# OPTIMIZATION OF *Plectranthus amboinicus* EXTRACTION USING MICROWAVE-ASSISTED TECHNIQUE

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To my beloved family, supervisor and friends

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# ABSTRACT

*Plectranthus amboinicus* (Lour.) Spreng is a medicinal herb with bioactive compounds which has been known to have pharmacological properties that can be found throughout the tropical countries and Middle East regions like India, Asia and Australia. It has an aromatic nature and has a capability in producing essential oil. Microwave-assisted extraction (MAE) has been known as a technique for extracting essential oil with lots of benefits such as decrease extraction time, less volume of solvent used, high yield of extract and lowered the environmental impact by emitting less $CO_2$ in the atmosphere compared to other conventional extraction methods such as soxhlet extraction method. The objective of this study was to determine the optimum extraction condition of essential oil from *P. amboinicus* by using MAE. This study focused on the comparison of the optimum extraction of *P. amboinicus* using ethanol and water by maximising the essential oil yield using response surface methodology (RSM). From the optimum extraction condition obtained, analyses on phenolic content, refractive index, bioactive compounds and antioxidants activity of the *P. amboinicus* extract were performed. Result showed the optimum condition for ethanol extract was at irradiation time (2 min), ethanol concentration (30%) and solvent-to-solid ratio (30 ml/g) with yield of extract obtained at 39.81% w/w and the optimum condition for water extract was at irradiation time (10 min), microwave power (529.58 w) and solvent-to-solid ratio (16.95 ml/g) with yield of extract obtained at 31.89% w/w. Meanwhile, the result analysis of total phenolic content of *P. amboinicus* for ethanol extract was 12.22 ± 0.93 mg GAE/g FW and for water extract was 9.61 ± 1.56 mg GAE/g FW while the antioxidants of the *P. amboinicus* extract of $IC_{50}$ value for ethanol extract was 4.63 mg/ml while the inhibition of DPPH radical scavenging activity was 5.48 ± 0.77 mg Trolox eq/g extract. Refractive index for *P. amboinicus* extract for ethanol extract was 1.3622 ± 0.0006 while for water extract was 1.3492 ± 0.0021 at 20°C. Analysis of bioactive in ethanol extract comprised of α-Terpinene, carvacrol, α-Terpineol, thymol, trans-α-Bergamotene whereas for water extract consisted of ρ-Cymene, α-Terpinene, α-Terpineol and thymol. The study has proven that MAE is a better alternative compared to conventional extraction method with 15% higher yield and the extract of *P. amboinicus* can be potentially applied in healthcare and pharmaceutical industry.

# ABSTRAK

*Plectranthus amboinicus* (Lour.) Spreng merupakan tumbuhan perubatan dengan sebatian bioaktif yang dilaporkan mempunyai sifat-sifat farmakologi yang boleh didapati di seluruh negara tropika dan rantau Timur Tengah seperti India, Asia dan Australia. Ia mempunyai sifat aromatik dan mempunyai keupayaan menghasilkan minyak pati. Pengekstrakan kaedah bantuan ketuhar gelombang mikro (MAE) telah dikenalipasti sebagai satu teknik untuk mengekstrak minyak pati dengan mengandungi pelbagai manfaat seperti pengurangan masa pengekstrakan, kurang isipadu pelarut yang digunakan, hasil ekstrak yang tinggi dan mengurangkan kesan alam sekitar dengan mengeluarkan kurang $CO_2$ di berbanding kaedah pengekstrakan konvensional yang lain seperti kaedah pengekstrakan soxhlet. Kajian ini bertujuan untuk menentukan keadaan optimum pengekstrakan minyak pati dari *P. amboinicus* dengan menggunakan kaedah MAE. Fokus kajian ini adalah untuk membandingkan pengekstrakan optimum *P. amboinicus* menggunakan etanol dan air dengan memaksimumkan hasil ekstrak minyak pati dengan menggunakan kaedah permukaan tindak balas (RSM). Daripada kondisi optimum pengekstrakan yang diperolehi, analisis bagi jumlah kandungan fenolik, indeks biasan, sebatian bioaktif dan aktiviti antioksida bagi ekstrak *P. amboinicus* telah dijalankan. Keputusan menunjukkan keadaan optimum untuk ekstrak etanol adalah pada iradiasi masa (2 min), kepekatan etanol (30%) dan nisbah pelarut kepada pepejal (30 ml/g) dengan hasil ekstrak yang didapati 39.81% w/w dan optimum ekstrak air adalah pada masa iradiasi (10 min), kuasa gelombang mikro (529.58 W) dan nisbah pelarut kepada pepejal adalah (16.95 ml/g) dengan hasil ekstrak yang didapati 31.89% w/w. Sementara itu, hasil analisis bagi jumlah kandungan fenolik *P. amboinicus* untuk ekstrak etanol ialah 12.22 ± 0.93 mg GAE/g FW dan bagi ekstrak air ialah 9.61 ± 1.56 mg GAE/g FW manakala antioksidan daripada ekstrak *P. amboinicus* untuk nilai $IC_{50}$ bagi ekstrak etanol adalah 4.63 mg/ml manakala perencatan aktiviti penipisan radikal DPPH adalah 5.48 ± mg 0.77 Trolox eq/g ekstrak. Indeks refraktif *P. amboinicus* untuk ekstrak etanol ialah 1.3622 ± 0.0006 manakala ekstrak air ialah 1.3492 ± 0.0021 pada 20 ° C. Analisis bioaktif dalam ekstrak etanol terdiri daripada α-Terpinene, carvacrol, α-Terpineol, thymol, trans-α-Bergamotene manakala bagi ekstrak air terdiri daripada Cymene, α-Terpinene, α-Terpineol dan thymol. Kajian ini telah membuktikan bahawa MAE adalah alternatif yang lebih baik berbanding kaedah pengekstrakan konvensional dengan hasil ekstrak 15% lebih tinggi dan ekstrak *P. amboinicus* berpotensi digunakan dalam industri penjagaan kesihatan dan farmaseutikal..

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| | | |
|---|---|---|
| *P. amboinicus* | - | *Plectranthus amboinicus* |
| MAE | - | Microwave –assisted Extraction |
| RSM | - | Response Surface Methodology |
| BBD | - | Box-Behnken Design |
| OFAT | - | One factor at a time |
| ANOVA | - | Analysis of Variance |
| S:S | - | Solid to Solvent |
| GC-MS | - | Gas Chromatography Mass Spectrometer |
| TPC | - | Total Phenolic Content |
| DPPH | - | 2,2-diphenyl-1-picryl-hydrazyl-hydrate |
| GAE | - | Gallic Acid Equivalent |

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# CHAPTER 1

# INTRODUCTION

## 1.1 Background of Study

Malaysia is located at Southeast Asian country where the land is covered by tropical rainforest. There are plenty of herbs that can be found in Malaysia either as a medicinal herbs or culinary herbs. This is due to the Malaysia's perfect climate which is hot and humid throughout the year. However, some herbs have not been scientifically studied for their properties, but the old folk had been using it as part of remedies in their daily life.

In Malaysia, *Plectranthus amboinicus* are known with various names such as pokok bangun-bangun, bebangun, sedingin or hati-hati hijau (Sabrina *et al.,* 2014). It is an indigenous plant that can be eaten raw or used as flavouring in dishes as it have a pleasant aroma with distinctive and refreshing odour. (Arumugam *et al.,* 2016). Besides, *P. amboinicus* is among the most cited species in the family Lamiaceae mainly for its medicinal properties (Lukhoba *et al.,* 2006) such as for digestive condition, skin condition, respiratory condition, infections and fever as well as genito-urinary conditions (Morton, 1992). This indicates that *P. amboinicus* have a

pharmacological properties, which is suitable for curing disease like cardiovascular, respiratory, skin, oral, digestive and urinary diseases.

Traditionally, the extraction of *P. amboinicus* is done by using hydrodistillation and soxhlet extraction method. It is commonly used as a standard for a comparing between different extraction method to extract and recover oil from natural products. Soxhlet extraction requires up to 16 hours of extraction time and uses more than 300 ml of solvent (Hadkar *et al.*, 2013). Therefore, an alternative extraction method is required to overcome the problem. Microwave extraction process was proven to reduce extraction time and solvent usage as well as improved extraction yield as it uses microwave energy to heat solvents rapidly and efficiently with an ability to have a homogeneous heating of natural product and solvent (Jain *et al.,* 2009).

In microwave assisted extraction (MAE) requires only 10 to 20 ml of solvent and 30 to 45 seconds of extraction time depending on the natural product used for the extraction (Hadkar *et al.,* 2013). Study by Wang & Weller (2006) has reported that MAE is a comparable extraction technique to other technique such as subcritical water extraction, supercritical carbon dioxide extraction and ultrasonic assisted extraction (UAE). This is because of the process simplicity, faster, more effective and low cost. Moreover, previous study by Garofulić *et al.* (2013) has proven that MAE was able to produce higher concentration of sour cherry polyphenol extracted with more efficiency and faster compared to conventional extraction method.

Essential oil of *P. amboinicus* contains high amount of bioactive compounds such as thymol, carvacrol , ρ-cymene, α-terpineol , β-caryophyllene, α-humulene, γ-terpinene and β-selinene (Hussein, 2017; Senthilkumar & Venkatesalu, 2014; Manjamalai et al., 2012; Lima *et al.*, 2011) . Study by Arumugam *et al.* (2016) stated that the presence of thymol and carvacrol indicates that the *P. amboinicus* extract has an antioxidants properties against stress-created in cell line-induced lung cancer either

for *(in vitro and in vivo)* models. Moreover, work by Bhatt & Negi (2012); Manjamalai *et al.* (2012); Khanum *et al.*, (2011); Kumaran & Joel karunakaran (2006) also claims that the presence of carvacrol and thymol show a significant inhibition in DPPH free radical formation. This shows that *P. amboinicus* has a good potential to be use in the formulation for healthcare and pharmaceutical industry application.

## 1.2 Problem Statement

Extraction is a crucial step in producing essential oil therefore finding the most suitable method that can produces a high yield and the desired active compound composition is a must. There are plenty of extraction methods available and the most commonly used by researchers and for commercialization are hydro distillation, steam distillation and soxhlet extraction. Commonly, it took 3 to 4 hours for extracting *P. amboinicus* by hydro distillation method using a Clevenger type apparatus while soxhlet extraction require up to 16 hours of extraction time and uses more than 300 ml of solvent with the small amount of yield produced. Hence, most conventional method took longer extraction time, this will affect the large scale production when the essential oil to be used for commercialization.

The application of microwave in extraction process was proven to decrease extraction time, volume of solvent used and lowered the environmental impact by emitting less $CO_2$ in atmosphere (Ferhat *et al.,* 2007; Lucchesi *et al.,* 2004), high yield of extract obtained and it also required a small amount of energy as compared to conventional extraction methods (Farhat *et al.,* 2017).

*P. amboinicus* contains valuable bioactive compounds where it can be widely applied in both traditional and modern therapies for improving human health with

relatively none or less effects. Antioxidants are minerals and vitamins that occur naturally in foods and can also synthesised by human bodies (Hopkins & Hüner, 2008). It consists of important compounds which maintains our health and has the capability to reduce the risk of disease Adom *et al.,* (2005) as well as avoid from oxidative damages by neutralizing the free radicals before they can attack the cells and avoid damage to lipids, proteins, enzymes, carbohydrates and DNA (Fang *et al.*, 2002).

Therefore, this study aims to focus on optimization of the extraction process of *P. amboinicus* using microwave-assisted extraction method and characterization of the total phenolic content, refractive index, bioactive compounds and antioxidants activity of the extract. Thus, research on it need to be done to show an alternative extraction method besides to shows the antioxidants activity in *P. amboinicus.*

## 1.3 Objectives of The Study

This study embarks on the following objectives:

1. To optimize the yield of essential oil extract from *P. amboinicus* by using microwave-assisted extraction.
2. To analyze the properties of *P. amboinicus* in terms of its total phenolic content, refractive index, bioactive compounds and antioxidants activity.

## 1.4 Scope of the study

1. Optimization of essential oil yield extracted from *P. amboinicus* using ethanol at different operation condition of irradiation time (2-10min), solvent concentration (30-80%) and solvent-to-solid (10-30 mL/g) using MAE by Response Surface Methodology (RSM).
2. Comparison of percentage yield extract using water at optimum condition. The optimum condition was obtained by optimization of *P. amboinicus* water extract at different operation condition of irradiation time (10-30 min), microwave power (400-700 W), and solvent-to-solid (10-30 mL/g) using MAE by RSM.
3. Determination of the essential oil characteristics in terms of the refractive index, bioactive compounds, total phenolic content and antioxidants activity of *P. amboinicus* extract from the optimized condition.

## 1.5 Significance of the study

The significance of this study is to provide an alternative to conventional extraction method by identifying the optimum condition of extraction using MAE. The optimum extraction condition of *P. amboinicus* extract could be one step to produce a high yield product with less extraction time and less solvent used. Besides, the characteristic of *P. amboinicus* extract such as its refractive index, bioactive compound and antioxidants activity will be analysed from the optimum condition obtain. Hence, with the information at hand, the study of using MAE could be used for the future reference in extraction method as well as for the commercial production of *P. amboinicus* extract.

# REFERENCES


Abdul Rahim, M. S. A., Salihon, J., Mohd Yusoff, M., & Martua Damanik, M. R. (2013). Antioxidative Activity and Phenols Content in Five Tropical Lamiaceae Plants. In *Journal Of Tropical Resources and Sustainable Science* (Vol. 1, pp. 49–54).

Adom, K. K., Mark E., S., & Rui Hai, L. (2005). Phytochemicals and Antioxidant Activity of Milled Fractions of Different Wheat Varieties. https://doi.org/10.1021/JF048456D

Afoakwah, A.N., ; Owusu, J.; Adomako, C. ; Teye, E. (2012). Microwave Assisted Extraction (MAE) of Antioxidant. *Global Journal of Bio-Science and Biotechnology*, *1*(2), 132–140.

Ahirwar, P., Tembhre, M., Singh, R., Ahmad Sheikh, M., & Akram, M. A. (2013). Screening of Ethanolic Leaf Extract of *Coleus amboinicus* for its Phytochemical composition, Antioxidant Property, Total Phenolic Content and Flavonoid Contents. *International Journal of Innovative Pharmaceutical Sciences and Research*, *4*(2), 143–146.

Alara, O. R., Abdurahman, N. H., & Olalere, O. A. (2018). Optimization of microwave-assisted extraction of flavonoids and antioxidants from *Vernonia amygdalina* leaf using response surface methodology. *Food and Bioproducts Processing*, *107*, 36–48.

Ali Khan Khattak, M. M., Taher, M., Rizal, D., Abdulrahman, S., Abu Bakar, I., & Yahaya, A. (2013). Torbangun (*Coleus amboinicus* Lour) Extracts Affect Microbial and Fungus Activities. *Journal of Nutrional Therapeutics*, *2*(January), 141–148.

Alupului, A., Călinescu, I., & Lavric, V. (2012). Microwave Extraction of active principles from medicinal plants. *UPB Scientific Bulletin, Series B: Chemistry and Materials Science*, *74*(2), 129–142.

Arjunan, N., Murugan, K., Madhiyazhagan, P., & Barnard, D. R. (2012). Mosquitocidal and water purification properties of Cynodon dactylon , Aloe vera , H*emidesmus indicus* and C*oleus amboinicus* leaf extracts against the mosquito vectors, 1435–1443.

Arumugam, G., Swamy, M. K., & Sinniah, U. R. (2016). *Plectranthus amboinicus* (Lour.) Spreng: Botanical, Phytochemical, Pharmacological and Nutritional Significance. *Molecules*, *21*(4).

Asha.D, A., Mathew, L., & K.S, R. (2015). Evaluation of HPTLC Fingerprints of Flavonoids and Antioxidant Activity of Selected Medicinal Plants of Lamiaceae Family. *International Journal of Pharmacognosy and Phytochemical Research*, *7*(2), 240–245.

Asiimwe, S., Karlsson, A. B., Azeem, M., Mugisha, K. M., Namutebi, A., & Gakunga, N. J. (2014). Chemical composition and Toxicological evaluation of the aqueous leaf extracts of *Plectranthus amboinicus* Lour . Spreng. *International Journal of Pharmaceutical Science Invention*, *3*(2), 19–27.

Baghdikian, B., Filly, A., Fabiano-Tixier, A. S., Petitcolas, E., Mabrouki, F., Chemat, F., & Ollivier, É. (2016). Extraction by solvent using microwave and ultrasound-assisted techniques followed by HPLC analysis of Harpagoside from *Harpagophytum procumbens* and comparison with conventional solvent extraction methods. *Comptes Rendus Chimie*, *19*(6), 692–698.

Bai, X., Qiu, A., & Guan, J. (2007). Optimization of Microwave-Assisted Extraction of Antihepatotoxic Triterpenoid from *Actinidia deliciosa* Root and Its Comparison with Conventional Extraction Methods. *Food Technology Biotechnology*, *45*(2), 174–180.

Bhatt, P., Joseph, G. S., Negi, P. S., & Varadaraj, M. C. (2013). Chemical composition and nutraceutical potential of Indian borage (*Plectranthus amboinicus*) stem extract. *Journal of Chemistry*, *2013*.

Bhatt, P., & Negi, P. S. (2012). Antioxidant and Antibacterial Activities in the Leaf Extracts of Indian Borage (*Plectranthus amboinicus*). *Food and Nutrition Sciences*, *2012*(February), 146–152.

Bodner, C. C., & Gereau, R. E. (1988). A contribution to Bontoc ethnobotany. *Economic Botany*, *42*(3), 307–369.

Bos, R., Hendriks, H., & van Os, F. H. L. (1983). The composition of the essential oil in the leaves of *Coleus aromaticus* Bentham and their importance as a component of the Species antiaphthosae Ph. Ned. Ed. V. *Pharmaceutisch Weekblad Scientific Edition*, *5*(4), 129–130.

Brachet, A., Christen, P., & Veuthey, J. L. (2002). Focused microwave-assisted extraction of cocaine and benzoylecgonine from coca leaves. *Phytochemical Analysis*, *13*(3), 162–169.

Brown, D., (1997). Grenada: Isle of Spices. Herbs, 22, 6–7.

Cano, J. H., & Volpato, G. (2004). Herbal mixtures in the traditional medicine of Eastern Cuba. *Journal of Ethnopharmacology*, *90*(2–3), 293–316.

Carbajal, D., Casaco, A., Arruzazabala, L., Gonzalez, R., & Fuentes, V. (1991). Pharmacological screening of plant decoctions commonly used in Cuban folk medicine. *Journal of Ethnopharmacology*, *33*(1–2), 21–24.

Castillo, R. A. M., & González, V. P. (1999). Plecthranthus amboinicus (Lour.) Spreng. *Revista Cubana de Plantas Medicinales*, *4*(3), 110–115. Retrieved from http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S1028-47961999000300006

Chan, C. H., Yusoff, R., Ngoh, G. C., & Kung, F. W. L. (2011). Microwave-assisted extractions of active ingredients from plants. *Journal of Chromatography A*, *1218*(37), 6213–6225.

Chemat, F., & Cravotto, G. (2013). Microwave-assisted Extraction for Bioactive Compounds. https://doi.org/10.1007/978-1-4614-4830-3

Craig & Mayenda, (1990). *P. amboinicus* (Lour.) Spreng. Herbarium specimen held at K. collected from the Pacific (Craig & Mayenda 25).

Cravotto, G., Boffa, L., Mantegna, S., Perego, P., Avogadro, M., & Cintas, P. (2008). Improved extraction of vegetable oils under high-intensity ultrasound and/or microwaves. *Ultrasonics Sonochemistry*, *15*(5), 898–902.

Dahmoune, F., Nayak, B., Moussi, K., Remini, H., & Madani, K. (2015). Optimization of microwave-assisted extraction of polyphenols from *Myrtus communis* L . leaves. *Food chemistry*, *166*, 585–595.

Dahmoune, F., Spigno, G., Moussi, K., Remini, H., Cherbal, A., & Madani, K. (2014a). *Pistacia lentiscus* leaves as a source of phenolic compounds : Microwave-assisted extraction optimized and compared with ultrasound-assisted and conventional solvent extraction. *Industrial Crops & Products*, *61*, 31–40.

Dean, J. R., & Xiong, G. (2000). Extraction of organic pollutants from environmental matrices: Selection of extraction technique. *TrAC - Trends in Analytical Chemistry*, *19*(9), 553–564.

De Padua, L. S. (1988). *Medicinal Plants*. (S. J. Banta & B. S. Vergara, Eds.). Island Publishing House, Inc, Manila, Philippines.

Dhruv Patel, R., Mahobia, N. K., Singh, M. P., Singh, A., A. Sheikh, N. W., Alam, G., & K. Singh, S. (2010). Antioxidant Potential of Leaves of *Plectranthus amboinicus* (Lour) Spreng. *Der Pharmacia Lettre*, *2*(4), 240–245.

Dymock, W. (1985). *The Vegetable Materia Medica of Western India* (2nd ed.). Education Society Press, Byculla, London.

El-Ahmady, S. H. (2014). Histochemical Application for the Identification of Thymol and Carvacrol Chemotypes of Various Essential Oil Producing Plants. *Journal of Essential Oil-Bearing Plants*, *17*(5), 880–885.

El-Hawary, S. S., El-Sofany, R. H., Abdel-Monem, A. R., Ashour, R. S., & Sleem, A. A. (2013). Seasonal variation in the composition of Plectranthus amboinicus ( Lour . ) Spreng essential oil and its biological activities. *American Journal of Essential Oils and Natural Products AJEONP*, *1*(2), 11–18. Retrieved from https://www.researchgate.net/profile/Rehab_Ashour/publication/310753721_Seasonal_variation_in_the_composition_of_Plectranthus_amboinicus_Lour_Spreng_essential_oil_and_its_biological_activities/links/5835dfce08aee4a98e80e1a7.pdf

Epling, (1981). *P. amboinicus* (Lour.) Spreng. Herbarium specimen held at K collected from the Pacific (*Epling* 18080).

Eskilsson, C. S., Björklund, E., Mathiasson, L., Karlsson, L., & Torstensson, A. (1999). Microwave-assisted extraction of felodipine tablets. *Journal of Chromatography A*, *840*(1), 59–70.

Fang, Y.-Z., Yang, S., & Wu, G. (2002). Free radicals, antioxidants, and nutrition. *Nutrition*, *18*(10), 872–879.

Farhat, A., Benmoussa, H., Bachoual, R., Nasfi, Z., Elfalleh, W., Romdhane, M., & Bouajila, J. (2017). Efficiency of the optimized microwave assisted extractions on the yield, chemical composition and biological activities of Tunisian Rosmarinus officinalis L. essential oil. *Food and Bioproducts Processing*, *105*, 224–233.

Ferhat, M. A., Tigrine-Kordjani, N., Chemat, S., Meklati, B. Y., & Chemat, F. (2007). Rapid Extraction of Volatile Compounds Using a New Simultaneous Microwave Distillation: Solvent Extraction Device. *Chromatographia*, *65*(3–4), 217–222.

Frame, A. D., Ríos-Olivares, E., De Jesús, L., Ortiz, D., Pagán, J., & Méndez, S. (1998). Plants from Puerto Rico with anti-Mycobacterium tuberculosis properties. *Puerto Rico Health Sciences Journal*, *17*(3), 243–252. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9883470

França, F., Lago, E. L., & Marsden, P. D. (1996). Plants used in the treatment of leishmanial ulcers due to Leishmania (Viannia) braziliensis in an endemic area of Bahia, Brazil. *Revista Da Sociedade Brasileira de Medicina Tropical*, *29*(3), 229–232.

Gallo, M., Ferracane, R., Graziani, G., Ritieni, A., & Fogliano, V. (2010). Microwave assisted extraction of phenolic compounds from four different spices. *Molecules*, *15*(9), 6365–6374.

Gao, M., Song, B.-Z., & Liu, C.-Z. (2006). Dynamic microwave-assisted extraction of flavonoids from Saussurea medusa Maxim cultured cells. *Biochemical Engineering Journal*, *32*(2), 79–83.

Garofulić, I. E., Dragović-uzelac, V., Jambrak, A. R., & Jukić, M. (2013). The Effect of Microwave Assisted Extraction on the Isolation of Anthocyanins and. *Journal of Food Engineering*, *117*, 437–442.

Githinji, C.W., (1990). Ethnobotanical and chemotaxonomic study of some Kenyan medicinal Labiate species. M.Sc. Thesis, University of Nairobi, Kenya, Africa.

Githinji, C. W., & Kokwaro, J. O. (1993). Ethnomedicinal study of major species in the family Labiatae from Kenya. *Journal of Ethnopharmacology*, *39*(3), 197–203.

Govindaraju, S., Indra Arulselvi, P., & Karthik, C. (2016). Evaluation of chemical composition and larvicidal activity of *Coleus aromaticus* essential oil, its major compound carvacrol against *Aedes aegypti, Culex quinquefasciatus and Anopheles stephensi* (Diptera: Culicidae). *International Journal of Mosquito Research IJMR*, *3*(33), 611.

Gurib-Fakim, A., Sewraj, M. D., Narod, F., & Menut, C. (1995). Aromatic Plants of Mauritius: Volatile Constituents of the Essential Oils of *Coleus aromaticus* Benth., *Triphasia trifolia* (Burm.f.) and *Eucalyptus kirtoniana* F. Muell. *Journal of Essential Oil Research*, *7*(2), 215–218.

Hadkar, U. ., Dhruv, N., Malode, Y., & Chavan, B. (2013). Microwave Assisted Extraction of Phytoconstituents. *Asian Journal of Phytomedicine and Clinical Research*, *2*(3), 73–86.

Harsha, V. H., Hebbar, S. S., Hegde, G. R., & Shripathi, V. (2002). Ethnomedical knowledge of plants used by Kunabi Tribe of Karnataka in India. *Fitoterapia*, *73*(4), 281–287.

Harsha, V. H., Hebbar, S. S., Shripathi, V., & Hegde, G. R. (2003). Ethnomedicobotany of Uttara Kannada District in Karnataka, India—plants in treatment of skin diseases. *Journal of Ethnopharmacology*, *84*(1), 37–40. https://doi.org/http://dx.doi.org/10.1016/S0378-8741(02)00261-1

Hattori, M., Nakabayashi, T., Lim, Y. A., Miyashiro, H., Kurokawa, M., Shiraki, K., Pilapitiya, U. (1995). Inhibitory effects of various ayurvedic and Panamanian medicinal plants on the infection of herpes simplex virus-1in vitro andin vivo. *Phytotherapy Research*, *9*(4), 270–276.

Hopkins, W. G., & Hüner, N. P. A. (2008). *Introduction to plant physiology* (Vol. 7). Wiley. Retrieved from https://books.google.com/books?id=Nz9FAQAAIAAJ&pgis=1

Hussein, K., Ahmed, A. H., & Algabali, S. (2017). Antibacterial / radical scavenging activities , content , chemotaxonomy and chemical components of volatile oils of two *Plectranthus amboinicus* (Lour.) Spreng. (Lamiaceae), grown in Yemen. *American Journal of Essential Oils and Natural Products 2017*, *5(2)*(November), 12–18.

Jain, S.K.; Lata, S. (1996) Amazonian uses of some plants growing in India. Indig. Knowl. Dev. Monit., 4, 21–23.

Jain Tripti, V Jain, R Pandey, A Vyas, SS Shukla (2009). Microwave assisted extraction for phytoconstituents – An overview. Asian J. Research Chem. 2(1): Jan.-March, 19-25.

Jin, Q., Liang, F., Zhang, H., Zhao, L., Huan, Y., & Daqian Song. (1999). Application of microwave techniques in analytical chemistry. *TrAC Trends in Analytical Chemistry*, *18*(7), 479–484.

Khanum, H., Ramalakshmi, K., Srinivas, P., & Borse, B. B. (2011). Synergistic Antioxidant Action of Oregano, Ajowan and Borage Extracts. *Food and Nutrition Sciences*, *2*(5), 387–392.

Kortepeter, M. G., & Parker, G. W. (1999). Potential biological weapons threats. *Emerging Infectious Diseases*, *5*(4), 523–527.

Krishnamurthy, P., & Wadhwani, A. (2012). *Antioxidant Enzyme. Antioxidant Enzyme*. https://doi.org/10.5772/2895

Kuok Loong, N. G., Wahida, P. F., & Chong, C. H. (2014). Optimisation of extraction of thymol from *Plectranthus amboinicus* leaves using response surface methodology. *Journal of Engineering Science and Technology*, *9*(Spec. Issue on eureca 2013), 79–88.

Kusuma, H. S., & Mahfud, M. (2015). Box-Behnken design for investigation of microwave-assisted extraction of patchouli oil. *AIP Conference Proceedings*, *1699*(January 2016).

Lasure, A., Van Poel, B., De Clerck, L. S., Bridts, C. H., Stevens, W. J., Rwangabo, P. C., Vlietinck, A. J. (1995). Screening of Rwandese plant extracts for their influence on lymphocyte proliferation. *Phytomedicine*, *1*(4), 303–307.

Letellier, M., & Budzinski, H. (1999). Microwave assisted extraction of organic compounds. *Analusis*, *27*(3), 259–270.

Letellier, M., Budzinski, H., Charrier, L., Capes, S., & Dorthe, A. M. (1999). Optimization by factorial design of focused microwave assisted extraction of polycyclic aromatic hydrocarbons from marine sediment. *Fresenius' Journal of Analytical Chemistry*, *364*(3), 228–237.

Li, M., Ngadi, M. O., & Ma, Y. (2014). Optimisation of pulsed ultrasonic and microwave-assisted extraction for curcuminoids by response surface methodology and kinetic study. *Food chemistry*, *165*, 29–34.

Lima, M. a a, Oliveira, F. F. M. De, Gomes, G. a, Patrícia, L. L., Santiago, G. M. P., Nagao-dias, A. T., Carvalho, M. G. De. (2011). Evaluation of larvicidal activity of the essential oils of plants species from Brazil against *Aedes aegypti* ( Diptera : Culicidae ). *African Journal of Biotechnology*, *10*(SEPTEMBER), 11716–11720.

Lopes, P. Q., Carneiro, F. B., de Sousa, A. L. B., Santos, S. G., Oliveira, E. E., & Soares, L. A. L. (2017). Technological evaluation of emulsions containing the volatile oil from leaves of *Plectranthus amboinicus* Lour. *Pharmacognosy Magazine*, *13*(49), 159–167.

Lu, J., Zhou, C., Rong, O., Xu, Y., Zhou, B., & Li, Z. (2013). Optimization of Microwave-assisted Extraction of Flavonoids from Cryptotaenia japonica Hassk using Response Surface Methodology. *Advance Journal of Food Science and Technologgy*, *5*(3), 310–317.

Lucchesi, M. E., Chemat, F., & Smadja, J. (2004). Solvent-Free Microwave Extraction: An Innovative Tool for Rapid Extraction of Essential Oil from Aromatic Herbs and Spices. *Journal of Microwave Power & Electromagnetic Energy*, *39*, 1–31.

Lukhoba, C., Gardens, R. B., Lukhoba, C. W., Simmonds, M. S. J., & Paton, A. J. (2006). Plectranthus : A review of ethnobotanical uses *Plectranthus* : A review of ethnobotanical uses. *Journal of Ethnopharmacology*, (October 2016).

Luque-García, J. L., & Luque De Castro, M. D. (2003). Where is microwave-based analytical equipment for solid sample pre-treatment going? *TrAC - Trends in Analytical Chemistry*, *22*(2), 90–98.

Malaczewska, J., Wójcik, R., Jung, L., & Siwicki, A. (2010). Effect of Biolex β -HP on selected parameters of specific and non-specific humoral and cellular immunity in rats. *Bulletin of the Veterinary Institute in Pulawy*, *54*, 75–80.

Mandal, V., Mohan, Y., & Hemalatha, S. (2007). Microwave assisted extraction - An innovative and promising extraction tool for medicinal plant research. *Pharmacognosy Review*, *1*(1), 7–18.

Manjamalai, A., Alexander, T., & Berlin Grace, V. M. (2012a). Bioactive evaluation of the essential oil of plectranthus amboinicus by GC-MS analysis and its role as a drug for microbial infections and inflammation. *International Journal of Pharmacy and Pharmaceutical Sciences*, *4*(3), 205–211.

Manjamalai, A., Alexander, T., & Berlin Grace, V. M. (2012b). Bioactive evaluation of the essential oil of *Plectranthus amboinicus* by GC-MS analysis and its role as a drug for microbial infections and inflammation. *International Journal of Pharmacy and Pharmaceutical Sciences*, *4*(SUPPL.3), 205–211.

Manjamalai, A., & Berlin Grace, V. M. (2012). Volatile constituents and antioxidant property of essential oil from *Plectranthus amboinicus* (Lour). *International Journal of Pharma and Bio Sciences*, *3*(4), 445–458.

Megha Rani N, Rao SN, P. S. (2013). Evaluation of analgesic activity of aqueous extracts of *Coleus amboinicus* leaves in wistar albino rats. *International Journal of Universal*, *2*(December), 535–547.

Megha Rani N, S. K. and S. N. R. (2016). Preliminary phytochemical analysis of fresh juice and aqueous extract of *Coleus amboinicus* linn leaves. *International Journal of Applied Biology and Pharmaceutical Technology*, *7*(1), 216–221.

Mello, P. A., Barin, J. S., & Guarnieri, R. A. (2014). Microwave Heating. In *Microwave-Assisted Sample Preparation for Trace Element Analysis* (pp. 59–75).

Meyerhoff, (1978b). *P. barbatus* Andr. Herbarium specimen held at K collected from Kenya (*Meyerhoff* 28M).

Meyerhoff, (1978c). *P. amboinicus* (Lour.) Spreng. Herbarium specimen held at K collected from Kenya (*Meyerhoff* 94M)

Morton, J. F. (1992). Country Borage ( *Coleus amboinicus* Lour.): *Journal of Herbs, Spices & Medicinal Plants*, *1*(1–2), 77–90.

Odalo, J. O., Omolo, M. O., Malebo, H., Angira, J., Njeru, P. M., Ndiege, I. O., & Hassanali, A. (2005). Repellency of essential oils of some plants from the Kenyan coast against *Anopheles gambiae*. *Acta Tropica*, *95*(3), 210–218.

Ong, H. C., & Nordiana, M. (1999). Malay ethno-medico botany in Machang, Kelantan, Malaysia. *Fitoterapia*, *70*(5), 502–513.

Pan, X., Niu, G., & Liu, H. (2003). Microwave-assisted extraction of tea polyphenols and tea caffeine from green tea lea v es. *Chemical Engineering and Processing*, *42*, 129–133.

Pino, J. A., Garcia, J., & Martinez, M. A. (1996). Comparative Chemical Composition of the Volatiles of *Coleus aromaticus* Produced by Steam Distillation, Solvent Extraction and Supercritical Carbon Dioxide Extraction. *Journal of Essential Oil Research*, *8*(4), 373–375.

Prajapati, N. Das. (2003). *A handbook of medicinal plants : a complete source book*. Agrobios (India).

Prasenjit, B., Hullatti, K. K., & M. L, V. K. (2011). Anthelmintic and Antioxidant Activity of Alcoholic Extracts of Different Parts of *Coleus Amboinicus* Lour. *International Journal*, *2*(1), 181–185.

Pritima, R. A., & R. Selvaraj, P. (2007). Antimicrobial activity of *Coleus aromaticus* (benth) against microbes of reproductive tract infections among women. *Afr. J. Infect. Diseases*, *1*(1), 18–24.

Prudent, D., Perineau, F., Bessiere, J. M., Michel, G. M., & Baccou, J. C. (1995). Analysis of the Essential Oil of Wild Oregano from Martinique ( *Coleus aromaticus* Benth.)—Evaluation of Its Bacteriostatic and Fungistatic Properties. *Journal of Essential Oil Research*, *7*(2), 165–173.

Purnomo, H., Setiawan, C., & Kusnadi, J. (2014). Application of Microwave-Assisted Extraction on Teak ( *Tectona grandis* ) Leaves Antioxidant Extraction. *International Journal of Pharmaceutical, Biological and Chemical Sciences*, *4*(3), 1012–1018.

Rao, M. R., Reddy, I. B., Ramana, T., Gopal, S. V. R., Raman, B. V, Pallavi, D. S., & Hyma, D. (2007). Radical scavenging and antioxidant activity of ethylacetate fraction of *Plectranthus aromaticus* leaves. *Bioresources Biotechnology Research Asia*, *4*(2), 581–588.

Rasineni, G. K., Siddavattam, D., & Reddy, A. R. (2008). Free radical quenching activity and polyphenols in three species of Coleus. *Journal of Medicinal Plants Research*, *2*(10), 285–291. Retrieved from http://www.academicjournals.org/JMPR

Roberts, M. (Margaret J., & Roberts, S. (1990). Indigenous healing plants. Southern Book Publisher.

Ruiz, A. R., De La Torre, R. A., Alonso, N., Villaescusa, A., Betancourt, J., & Vizoso, A. (1996). Screening of medicinal plants for induction of somatic segregation activity in *Aspergillus nidulans*. *Journal of Ethnopharmacology*, *52*(3), 123–127.

Sabrina, E., Razali, Mirfat, & Shukri, M. (2014). Antimicrobial Activity and Bioactive Evaluation of *Plectranthus amboinicus* Essential Oil. *American Journal of Research Communication*, *2*(12), 121–127.

Sadeghi, A., Hakimzadeh, V., & Karimifar, B. (2017). Microwave Assisted Extraction of Bioactive Compounds from Food : A Review. *International Journal of Food Science and Nutrition Engineering*, *7*(1), 19–27.

Santos, F. A. V, Serra, C. G., Bezerra, R. J. A. C., Figueredo, F. G., Matias, F. F., Menezes, I. R. A., … Coutinho, H. D. M. (2016). Antibacterial activity of *Plectranthus amboinicus* Lour ( Lamiaceae ) essential oil against Streptococcus mutans. *European Journal of Integrative Medicine*, *8*, 293–297.

Santos, F. a V, Serra, C. G., Bezerra, R. J. a C., Figueredo, F. G., Edinardo, Matias, F. F., … Coutinho, H. D. M. (2016). Antibacterial activity of *Plectranthus amboinicus* Lour (Lamiaceae) essential oil against Streptococcus mutans. *European Journal of Integrative Medicine*, *8*(3), 293–297.

Schaneberg, B. T., & Khan, I. A. (2002). Comparison of Extraction Methods for Marker Compounds in the Essential Oil of Lemon Grass by GC. *Journal of Agricultural and Food Chemistry*, *50*(6), 1345–1349.

Senthilkumar, A., & Venkatesalu, V. (2010). Chemical composition and larvicidal activity of the essential oil of *Plectranthus amboinicus* (Lour.) Spreng against Anopheles stephensi: A malarial vector mosquito. *Parasitology Research*, *107*(5), 1275–1278.

Senthilkumar, A., & Venkatesalu, V. (2014). Chemical composition and larvicidal activity of the essential oil of *Plectranthus amboinicus* ( Lour .) Spreng against Anoph, (May). https://doi.org/10.1007/s00436-010-1996-6

Setyaningsih, W., Saputro, I. E., Palma, M., & Barroso, C. G. (2015). Optimisation and validation of the microwave-assisted extraction of phenolic compounds from rice grains. *Food chemistry*, *169*, 141–149.

Silitonga, M., Ilyas, S., Hutahaean, S., & Sipahutar, H. (2014). Levels of Apigenin and Immunostimulatory Activity of Leaf Extracts of Bangun-bangun (*Plectranthus Amboinicus* Lour). *International Journal of Biology*, *7*(1).

Spigno, G., & De Faveri, D. M. (2009). Microwave-assisted extraction of tea phenols: A phenomenological study. *Journal of Food Engineering*, *93*(2), 210–217.

Stefanidis, G. D., Muñoz, A. N., Sturm, G. S. J., & Stankiewicz, A. (2014). A helicopter view of microwave application to chemical processes: Reactions, separations, and equipment concepts. *Reviews in Chemical Engineering*, *30*(3), 233–259.

Swamy, M. K., Arumugam, G., Kaur, R., Ghasemzadeh, A., Yusoff, M. M., & Sinniah, U. R. (2017). GC-MS Based Metabolite Profiling, Antioxidant and Antimicrobial Properties of Different Solvent Extracts of Malaysian Plectranthus amboinicus Leaves. *Evidence-Based Complementary and Alternative Medicine*, *2017*.

Teng, H., & Lee, W. Y. (2013). Optimization of microwave-assisted extraction of polyphenols from mulberry fruits (Morus alba L.) using response surface methodology. *Journal of the Korean Society for Applied Biological Chemistry*, *56*(3), 317–324.

Vijayakumar, S., Vinoj, G., Malaikozhundan, B., Shanthi, S., & Vaseeharan, B. (2015). *Plectranthus amboinicus* leaf extract mediated synthesis of zinc oxide nanoparticles and its control of methicillin resistant *Staphylococcus aureus* biofilm and blood sucking mosquito larvae. *Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy*, *137*, 886–891.

Wadikar, D. D., & Premavalli, K. S. (2011). Appetizer administration stimulates food consumption, weight gain and leptin levels in male Wistar rats. *Appetite*, *57*(1), 131–133.

Wang, L., & Weller, C. L. (2006). Recent advances in extraction of nutraceuticals from plants. *Trends in Food Science and Technology*, *17*(6), 300–312.

Wong, C.-C., Li, H.-B., Cheng, K.-W., & Chen, F. (2006). A systematic survey of antioxidant activity of 30 Chinese medicinal plants using the ferric reducing antioxidant power assay. *Food Chemistry*, *97*(4), 705–711.

Xiong, W., Chen, X., Lv, G., Hu, D., Zhao, J., & Li, S. (2016). Optimization of microwave-assisted extraction of bioactive alkaloids from lotus plumule using response surface methodology. *Journal of Pharmaceutical Analysis*, *6*(6), 382–388.

Xu, J., Zhou, F., Ji, B.-P., Pei, R.-S., & Xu, N. (2008). The antibacterial mechanism of carvacrol and thymol against *Escherichia coli*. *Letters in Applied Microbiology*, *47*(3), 174–179.

Yang, L., Jiang, J. G., Li, W. F., Chen, J., Wang, D. Y., & Zhu, L. (2009). Optimum extraction process of polyphenols from the bark of Phyllanthus emblica L. based on the response surface methodology. *Journal of Separation Science*, *32*(9), 1437–1444.

Yuncker, (1953). *P. amboinicus* (Lour.) Spreng. Herbarium specimen held at BM colected from Tonga (*Yuncker* 15232).

Zheng, X., Wang, X., Lan, Y., Shi, J., Xue, S. J., & Liu, C. (2009). Application of response surface methodology to optimize microwave-assisted extraction of silymarin from milk thistle seeds. *Separation and Purification Technology*, *70*(1), 34–40.

Zhu, X., Su, Q., Cai, J., & Yang, J. (2006). Optimization of microwave-assisted solvent extraction for volatile organic acids in tobacco and its comparison with conventional extraction methods. *Analytica Chimica Acta*, *579*, 88–94.

Zlotorzynski, A. (1995). The Application of Microwave Radiation to Analytical and Environmental Chemistry. *Critical Reviews in Analytical Chemistry*, *25*(1), 43–76.