EFFECTIVE METHOD FOR THE PURIFICATION OF THE LIPID COMPONENTS IN CHILLI POWDER

I Abdul Wahab[1*], S A Ali Shah[1]

[1]*Faculty of Pharmacy, Universiti Teknologi MARA UiTM, 42300 Bandar Puncak Alam, Selangor Darul Ehsan*

[*]*Corresponding author: ibtisam@uitm.edu.my*

Abstract

Local chilli pepper (*Capsicum*) plants contain rich resources of ingredients which can be used in the development and synthesis of pharmaceutical products. Therefore, phytochemical analysis is required in order to extract and identify the important bioactive compounds, derived from the chilli, to be used as therapeutic elements for humans. In this study, the compositions of *Capsicum* species were investigated by using chromatographic and spectroscopic methods. Following the organic extraction, the sample was investigated via analytical thin layer chromatography. It is believed that this technique is effective in separating the extract for preliminary qualitative analysis. The nonpolar constituents were later isolated by using preparative scale. From the findings, lipid fraction could be identified. In summary, proton Nuclear Magnetic Resonance (NMR) spectroscopy could provide useful spectral data on the composition of the triacylglycerol of this spice.

Keyword: Analysis, *Capsicum*, Chemical, Lipid

Introduction

Chillies are widely consumed. The common names of chilli are capsicum, pepper, hot pepper, cayenne, red pepper, green pepper, paprika pepper, sweet pepper and bell pepper (Bhuvaneshwari et al., 2013). The chilli is categorized in the plant genus, *Capsicum*, belonging to the Solanaceae family. The scientific name of the pungent varieties of chillies is called *Capsicum fructescens*. This crop is one of the popular vegetables within housing areas (Aswad Khalid et al., 2015), which could be planted and enjoyed among local communities (Ismail et al., 2017). Meanwhile, the species of *Capsicum annum* encompasses a variety of shapes and sizes of peppers, including the mild, non-pungent and hot varieties, such as the bell peppers, sweet peppers and green peppers (Cordell & Araujo, 1993). A sustainable development plan for chilli is launched to improve its cultivation and competitiveness. The fertigation of chillies is locally practiced (MARDI, 2017). The chemical content or the secondary metabolites retaining the pungency of chilli peppers are the nitrogenous compounds called capsaicinoids (Ryu et al., 2017; Naves et al., 2019). The pungency varies in different varieties of chillies. They are the spices in cuisines, which can be used in both fresh and dried forms (Ng & Ab. Karim, 2016).

Capsicum is utilized for various therapeutic purposes such as asthma, coughs, sore throats, to relieve toothaches (Goci et al., 2013) and for treating scald (Ripen & Noweg, 2017). It is also used as a counter-irritant balm for external application. This is due to its alkaloidic content, in addition to their role as a food additive. These capsaicinoids are composed of vanillylamide and an acyl chain. They are classified by the acyl chain structure, into a capsaicin group, dihydrocapsaicin group and N-vanillyl-n-acrylamide group. Capsaicin is the main capsaicinoid in chilli peppers, followed by dihydrocapsaicin. Both compounds present 90% of the total pungency of pepper fruits. These two compounds are also more potent to the pungent taste and

insensitive nerves compared with the minor capsaicinoids: nordihydrocapsaicin, homo dihydrocapsaicin and homocapsaicin (**Figure 1**). The red pepper as well as capsaicin, has the ability to control metabolic syndrome and its related disorders, such as obesity, diabetes and its complication. However, more research needs to be performed to validate its advantages especially in human (Sanati et al., 2018).

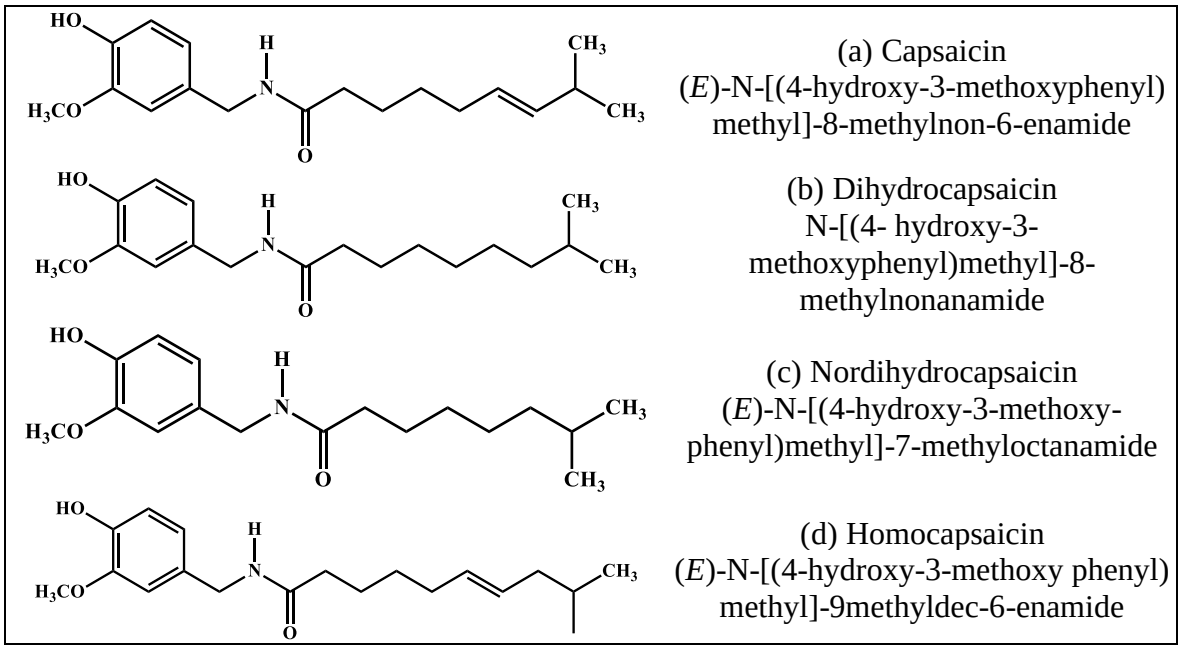


Figure 1 Chemical structures of a) capsaicin, b) dihydrocapsaicin, c) nordihydrocapsaicin d) homocapsaicin molecules (Antonio et al., 2018)

Chromatographic methods, in particular thin layer chromatography (TLC), high performance liquid chromatography (HPLC) (Hamada et al., 2019) and gas chromatography (GC) (Simonovska et al., 2019), are selected. They were used for the qualitative and quantitative determination of the chemical principles from the capsicum and pharmaceutical products, for example, the capsaicin gels. A minimum of 1 μg/mL capsaicinoids can be detected by using HPLC. This technique could be easily applied to drug manufacturing and quality control of chilli pepper products, such as kimchi and chilli pepper sauce (Ryu et al., 2017). Other chemicals of the chilli would include the phenolics, terpenoids, steroidal glycosides, carotenoid, fat, protein, vitamin C, vitamin A, volatile oils, minerals and fibres. On the other hand, ^{1}H Nuclear Magnetic Resonance (NMR) spectroscopy was employed to provide information about the composition and relative content of fatty acid residues in triacylglycerols (Simonovska et al., 2019). In this study, the chilli compositions were investigated by using chromatographic and spectroscopic applications.

Materials and Methods

General

Dried red chilli peppers were purchased from the local market. All chemical reagents were analytical grade solvents: ethanol, *n*-hexane, as well as, ethyl acetate, which were supplied from Merck (Germany). Pre-coated silica gel 60 F_{254} sheets and plates (Merck, Darmstadt, Germany) and TLC twin trough development chambers were used. Deuterated chloroform

($CDCl_3$, 99.8% D) with tetramethylsilane (TMS), was purchased from Deutero GmbH (Germany).

Extract preparation

The ethanol and *n*-hexane extracts were obtained from the maceration of the chilli peppers. 20 g of the capsicum powders were introduced in closed amber bottles with 50 ml of solvent. The plant materials were let to macerate for 48 hours in the room temperature. These samples were prepared for TLC identification.

Thin layer chromatography (TLC) method

Thin layer chromatography for qualitative separation of the main compounds in the extracts was performed on an aluminium sheet with 0.25 mm thick layer of silica gel 60. The samples (5 µL) were applied as spots. The sheet was developed up to 70 mm in a development chamber. After development with ethyl acetate and *n*-hexane (1:1, v/v) mixture as a mobile phase, the plates were air dried to visualize the components. Later, the ethanol extract was selected for preparative TLC.

Nuclear magnetic resonance (NMR) spectroscopy

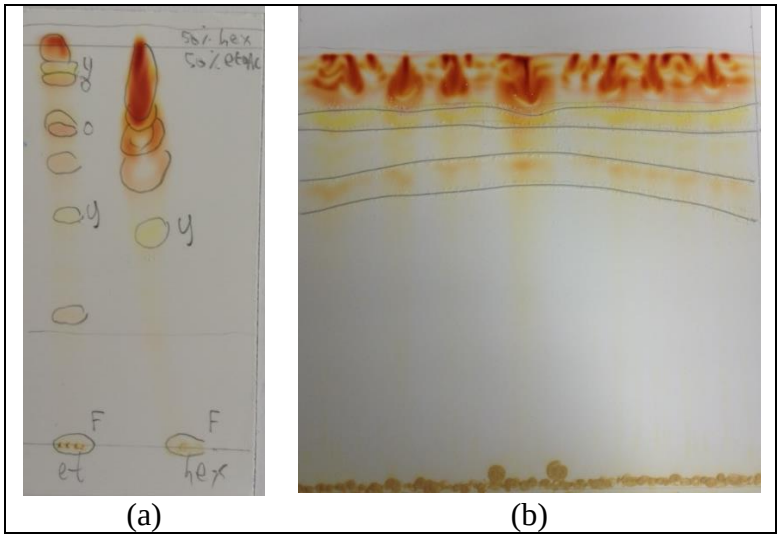
NMR spectra were recorded on a Bruker Ultrashield^{TM} 500 MHz spectrometer at ambient temperature. 10 mg of samples were dissolved in $CDCl_3$. The data were reported in ppm.

Results and Discussion

The best solvent extraction for the capsicum sample was ethanol (Goci et al., 2013). Thin layer chromatography (TLC) is a quick and effective method of separating the components of the capsicum extract for qualitative analysis (Ekwere Mercy, 2015). For the compound development, it was found that with the mobile phase; ethyl acetate and hexane (1:1, v/v), the compound separation occurred. This could be a good resolution for components with specific retardation factor (Rf) values. The TLC plates for analysis of the extracts are shown in **Figure 2**. These biomolecules are separated by their relative interactions with the stationary and mobile phase. For each TLC, the Rf values could be measured. Dominant spots and 3 major bands (Band 1-3) were observed at Rf 0.6 – 0.9.

Nevertheless, only one compound from Band 3, gave significant NMR data. From the ^{1}H NMR (500 MHz, $CDCl_3$) spectrum (**Figure 3** and **4**), NMR signals at δ_H 4.14 - 4.29 ppm with integral for a total of 4H ($2xCH_2$), could be assignable to the two αCH_2 groups of the glycerol molecule. The above signals can be used for calibration of the integral areas of the fatty acid signals in the ^{1}H NMR spectrum. In addition, the signal at δ_H 5.25 ppm for 1 H, could be originated from the βCH (Simonovska et al., 2019). Next, the chemical shifts at δ_H 5.35 ppm could correspond to the total amount of olefinic protons in the unsaturated fatty acids. Only polyunsaturated fatty acids would give signals at δ_H 2.77 ppm, corresponding to the chemical shifts of the methylene hydrogens between two double bonds.

The proton signals at δ_H 2.32 ppm could be due to $CO\text{-}CH_2$ groups, indicating for both saturated and unsaturated fatty acids. The same is valid for the signals of $CO\text{-}CH_2\text{-}CH_2$- groups of saturated and unsaturated fatty acids at δ_H 1.61 ppm. The ^{1}H NMR signals at δ_H 2.04 ppm are characteristic for the allylic methylene protons of all unsaturated fatty acids, including monoenoic and polyunsaturated. The proton signals in the region δ_H 1.2 - 1.4 ppm belong to saturated CH_2 chains. Meanwhile, the proton signals of the terminal methyl groups appear at δ_H 0.88 ppm (**Table 1**). The suggested structure for the fatty acids composition of this triacylglycerol is displayed in **Figure 5**.



(a) (b)

Figure 2 The (a) analytical separation of the ethanol and n-hexane extracts, and (b) the preparative scale for the ethanolic extract, showing 3 major bands, Rf = 0.6, 0.8 and 0.9, respectively

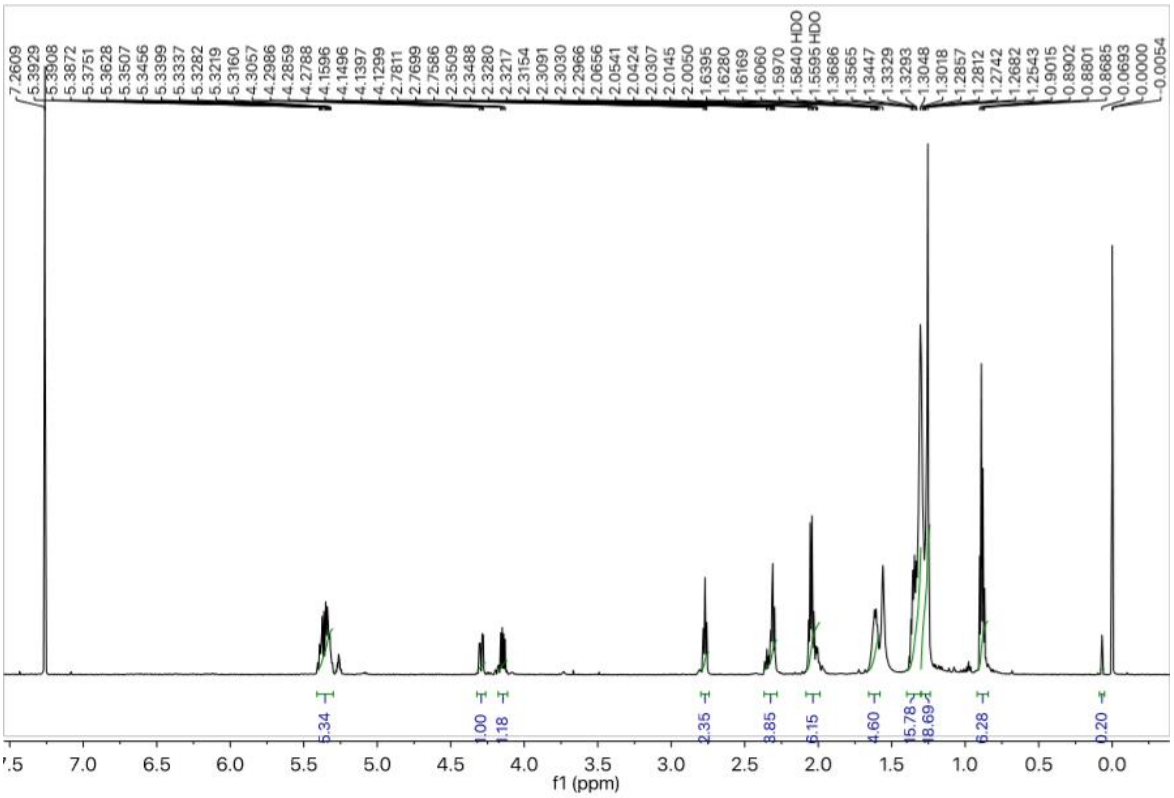


Figure 3 The ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of the compound from the ethanolic extract (Rf = 0.9)

Table 1 The ^{1}H-NMR signals and the functional groups of the extract's component.

δ_H, ppm	Multiplicity *	Integration	Functional groups
5.35	*m*	10 units of C=C	representing a total amount of olefinic protons (possibly 10 units of -CH=CH-), in the unsaturated fatty acids
5.25	*m*	1 H	βCH or -C**H**(OH)(CH_2)O-
4.29	*dd*, J = 9.90, 3.55 Hz	2 H	αCH_2 groups of the glycerol molecule, or -O(C**H**$_2$)$_2$CH(OH)
4.14	*dd*, J = 9.95, 5.00 Hz	2 H	αCH_2 groups of the glycerol molecule, or -O(C**H**$_2$)$_2$CH(OH)
2.77	*t*, J = 5.65 Hz	4 H	Methylene hydrogens (-C**H**$_2$), between two double bonds, only in unsaturated fatty acids
2.32	*m*	6 H	-C**H**$_2$COO- (3 units of CH_2) groups, indicating for both saturated and unsaturated fatty acids
2.04	*m*	12 H	-C**H**$_2$-CH=CH- (6 units of CH_2), the allylic methylene protons of unsaturated fatty acids
1.61	*m*	8 H	The signals of –C**H**$_2$$CH_2$COO- (4 units of CH_2) groups of saturated and unsaturated fatty acids
1.25 - 1.38	*m*	CH_2 units	The proton signals in the region belong to saturated –C**H**$_2$– chains
0.88	*t*, J = 5.65 Hz	12 CH_3	The proton signals of the terminal methyl groups, C**H**$_3$$CH_2$-

* dd = doublet of doublets, m = multiplet, t = triplet

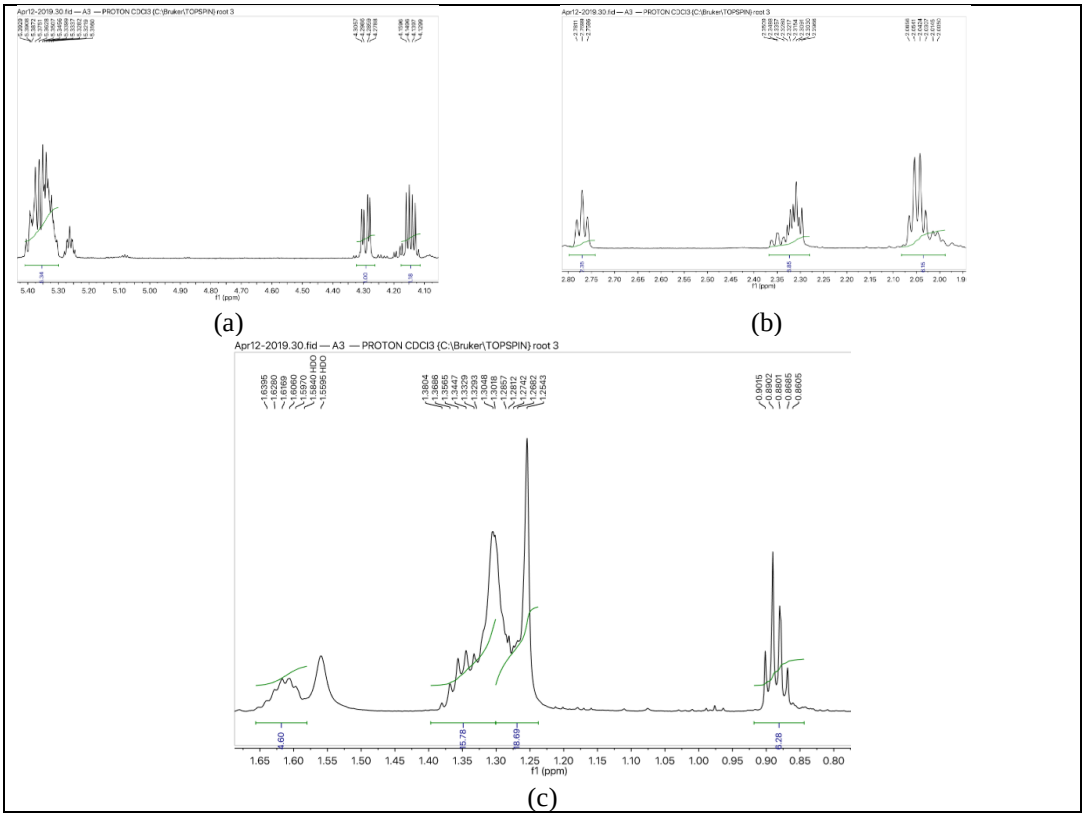


Figure 4 The ^{1}H NMR [500 MHz, $CDCl_3$, (a) δ_H = 4.1 – 5.4 ppm; (b) δ_H = 1.9 – 2.8 ppm; (c) δ_H = 0.8 – 1.7 ppm] spectrum of the nonpolar compound from the ethanol extract (Rf = 0.9)

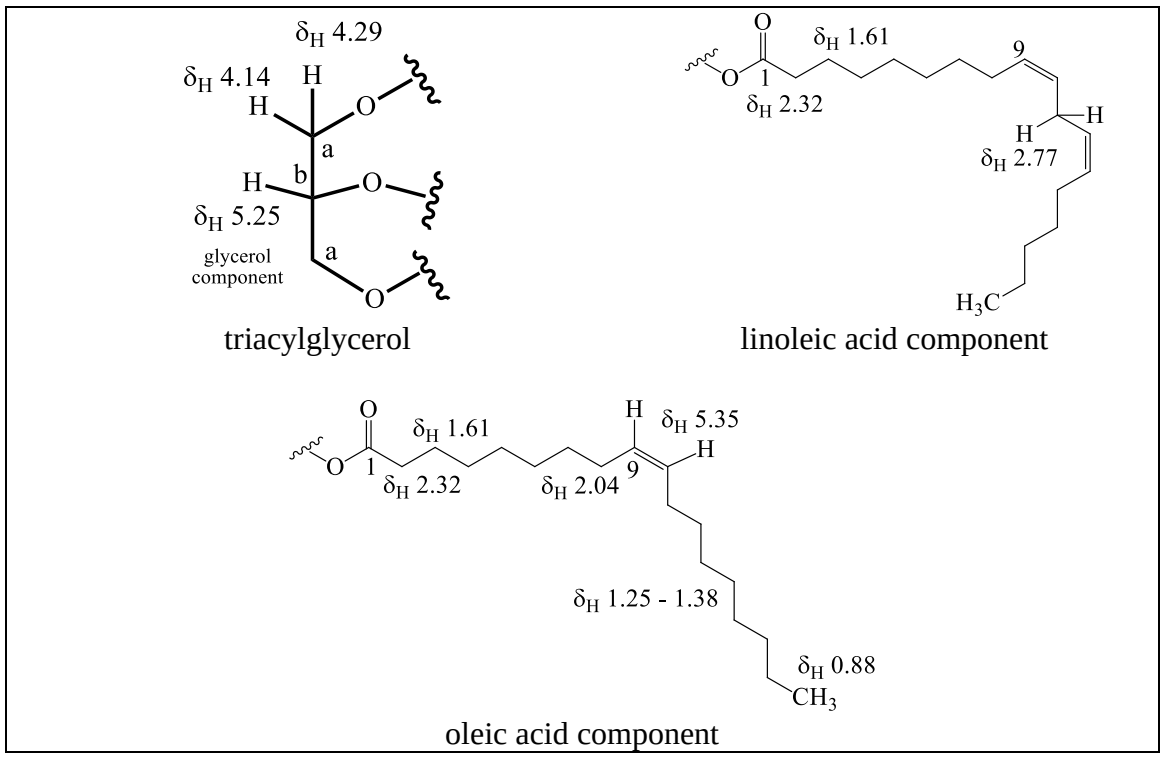


Figure 5 The suggested chemical structure of the triacylglycerol in the chilli extract

Conclusion

The result was obtained from the fruit. Further investigation could be organised in order to analyse the respective organs, including the pericarp, placenta, seed and the stalk, separately from the chilli. It is found that TLC method is adequate for preliminary analysis of the powdered sample. The preparative scale also serves as a purification step of the nonpolar component of this heritage spice. The methodology for the determination of the fatty acid composition through ^{1}H-NMR was concluded as a simple and rapid technique. Data on the compound's functionality and proportion were unwavered. However, advanced two-dimentional NMR could be utilised for a detailed stereochemistry (e.g. for δ_H = 5.25 ppm) of the molecule.

Acknowledgement

The authors wish to thank the Faculty of Pharmacy and Atta-ur-Rahman Institute for Natural Products Discovery (AuRIns), UiTM Puncak Alam, for the support.

Conflict of interests

The authors declare no conflict of interest.

References

Antonio, A. S., Wiedemann, L. S. M., & Veiga Junior, V. F. (2018). The genus *Capsicum*: a phytochemical review of bioactive secondary metabolites. *RSC Adv*., *8*, 25767 - 25784.

Aswad Khalid, M., K., Abd Rashid, M. S., & Zakaria, A. Z. (2015). In Search of Malay Landscape Design: Characteristic and Identification of Traditional Landscape at Sungai Perak. Proceedings of Postgraduate Conference on Global Green Issues, UiTM Perak, 7-8 October

2015, 58 – 65.

Bhuvaneshwari, S., Prasanna Karthik, S., & Kuruvilla, A. (2013). Estimation of Capsaicin Content of Two Varieties of Green Chilies by TLC Method. *International J. of Pharmaceutical & Biological Archives*, *4*(6), 1186 – 1188.

Cordell, G. A. & Araujo, O. E. (1993). Capsaicin: Identification, nomenclature and pharmacotherapy. *Annals of Pharmacotherapy*, *27*, 330-336.

Ekwere Mercy, R. (2015). Thin layer chromatographic analysis of capsaicin and moisture contents from three varieties of capsicum peppers. *J Chromatogr Sep Tech*, *6*(4), 68.

Goci, E., Haloçi, E. Vide, K., & Malaj, L. (2013). Application and Comparison of Three Different Extraction Methods of Capsaicin from *Capsicum* Fruits. *Albanian Journal of Pharmaceutical Sciences*, *1*(1), 16 – 19.

Hamada, N., Hashi, Y., Yamaki, S., Guo, Y., Zhang, L., Li, H., & Lin, J.-M. (2019). Construction of on-line supercritical fluid extraction with reverse phase liquid chromatography-tandem mass spectrometry for the determination of capsaicin. *Chinese Chemical Letters*, *30*, 99-102.

Ismail, A. A., Samsuddin, I., Zainonabidin, A. & Mohd Ali, H. (2017). A Review on the Benefits of Horticultural Therapy for Retirement Community in Malaysia. *Malaysian Journal of Sustainable Environment*, *2*(1), 1-18.

MARDI (Malaysian Agricultural Research and Development Institute) (2017). Accessed via https://blogmardi.wordpress.com/2017/02/02/sustainable-food-production-through-fertigation-system/ [1st July 2019].

Naves, E. R., de Ávila Silva, L., Sulpice, R., Araújo, W. L., Nunes-Nesi, A., Peres, L. E. P., Zsögön, A. (2019). Capsaicinoids: Pungency beyond Capsicum, *Trends in Plant Science*, *24*(2), 109-120.

Ng, C. Y. & Ab. Karim, S. (2016). Historical and contemporary perspectives of the Nyonya food culture in Malaysia. *J. of Ethnic Foods*, *3*(2), 93-106.

Ripen, J. E. & Noweg, G. T. (2017). Documenting Ethnobotanical Knowledge of Rural Community for Sustainable Benefits. *International Journal of Service Management and Sustainability (IJSMS)*, *2*(1), 1-16.

Ryu, W.–K., Kim, H.–W., Kim, G.-D., & Rhee H.-I. (2017). Rapid determination of capsaicinoids by colorimetric method. *Journal of Food and Drug Analysis*, 798 – 803.

Sanati, S., Razavi, B. M. & Hosseinzadeh, H. (2018). A review of the effects of *Capsicum annuum* L. and its constituent, capsaicin, in metabolic syndrome. *Iran J Basic Med Sci*., *21*, 439-448.

Simonovska, J. M., Yancheva, D. Y., Mikhova, B. P., Momchilova, S. M., Knez, Ž. F., Primožić, M. J., Kavrakovski, Z. S., & Rafajlovska, V. G. (2019). Characterization of extracts from red hot pepper (*Capsicum annuum* L.), *Bulgarian Chemical Communications*, *51*(1), 103 – 112.