., & Zarate, C. a. (2013). Glutamate and its receptors in the pathophysiology and treatment of major depressive disorder. *Journal of Neural Transmission (Vienna, Austria : 1996)*, 907–924. http://doi.org/10.1007/s00702-013-1130-x

Okamoto, H., Agetsuma, M., & Aizawa, H. (2012). Genetic dissection of the zebrafish habenula, a possible switching board for selection of behavioral strategy to cope with fear and anxiety. *Developmental Neurobiology*, *72*(3), 386–394. http://doi.org/10.1002/dneu.20913

Orhan, I. E. (2012). Centella asiatica (L.) Urban: From traditional medicine to modern medicine with neuroprotective potential. *Evidence-Based Complementary and Alternative Medicine*, *2012*, 1–8. http://doi.org/10.1155/2012/946259

Oyedeji, O. A., & Afolayan, A. J. (2005). Chemical composition and antibacterial activity of the essential oil of Centella asiatica growing in South Africa. *Pharmaceutical Biology*, *43*(3), 249–252. http://doi.org/10.1080/13880200590928843

Ozerov, A. A., Bagmetova, V. V., Chernysheva, Y. V., & Tyurenkov, I. N. (2016). Comparison of the Efficiency of Adeprophen and Antidepressants of Various Groups on the Model of Reserpine-Induced Depression in Rats. *Bulletin of Experimental Biology and Medicine*, *160*(5), 649–652. http://doi.org/10.1007/s10517-016-3240-6

Pagnussat, N., Piato, A. L., Schaefer, I. C., Blank, M., Tamborski, A. R., Guerim, L. D., … Lara, D. R. (2013). One for All and All for One: The Importance of Shoaling on Behavioral and Stress Responses in Zebrafish. *Zebrafish*, *10*(3), 1–5. http://doi.org/10.1089/zeb.2013.0867

Pavlidis, M., Theodoridi, A., & Tsalafouta, A. (2015a). Neuroendocrine regulation of the stress response in adult zebrafish, Danio rerio. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *60*, 121–131. http://doi.org/10.1016/j.pnpbp.2015.02.014

Pavlidis, M., Theodoridi, A., & Tsalafouta, A. (2015b). Neuroendocrine regulation of the stress response in adult zebrafish, Danio rerio. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *60*, 121–131. http://doi.org/10.1016/j.pnpbp.2015.02.014

Phillip, T. (1959). THE EFFECT OF RESERPINE ON HISTAMINE AND SEROTONIN. *Journal of Allergy*, *30*(5), 408–414.

Porter, J., & Hillhouse, T. M. (2016). A brief history of the development of antidepressant drugs: From monoamines to glutamate Todd. *Exp Clin Psychopharmacol.*, *23*(1), 1–21. http://doi.org/10.1037/a0038550.A

Qi, X., Salem, M., Zhou, W., Sato-Shimizu, M., Ye, G., Smitz, J., & Peng, C. (2016). Neurokinin B Exerts Direct Effects on the Ovary to Stimulate Estradiol Production. *Endocrinology*, *157*(9), 3355–3365. http://doi.org/10.1210/en.2016-1354

Quinones, M. P., & Kaddurah-Daouk, R. (2009). Metabolomics tools for identifying biomarkers for neuropsychiatric diseases. *Neurobiology of Disease*, *35*(2), 165–176. http://doi.org/10.1016/j.nbd.2009.02.019

R&D Systems, I. (2013). *Cortisol Assay*.

Raterink, R.-J., Lindenburg, P. W., Vreeken, R. J., Ramautar, R., & Hankemeier, T. (2014). Recent developments in sample-pretreatment techniques for mass spectrometry-based metabolomics. *TrAC Trends in Analytical Chemistry*, *61*, 157–167. http://doi.org/10.1016/j.trac.2014.06.003

Richetti, S. K., Blank, M., Capiotti, K. M., Piato, A. L., Bogo, M. R., Vianna, M. R., & Bonan, C. D. (2011). Quercetin and rutin prevent scopolamine-induced memory impairment in zebrafish. *Behavioural Brain Research*, *217*(1), 10–15. http://doi.org/10.1016/j.bbr.2010.09.027

Riley, C. A., & Renshaw, P. F. (2018). Brain choline in major depression : A review of the literature. *Psychiatry Research: Neuroimaging*, *271*(October 2017), 142–153. http://doi.org/10.1016/j.pscychresns.2017.11.009

Rotroff, D. M., Corum, D. G., Motsinger-Reif, A., Fiehn, O., Bottrel, N., Drevets, W. C., … Kaddurah-Daouk, R. (2016). Metabolomic signatures of drug response phenotypes for ketamine and esketamine in subjects with refractory major depressive disorder: New mechanistic insights for rapid acting antidepressants. *Translational Psychiatry*, *6*(9), 1–10. http://doi.org/10.1038/tp.2016.145

Roy, D., Steyer, G. J., Gargesha, M., Stone, M. E., & Wilson, L. (2009). Caffeoylquinic acids in Centella asiatica protect against β- amyloid toxicity. *J Alzheimers*, *292*(3), 342–351. http://doi.org/10.1002/ar.20849.3D

Ryan, D., & Robards, K. (2006). Metabolomics: The greatest omics of them all? *Analytical Chemistry*, *78*(23), 7954–7958. http://doi.org/10.1021/ac0614341

Saadallah Ramadan, Alexander Lin, and P. S. (2014). Glutamate and Glutamine: A Review of In Vivo MRS in the Human Brain. *NMR Biomed.*, *26*(12), 1–36. http://doi.org/10.1002/nbm.3045.Glutamate

Sampath, U., & Janardhanam, V. A. (2013). Asiaticoside , a trisaccaride triterpene induces biochemical and molecular variations in brain of mice with parkinsonism, 1–10. http://doi.org/10.1186/2047-9158-2-23

Samuel, A. J. S. J., Kalusalingam, A., Chellappan, D. K., Gopinath, R., Radhamani, S., Husain, H. A., … Promwichit, P. (2010). Ethnomedical survey of plants used by the Orang Asli in Kampung Bawong, Perak, West Malaysia. *Journal of Ethnobiology and Ethnomedicine*, *6*, 1–6. http://doi.org/10.1186/1746-4269-6-5

Santos-Fandila, A., Vázquez, E., Barranco, A., Zafra-Gómez, A., Navalón, A., Rueda, R., & Ramírez, M. (2015). Analysis of 17 neurotransmitters, metabolites and precursors in zebrafish through the life cycle using ultrahigh performance liquid chromatography-tandem mass spectrometry. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, *1001*(August), 191–201. http://doi.org/10.1016/j.jchromb.2015.07.040

Saroya, R., Smith, R., Seymour, C., & Mothersill, C. (2010). Injection of resperpine into zebrafish, prevents fish to fish communication of radiation-induced bystander signals: Confirmation in vivo of a role for serotonin in the mechanism. *Dose-Response*, *8*(3), 317–330. http://doi.org/10.2203/dose-response.09-043.Saroya

Schaneberg, B. T., Mikell, J. R., Bedir, E., & Khan, I. A. (2003). An improved HPLC method for quantitative determination of six triterpenes in Centella asiatica extracts and commercial products. *Pharmazie*, *58*(6), 381–384. http://doi.org/10.1613/jair.301

Schneider, H. (2011). *Measuring agonistic behavior in zebrafish*. *Neuromethods* (Vol. 51). http://doi.org/10.1007/978-1-60761-953-6_10

Selvi, P. T., Kumar, M. S., Rajesh, R., & Kathiravan, T. (2012). Antidepressant activity of ethanolic extract of leaves of Centella asiatica. Linn by in vivo methods. *Asian Journal of Research in Pharmaceutical Science*, *2*(2), 76–79.

Setoyama, D., Kato, T. A., Hashimoto, R., Kunugi, H., Hattori, K., Hayakawa, K., … Kanba, S. (2016). Plasma metabolites predict severity of depression and suicidal ideation in psychiatric patients-a multicenter pilot analysis. *PLoS ONE*, *11*(12), 1–16. http://doi.org/10.1371/journal.pone.0165267

Shabir, B. Y. G. (2004). Step-by-step analytical methods validation and protocol in the quality system compliance industry. *Journal of Validation Technology*, *10*, 210–218.

Shan, L., Bao, A., & Swaab, D. F. (2015). The human histaminergic system in neuropsychiatric disorders. *Trends in Neurosciences*, *38*(3), 167–177. http://doi.org/10.1016/j.tins.2014.12.008

Shao, W., Chen, J., Fan, S., Lei, Y., Xu, H., Zhou, J., … Xie, P. (2015). Combined Metabolomics and Proteomics Analysis of Major Depression in an Animal Model: Perturbed Energy Metabolism in the Chronic Mild Stressed Rat Cerebellum. *OMICS: A Journal of Integrative Biology*, *19*(7), 383–392. http://doi.org/10.1089/omi.2014.0164

Shao, Y., Ou-Yang, D. W., Gao, W., Cheng, L., Weng, X. X., & Kong, D. Y. (2014). Three new pentacyclic triterpenoids from centella asiatica. *Helvetica Chimica Acta*, *97*, 992–998. http://doi.org/10.1002/hlca.201300382

Shen, Y., Liu, A., Ye, M., Wang, L., Chen, J., Wang, X., & Han, C. (2009). Analysis of Biologically Active Constituents in Centella asiatica by Microwave-Assisted Extraction Combined with LC – MS, (3), 431–438. http://doi.org/10.1365/s10337-009-1152-6

Shi, B., Tian, J., Xiang, H., Guo, X., & Zhang, L. (2013). A 1 H-NMR plasma metabonomic study of acute and chronic stress models of depression in rats. *Behavioural Brain Research*, *241*(92), 86–91. http://doi.org/10.1016/j.bbr.2012.11.036

Shin, I. J., Son, S. U., Park, H., Kim, Y., Park, S. H., Swanberg, K., … Maeng, S. (2014). Preclinical evidence of rapid-onset antidepressant-like effect in radix polygalae extract. *PLoS ONE*, *9*(2). http://doi.org/10.1371/journal.pone.0088617

Shukla, A., Rasik, A. M., Jain, G. K., Shankar, R., Kulshrestha, D. K., & Dhawan, B. N. (1999). In vitro and in vivo wound healing activity of asiaticoside isolated from Centella asiatica. *Journal of Ethnopharmacology*, *65*(1), 1–11. http://doi.org/10.1016/S0378-8741(98)00141-X

Si, T., & Yu, X. (2016). Current problems in the research and development of more effective antidepressants, *28*(3), 160–165.

Sillaber, I., Holsboer, F., & Turck, C. W. (2011). Metabolite profiling of antidepressant drug action reveals novel drug targets beyond monoamine elevation. *Transl Psychiatry*, (November), 1–9. http://doi.org/10.1038/tp.2011.56

Singer, M. L., Oreschak, K., Rhinehart, Z., & Robison, B. D. (2016). Anxiolytic effects of fluoxetine and nicotine exposure on exploratory behavior in zebrafish. *PeerJ*, *4*, 1–17. http://doi.org/10.7287/peerj.preprints.1718v2

Skalisz, L. (2002). Evaluation of the face validity of reserpine administration as an animal model of depression–Parkinson's disease association. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *26*(5), 879–883. http://doi.org/10.1016/S0278-5846(01)00333-5

Skalisz, L. L., Beijamini, V., Joca, S. L., Vital, M. A. B. F., Cunha, C. Da, & Andreatini, R. (2002). Evaluation of the face validity of reserpine administration as an animal model of depression – Parkinson ' s disease association, *26*, 879–883.

Socała, K., Nieoczym, D., Pieróg, M., Szuster-Ciesielska, A., Wyska, E., & Wlaź, P. (2016). Antidepressant-like activity of sildenafil following acute and subchronic treatment in the forced swim test in mice: effects of restraint stress and monoamine depletion. *Metabolic Brain Disease*, 1–10. http://doi.org/10.1007/s11011-016-9852-8

Soumyanath, a, Zhong, Y. P., Henson, E., Wadsworth, T., Bishop, J., Gold, B. G., & Quinn, J. F. (2012). Centella asiatica Extract Improves Behavioral Deficits in a Mouse Model of Alzheimer's Disease: Investigation of a Possible Mechanism of Action. *Int J Alzheimers Dis*, *2012*, 1–9. http://doi.org/10.1155/2012/381974

Stewart, A., Cachat, J. M., Suciu, C., Hart, P. C., Gaikwad, S., Utterback, E., … Kalueff, A. V. (2011). Chapter 14 Intraperitoneal Injection as a Method of Psychotropic Drug Delivery in Adult Zebrafish. *Zebrafish Neurobehavioral Protocols*, 169–179. http://doi.org/10.1007/978-1-60761-953-6

Stewart, A. M., Cachat, J., Gaikwad, S., Robinson, K. S. L., Gebhardt, M., & Kalueff, A. V. (2013). Perspectives on experimental models of serotonin syndrome in zebrafish. *Neurochemistry International*, *62*(6), 893–902. http://doi.org/10.1016/j.neuint.2013.02.018

Subathra, M., Shila, S., Devi, M. A., & Panneerselvam, C. (2005). Emerging role of Centella asiatica in improving age-related neurological antioxidant status. *Experimental Gerontology*, *40*(8–9), 707–15. http://doi.org/10.1016/j.exger.2005.06.001

Sulakhiya, K., Kumar, P., Jangra, A., Dwivedi, S., Hazarika, N. K., Baruah, C. C., & Lahkar, M. (2014). Honokiol abrogates lipopolysaccharide-induced depressive like behavior by impeding neuroinflammation and oxido-nitrosative stress in mice. *European Journal of Pharmacology*, *744*, 124–31. http://doi.org/10.1016/j.ejphar.2014.09.049

Sumpter, J. P., Donnachie, R. L., & Johnson, A. C. (2014). The apparently very variable potency of the anti-depressant fluoxetine. *Aquatic Toxicology*, *151*, 57–60. http://doi.org/10.1016/j.aquatox.2013.12.010

Szczesniak, O., Hestad, K. A., Hanssen, J. F., & Rudi, K. (2016). Isovaleric acid in stool correlates with human depression. *Nutritional Neuroscience*, *0*(0), 1–5. http://doi.org/10.1179/1476830515Y.0000000007

Tian, J., Peng, G., Gao, X., Zhou, Y., Xing, J., Qin, X., & Du, G. (2014). Dynamic analysis of the endogenous metabolites in depressed patients treated with TCM formula Xiaoyaosan using urinary 1H NMR-based metabolomics. *Journal of Ethnopharmacology*, *158*, 1–10. http://doi.org/10.1016/j.jep.2014.10.005

Tiwari, P., Verma, R., Ahirwar, D., Chandy, A., & Dwivedi, S. (2014). Evaluation of anxiolytic effect of Syzygium aromaticum: A traditional herb of India. *Asian Pacific Journal of Tropical Disease*, *4*(S1), 77–80. http://doi.org/10.1016/S2222-1808(14)60418-7

Van Der Werf, M. J., Jellema, R. H., & Hankemeier, T. (2005). Microbial metabolomics: Replacing trial-and-error by the unbiased selection and ranking of targets. *Journal of Industrial Microbiology and Biotechnology*, *32*(6), 234–252. http://doi.org/10.1007/s10295-005-0231-4

Villa, R. F., Ferrari, F., Bagini, L., Gorini, A., Brunello, N., & Tascedda, F. (2017). Mitochondrial energy metabolism of rat hippocampus after treatment with the antidepressants desipramine and fluoxetine. *Neuropharmacology*, *121*, 30–38. http://doi.org/10.1016/j.neuropharm.2017.04.025

Wagner, G., Schultes, M. T., Titscher, V., Teufer, B., Klerings, I., & Gartlehner, G. (2018). Efficacy and safety of levomilnacipran, vilazodone and vortioxetine compared with other second-generation antidepressants for major depressive disorder in adults: A systematic review and network meta-analysis. *Journal of Affective Disorders*, *228*(October 2017), 1–12. http://doi.org/10.1016/j.jad.2017.11.056

Wanasuntronwong, A., Tantisira, M. H., Tantisira, B., & Watanabe, H. (2012). Anxiolytic effects of standardized extract of Centella asiatica (ECa 233) after chronic immobilization stress in mice. *Journal of Ethnopharmacology*, *143*(2), 579–585. http://doi.org/10.1016/j.jep.2012.07.010

Wang, W., Guo, H., Zhang, S.-X., Li, J., Cheng, K., Bai, S.-J., … Xie, P. (2016). Targeted Metabolomic Pathway Analysis and Validation Revealed Glutamatergic Disorder in the Prefrontal Cortex among Chronic Social Defeat Stress Mice Model of Depression. *Journal of Proteome Research*, 1–28. http://doi.org/10.1021/acs.jproteome.6b00577

Wang, Y., Han, T., Zhu, Y., Zheng, C. J., Ming, Q. L., Rahman, K., & Qin, L. P. (2010). Antidepressant properties of bioactive fractions from the extract of crocus sativus L. *Journal of Natural Medicines*, *64*(1), 24–30. http://doi.org/10.1007/s11418-009-0360-6

Watanabe, R., Kakeda, S., Watanabe, K., Liu, X., Katsuki, A., Umeno-Nakano, W., … Korogi, Y. (2017). Relationship between the hippocampal shape abnormality and serum cortisol levels in first-episode and drug-naïve major depressive disorder patients. *Depression and Anxiety*, (October 2016), 1–9. http://doi.org/10.1002/da.22604

Wattanathorn, J., Mator, L., Muchimapura, S., Tongun, T., Pasuriwong, O., Piyawatkul, N., … Singkhoraard, J. (2008). Positive modulation of cognition and mood in the healthy elderly volunteer following the administration of Centella asiatica. *Journal of Ethnopharmacology*, *116*(2), 325–32. http://doi.org/10.1016/j.jep.2007.11.038

Wijeweera, P., Arnason, J. T., Koszycki, D., & Merali, Z. (2006). Evaluation of anxiolytic properties of Gotukola--(Centella asiatica) extracts and asiaticoside in rat behavioral models. *Phytomedicine : International Journal of Phytotherapy and Phytopharmacology*, *13*(9–10), 668–76. http://doi.org/10.1016/j.phymed.2006.01.011

Wilson, J. M., Bunte, R. M., & Carty, A. J. (2009). Evaluation of rapid cooling and tricaine methanesulfonate (MS222) as methods of euthanasia in zebrafish (Danio rerio). *Journal of the American Association for Laboratory Animal Science : JAALAS*, *48*(6), 785–9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/19930828%5Cnhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC2786934

Wong, K., Elegante, M., Bartels, B., Elkhayat, S., Tien, D., Roy, S., … Kalueff, A. V. (2010). Analyzing habituation responses to novelty in zebrafish (Danio rerio). *Behavioural Brain Research*, *208*(2), 450–457. http://doi.org/10.1016/j.bbr.2009.12.023

Woo, H.-I., Chun, M.-R., Yang, J.-S., Lim, S.-W., Kim, M.-J., Kim, S.-W., … Lee, S.-Y. (2015). Plasma amino acid profiling in major depressive disorder treated with selective serotonin reuptake inhibitors. *CNS Neuroscience & Therapeutics*, *21*(5), 417–24. http://doi.org/10.1111/cns.12372

Wu, H., Wang, P., Liu, M., Tang, L., Fang, J., Zhao, Y., … Yang, H. (2015). A 1H-NMR-Based Metabonomic Study on the Anti-Depressive Effect of the Total Alkaloid of Corydalis Rhizoma. *Molecules*, *20*(6), 10047–10064. http://doi.org/10.3390/molecules200610047

Wu, Y., Fu, Y., Rao, C., Li, W., Liang, Z., Zhou, C., … Xie, P. (2016). Metabolomic analysis reveals metabolic disturbances in the prefrontal cortex of the lipopolysaccharide-induced mouse model of depression. *Behavioural Brain Research*, *308*, 115–127. http://doi.org/10.1016/j.bbr.2016.04.032

Xia, B., Bai, L., Li, X., Xiong, J., Xu, P., & Xue, M. (2015). Structural Analysis of Metabolites of Asiatic Acid and Its Analogue Madecassic Acid in Zebrafish Using LC/IT-MSn. *Molecules (Basel, Switzerland)*, *20*(2), 3001–19. http://doi.org/10.3390/molecules20023001

Xing, H., Su, B., Wang, Y., Yang, Y., Ren, Q., Xiao, W., & Lu, X. (2009). Separation and Determination of Asiaticoside, Asiaticoside-B and Madecassoside in Centella asiatica Total Triterpenoid Saponins by HPLC. *Journal of Liquid Chromatography & Related Technologies*, *32*(13), 1891–1900. http://doi.org/10.1080/10826070903091597

Xu, H., Wang, Z., Zhu, L., Sui, Z., Bi, W., Liu, R., … Li, Q. (2018). Targeted Neurotransmitters Profiling Identifies Metabolic Signatures in Rat Brain by LC-MS/MS: Application in Insomnia, Depression and Alzheimer's Disease. *Molecules*, *23*(2375), 1–14. http://doi.org/10.3390/molecules23092375

Xu, J., Xu, H., Liu, Y., He, H., & Li, G. (2015). Vanillin-induced amelioration of depression-like behaviors in rats by modulating monoamine neurotransmitters in the brain. *Psychiatry Research*, *225*(3), 509–514. http://doi.org/10.1016/j.psychres.2014.11.056

Yang, B., Xu, Y., Hu, Y., Luo, Y., Lu, X., Tsui, C. K., … Liang, X. (2016). Madecassic Acid protects against hypoxia-induced oxidative stress in retinal microvascular endothelial cells via ROS-mediated endoplasmic reticulum stress. *Biomedicine and Pharmacotherapy*, *84*, 845–852. http://doi.org/10.1016/j.biopha.2016.10.015

Yehuda, R., Teicher, M. H., Trestman, R. L., Levengood, R. A., & Siever, L. J. (1996). Cortisol regulation in posttraumatic stress disorder and major depression: A chronobiological analysis. *Biological Psychiatry*, *40*(2), 79–88. http://doi.org/10.1016/0006-3223(95)00451-3

Yi, L. T., Li, J., Li, H. C., Zhou, Y., Su, B. F., Yang, K. F., … Zhang, Y. T. (2012). Ethanol extracts from Hemerocallis citrina attenuate the decreases of brain-derived neurotrophic factor, TrkB levels in rat induced by corticosterone administration. *Journal of Ethnopharmacology*, *144*(2), 328–334. http://doi.org/10.1016/j.jep.2012.09.016

Yuan, Y., Zhang, H., Sun, F., Sun, S., Zhu, Z., & Chai, Y. (2015). Biopharmaceutical and pharmacokinetic characterization of asiatic acid in Centella asiatica as determined by a sensitive and robust HPLC-MS method. *Journal of Ethnopharmacology*, *163*, 31–8. http://doi.org/10.1016/j.jep.2015.01.006

Zenk, M. H., & Juenger, M. (2007). Evolution and current status of the phytochemistry of nitrogenous compounds. *Phytochemistry*, *68*(22–24), 2757–2772. http://doi.org/10.1016/j.phytochem.2007.07.009

Zhang, R., Zhang, T., Ali, A. M., Al Washih, M., Pickard, B., & Watson, D. G. (2016). Metabolomic Profiling of Post-Mortem Brain Reveals Changes in Amino Acid and Glucose Metabolism in Mental Illness Compared with Controls. *Computational and Structural Biotechnology Journal*, *14*, 106–116. http://doi.org/10.1016/j.csbj.2016.02.003

Zhang, Y., Yuan, S., Pu, J., Yang, L., Zhou, X., Liu, L., & Jiang, X. (2018). Integrated Metabolomics and Proteomics Analysis of Hippocampus in a Rat Model of Depression. *Neuroscience*, *371*, 207–220. http://doi.org/10.1016/j.neuroscience.2017.12.001

Zhao, J., Jung, Y.-H., Jang, C.-G., Chun, K.-H., Kwon, S. W., & Lee, J. (2015). Metabolomic identification of biochemical changes induced by fluoxetine and imipramine in a chronic mild stress mouse model of depression. *Scientific Reports*, *5*(8890), 1–8. http://doi.org/10.1038/srep08890

Zhao, Z., Wang, W., Guo, H., & Zhou, D. (2008). Antidepressant-like effect of liquiritin from Glycyrrhiza uralensis in chronic variable stress induced depression model rats. *Behavioural Brain Research*, *194*(1), 108–113. http://doi.org/10.1016/j.bbr.2008.06.030

Zheng, M., Fan, Y., Shi, D., & Liu, C. (2013). Antidepressant-like effect of flavonoids extracted from Apocynum venetum leaves on brain monoamine levels and dopaminergic system. *Journal of Ethnopharmacology*, *147*(1), 108–113. http://doi.org/10.1016/j.jep.2013.02.015

Zheng, P., Wang, Y., Chen, L., Yang, D., Meng, H., Zhou, D., … Xie, P. (2013). Identification and Validation of Urinary Metabolite Biomarkers for Major Depressive Disorder. *Molecular & Cellular Proteomics*, *12*(1), 207–214. http://doi.org/10.1074/mcp.M112.021816

Zheng, P., Zeng, B., Zhou, C., Liu, M., Fang, Z., Xu, X., … Xie, P. (2016). Gut microbiome remodeling induces depressive-like behaviors through a pathway mediated by the host's metabolism. *Molecular Psychiatry*, *21*(6), 786–796. http://doi.org/10.1038/mp.2016.44

Zhou, X., Liu, L., Zhang, Y., Pu, J., Yang, L., Zhou, C., … Xie, P. (2016). Metabolomics identifies perturbations in amino acid metabolism in the prefrontal cortex of the learned helplessness rat model of depression. *Neuroscience*. http://doi.org/10.1016/j.neuroscience.2016.11.038