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Effect of extraction time and temperature on the extraction of phenolic compounds from *Orthosiphon stamineus* leaves

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ABSTRACT

HCWE is gaining popularity since it is a capable technique for extracting different classes of compounds and bioactive (mainly phenolic compounds) from plant sources. In the present work, Misai Kucing leaves or *Orthosiphon stamineus* (*O. stamineus*) was employed for the extraction of phenolic compounds using HCWE. The effect of different temperature and time on the Total Phenolic Compounds (TPC) of O. stamineus was investigated in this study. The HCWE operating variables were temperatures (100,120,140, 160, 180 and 200ºC) and time (10, 20, 30 minutes) at a fixed sample to solvent ratio of 1:29 (w/v) and constant pressure of 1500 psi. TPC of water extract of *O. stamineus* leaves were measured using Folin- Ciocalteu test. Results indicated that 20 minutes of extraction time and temperature of 160ºC gave the highest TPC value of 97.49 mg gallic acid g^{-1} dry sample. Antioxidant activity is the highest at 120ºC (92.15%). By using ANOVA statistical analysis, temperature show a significant ($p<0.05$) impact on TPC while time does not show any significant ($p<0.05$) impact on TPC of *O. stamineus*. This study shows that temperature has more impact on TPC of *O. stamineus* while time does not influence the TPC obtained. There is no simple relationship between antioxidant activity and TPC of *O. stamineus*. HCWE using water is a green extraction method that was able to extract phenolic compounds from *O. stamineus* leaves at higher temperature and has possibilities of being used at industrial scale.

INTRODUCTION

At present, the treatment use to heal most of the chronic diseases depends on synthetic drugs, which have long-term side effect to our body. Thus there is an increasing awareness to use herbal products to cure disease with minimal or no side efffect. Herbal plants are of considerable importance due to their therapeutic phytochemicals that may contribute to the development of novel drugs. Phytochemicals, especially phenolic in herbs are major bioactive compounds known for health benefits (Venugopal & Liu, 2012). According to Jamia (2006), it has been estimated that the market for herbal and natural products in Malaysia is worth USD 1.4 million. The medicinal property of the herbs is mainly due to the presence of phenolic compounds that hold various biological effects, including antiseptic, anti-cancer, antiviral, anti-inflammatory, and antioxidant activity (Serrano *et al*., 2009; Quideau *et al*., 2011).

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Orthosiphon stamineus or cat whisker belongs to the family of Lamiacea and popularly known as Misai Kucing in Malaysia is an important herbal species that is usually grown in South East Asia region (Khamsah *et al*., 2006). Different parts of *O. stamineus* are used traditionally as a medicine to treat various diseases such as diabetes, hypertension and rheumatism (Ahamed *et al.,* 2010). The leaves of *O. stamineus* are commonly used for herbal tea, well known as "Java tea" (Jaganath *et al*., 2000). Phytochemical and pharmacological studies of *O. stamineus* have been carried out since 1930 (Tezuka *et al.,* 2000). Scientific studies have established that the leaves demonstrate antioxidant, antibacterial and other pharmacological properties. It has been reported that the leaves exhibit the highest antioxidant properties than the other parts of the plant due to its greater phenolic fractions (Tezuka *et al.,* 2000; Akowuah *et al.,* 2005). Twenty phenolic compounds were isolated from *O. stamineus* including Rosmarinic acid, Sinensetin and Eupatorin which are the major phenolic compounds in the leaves of this plant. Various commercial and healthcare products related to *O. stamineus* with antioxidant activity claims on it due to the abundance of bioactive compounds in this plant. Phenolic compounds of *O. stamineus* have been extracted using several conventional techniques such as soxhlet, reflux and maceration. Saidan *et al.* (2015) reported that the Total phenolic content obtained from *O. stamineus* extracted using soxhlet, reflux and maceration were 358.2, 246.2 and 296.7 mg/g GAE respectively. Conventional extraction techniques usually use organic solvent such as acetone, methanol and ethanol that can cause toxicity. Lately, there has been an increasing interest in the use of green technology that precludes toxicity associated to the solvents. Hot Compressed Water Extraction (HCWE) meets the requirement to be considered as green technology. To the best of our knowledge, the phenolic extraction from *O. stamineus* using HCWE has never been reported.

Hot Compressed Water Extraction (HCWE) is becoming popular due to its advantages over conventional extraction technique. Extraction plays an important role in the development of processed foods and nutraceutical food ingredients. Extraction is defined as a separation process that act as to separate a solute from one phase into another. Thermodynamically, HCWE specifically refers as an extraction technique using liquid water as a solvent at temperatures above the atmospheric boiling point of water (100^0C), but below the critical point of water (374^0C) (N.A Morad *et al*., 2011). HCWE provides an environmentally sustainable extraction method because it avoids the use of organic solvent, giving high quality extracts, high extraction yield and faster extraction procedures (Chemat *et al*, 2012). HCWE also has established its capability to extract different classes of compounds by manipulating the temperature being used, with more polar compounds extracted at lower temperatures while the less polar compounds were extracted at higher temperatures. HCWE has been successfully employed to extract antioxidant, phenolic compounds and functional compounds from natural matrices such as oregano (Rodríguez *et al.,* 2006), rosemary (Herrero *et al.,* 2010), grape pomace (Hiba *et al.,* 2014) and others.

Although the bioactive compounds in *O. stamineus* have been widely extracted using other extraction techniques, the use of HCWE in the extraction of phenolic compounds from *O. stamineus* is not investigated and reported. Thus, the goal of the present investigation was to study the effect of temperature and time on total phenolic content of *O. stamineus.*

Experimental:

Chemicals and reagents:

2,2'-diphenyl-1-picrylhydrazyl (DPPH), analytical grade sodium carbonate (Na_2CO_3) and Follin-ciocalteu's phenol reagent were purchased from Sigma-Aldrich (USA). Gallic acid standard was purchased from Friendemann Schmidt Chemical (WA) and the analytical grade absolute ethanol was purchased from Fisher Scientific Co. (UK).

Plant materials:

10 kg of matured and fresh *O. stamineus* plant were bought from the supplier, Hussain Herba Enterprise at Sungai Udang, Melaka, Malaysia.

Sample preparation:

The harvested *O. stamineus* plant was washed thoroughly under running tap water and the leaves were removed from the whole plants. The leaves then were dried in the laboratory oven (Model FDD 1000) at 45ºC until its moisture content was less than 10%. The moisture content was measured using a moisture meter (Model OHAUS MB25). The dried leaves were ground into powder, vacuum packed in plastic bags and stored at room temperature (25ºC) for further extraction.

Hot compressed water extraction:

To perform the extractions, an Accelerated Solvent Extraction System ASE 200 equipped with a solvent controller unit from Dionex Corporation (Sunnycale, CA, USA) was used. Extractions were performed at six temperatures (100,120,140,160,180 and 200ºC) and three static extraction times (10, 20, 30 min). An extraction cell heating-up step was applied for a given time, which changed according to extraction temperature (i.e., 5 min

when the extraction temperature was set at 100ºC, 9 min at 200ºC). All extractions were performed in 100ml extraction cell containing five gram of sample and 3g of diatomaceous earth. The extraction procedure was as follow: (i) sample was loaded into the cell, (ii) the loaded cell was auto-filled with solvent at a pressure of 1500 psi, (iii) initial heat-up time was applied, (iv) static extraction with all system valves closed was performed for 10, 20 or 30 min at the respective set temperature, (v) the cell was rinsed with 60% cell volume using extraction solvent, (vi) solvent was purged from the cell with N_2 gas and (vii) depressurization of the system took place. Between each extraction, a rinse of the complete system was made in order to overcome any extract carry-over. The collected extracts were kept protected from light, at -20ºC until further analysis.

Determination of Total Phenolic Compounds (TPC):

The Folin-Ciocalteu method for determination of TPC was adapted from Matshediso *et al.* (2015) with some modification. The plant extract was diluted with distilled water in the ratio of 1:20 and filtered with filter paper. 0.5 ml of the diluted plant extract was added to a test tube containing 2.5 ml Folin-Ciocalteu reagent (diluted with distilled water with a ratio of 1:10) and was left to stand at room temperature for 3 to 5 min. 2 ml of sodium carbonate (75g/L) was added into the test tube and the mixture was incubated at room temperature (25ºC) for 2 hours. The resulting blue complex indicating the presence of TPC was measured at 765 nm using a UV/VIS spectrophotometer (SP-3000nano, Optima). Gallic acid was used as the standard for total phenols. The analysis was done in triplicate.

Determination of free radical scavenging activity using DPPH assay:

The method for estimating free radical scavenging activity was adapted from A. Sharifi *et al.* (2013) with some modification. 3.5 ml of 0.1 mM ethanolic 2,2'-diphenyl-1-picrylhydrazyl (DPPH) was mixed with 0.5 ml plant extract (diluted 10:40 with ethanol). The mixture was allowed to stand for 30 min at room temperature (25^{O}C) after which its absorbance was measured at 517 nm using a spectrophotometer, against ethanol as blank. The free radical scavenging activity (FRSA) of the tested sample was evaluated by comparing its absorbance with the control. Each measurement was done in triplicate. FRSA was calculated using the following formula:

$$\text{FRSA} = \left[\frac{Ac - As}{Ac}\right] \times 100$$

where Ac = absorbance of control and A_S = absorbance of tested sample.

Data analysis:

Statistical analysis of the data was computed using Microsoft Excel 2007. One-way analysis of variance (ANOVA) was employed to measure statistical differences between extractions. The significant difference was considered at a value of $p < 0.05$.

RESULTS AND DISCUSSION

The effect of different temperatures at 100, 120, 140, 160, 180 and 200ºC and times at 10, 20 and 30 min in HCWE on TPC of *O. stamineus* were investigated in this study. Table 1 and Figure 1 show the results obtained for TPC (expressed as mg gallic acid/ g dry sample) for all the temperatures and times studied. As can be seen by comparing the three different times used for each extraction temperature used, results show that time has no significant ($p>0.05$) impact on TPC of *O. stamineus*. The findings of this study using HCWE concur with previous study published in the literature where the end result of Pressurized Liquid Extractions of similar material was not influenced by the extraction time used (Herrero *et al*., 2006). In this study, 20 min of extraction time gave the highest value of TPC compared to 10 and 30 min for all the temperatures used. TPC increases slightly from 10 to 20 min and decreases at 30 min, the change was significant at 140ºC and above. It was noted that the TPC values at 10, 20 and 30 mins at 140ºC were 71.22^{c}, 80.05^{c} and 77.90^{c} whilst at 200ºC the values were 85.46^{f}, 90.90^{f} and 86.99^{f} mg gallic acid/g dry sample respectively. This observation indicates that after a certain time, extraction of TPC may be controlled by the final equilibrium that will be achieved between the solute concentrations in the plant matrix and in the solvent (Silva *et al.*, 2007). Hence, extraction of TPC for a prolonged time was not useful and it can cause degradation of phenolic compounds (Wang *et al.*, 2014). This was confirmed in the findings of this work where prolonged extraction time of 30 min gave reduced TPC values.

Table 1: Values of Total Phenolic Content (TPC) (as mg gallic acid/g dry sample) obtained for extractions at different times (10, 20 and 30 min) and temperatures (100,120,140,160,180 and 200ºC)

Time (minutes)	Temperature (ºC)	Total Phenolic Content, TPC (mg gallic acid/g dry sample)
	100	55.08 ± 0.0015^{a}
	120	62.38 ± 0.0015^{b}
10	140	71.22 ± 0.0015^{c}

	160	94.61 ± 0.0015[d]
	180	78.85 ± 0.0015[e]
	200	85.46 ± 0.0015[f]
20	100	55.48 ± 0.0022[a]
	120	62.81 ± 0.0022[b]
	140	80.05 ± 0.0022[c]
	160	97.49 ± 0.0022[d]
	180	82.48 ± 0.0022[e]
	200	90.90 ± 0.0022[f]
30	100	55.84 ± 0.0025[a]
	120	59.86± 0.0025[b]
	140	77.90 ± 0.0025[c]
	160	95.66 ± 0.0025[d]
	180	81.57 ± 0.0025[e]
	200	86.99 ± 0.0025[f]

All analyses are value of TPC (mg gallic acid/g dry sample) ± average absolute deviation (AAD). TPC not sharing a common superscript letters were significantly different at $p<0.05$.

From Figure 1, it can be seen that the trend of TPC for all temperatures at different time are similar, where 160ºC recorded the highest value and 100ºC gave the lowest value of total phenolic content. However, the highest TPC was recorded at 20 minutes for all of the temperatures. At 20 min, the peak value was observed at 160ºC with TPC values of 94.61, 97.49 and 95.66 mg gallic acid/g dry sample at 10, 20 and 30 min respectively. At 20 min, TPC increases as the temperature increases from 100ºC (55 mg gallic acid/ g dry sample) to 160ºC (97.49 mg gallic acid/g dry sample). TPC increases due to the changes in the water properties at high temperature. At high extraction temperature, the solubility and mass transfer of compounds also increases. The viscosity of the water is reduced, permitting a better penetration of the solvent into the matrix and releasing more bound phenolic due to the disruption of cellular constituent, thus enhancing the extraction rate (C. C. Teo *et al.*, 2010; Ong *et al.*, 2006).

The decrease in the TPC at 180ºC (82.48 mg gallic acid/ g dry sample) might be due to some degradation of phenolic compounds at high temperature. Hossain *et al*., (2011) studied the optimisation of accelerated solvent extraction of antioxidant compounds from oregano, rosemary and marjoram using response surface methodology found that thermal degradation of rosmarinic acid, the major phenolic content in *O. stamineus* leaves could only be detected at temperature above 150ºC using methanol as solvent. However, there is slight increase in the value of extracted phenolic content at 200ºC (90.90 mg gallic acid/ g dry sample) being interpreted as an increase in the extraction of non-polar compounds due to the changes in dielectric constant (ε) of water near critical point (Rodríguez *et al.*, 2006). At ambient conditions, water is a polar liquid with high dielectric constant (ε) which is favorable to extract polar compounds due to the structure of its hydrogen bond (Teo *et al.*, 2010). However, the applications of high temperature and pressure in HCWE change the dielectric constant of water from 80 at 25ºC to 14 at 350ºC thus increase the selectivity of water to extract non-polar compounds at higher temperature (Sirinivas *et al.*, 2009; Teo *et al*., 2010; Plaza & Turner, 2015). The result of this study which indicated a peak in TPC value of *O. stamineus* extract at 160ºC and reduced values at higher temperatures agree well with the previous result reported by Rodríguez *et al.* (2006). In their study, TPC of individual extract of oregano obtained was the highest at 150ºC but decreases as the temperature increases at 200ºC.

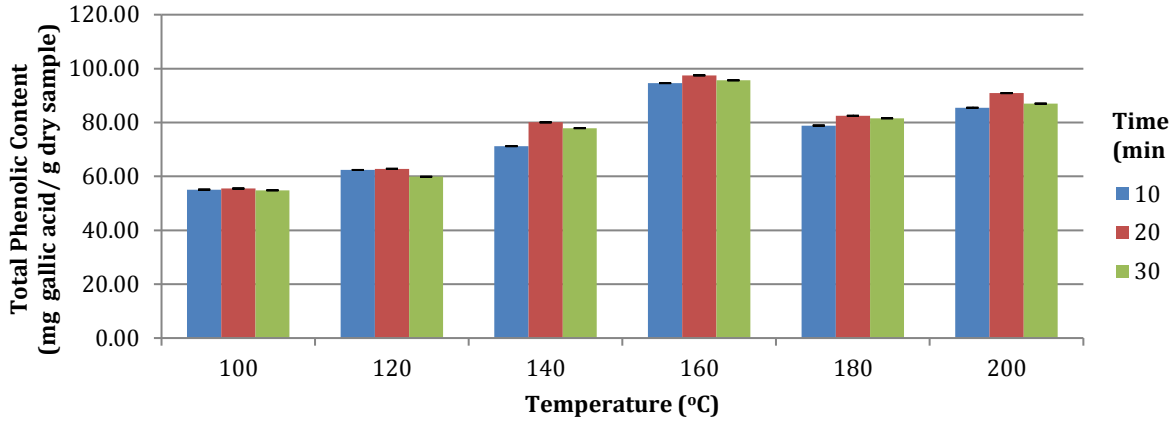

Fig. 1: Total Phenolic Content of *O. stamineus* leaves at different extraction temperatures and times

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Free radical scavenging was determined by DPPH assay. The purple color DPPH assay is normally used to verify the free radical scavenging ability of various food components. It is a stable free radical, which is reduced to α,α-diphenyl-β-picryl hydrazine which turned yellow when reacted with antioxidant. As 20 min gave the best TPC amongst the times tested, this extraction duration was chosen to evaluate the antioxidant activity of *O. stamineus* leaves extract. Figure 2 shows the results obtained for antioxidant activity (referred as percentage of inhibition) of *O. stamineus* leaves extracts at different temperature at a constant extraction time of 20 min. 120ºC gave the highest percentage of inhibition (92.15%) and 100ºC shows the lowest antioxidant activity (85.34%). From Figure 3, it can be concluded that the antioxidant activity of *O. stamineus* does not correlate with the TPC. Kahkonen *et al.,* (1999) also reported the same results on correlation between antioxidant activity of plant extracts and its TPC. Similar results have been further established by Rodríguez *et al.,* (2006) where the antioxidant activity of oregano water extracts were the highest at mild temperature 100ºC and does not show correlation with TPC.

Strong antioxidant activity at 120ºC can be explained by the different types of phenolic content that can be extracted which possess a different relationship structure-antioxidant activity (Mar´in *et al*., 2002). Previous study has identified pentacyclic triterpenes and stamine-type diterpernes presence in the leaves of *O. stamineus* which also showed free radical scavenging activity since free radical scavenging activity is not only contributed by phenolic compounds (Akowuah *et al.,* 2004; Tezuka *et al.,* 2000). Thus, the slightly higher antioxidant activity at 120ºC found in this study may be favorable and contributed by these compounds.

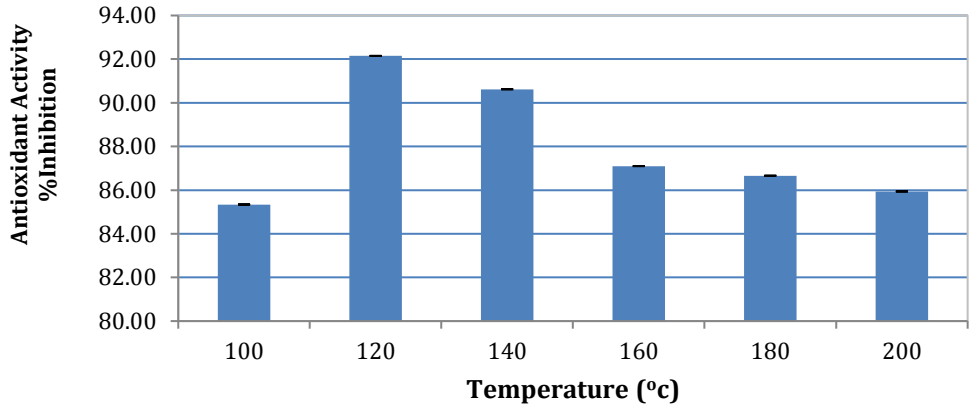

Fig. 2: Antioxidant activity of *O. stamineus* leaves at fixed time of 20 min and at different temperatures.

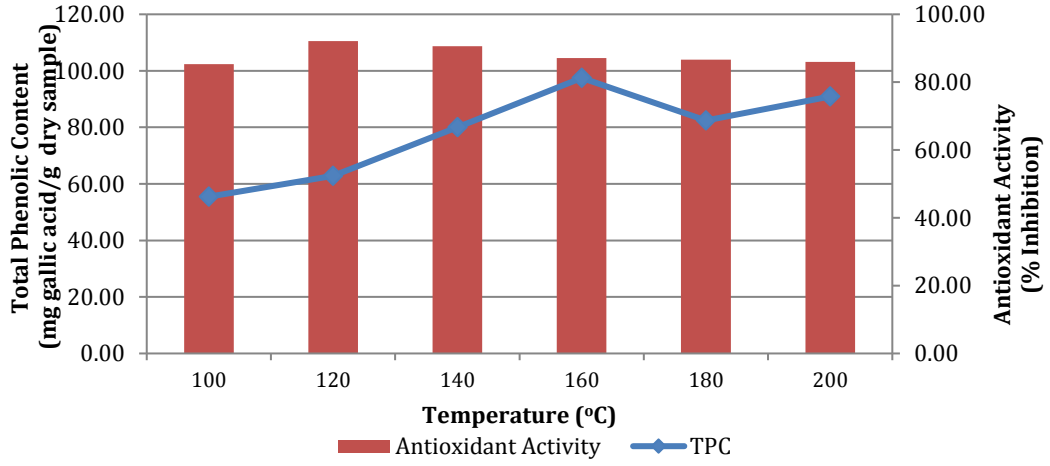

Fig. 3: Antioxidant activity and Total Phenolic Content of *O. stamineus* leaves at fixed time of 20 min and at different temperatures.

Conclusion:

In the present study, HCWE has been successfully used to extract polyphenols with antioxidant properties from the leaves of *O. stamineus* by manipulating its temperatures. This work shows that at 20 min and at

temperature of 160ºC and 120 ºC gives the highest value of TPC (97.49 mg gallic acid/g dry sample) and antioxidant activity (92.15%) respectively. Temperature show a significant impact ($p<0.05$) to the value of TPC recorded but time does not show any significant ($p>0.05$) impact on TPC of *O. stamineus*. However, there is no simple relationship between antioxidant activity and TPC of *O. stamineus*. HCWE is an alternative approach to extract valuable compounds from plant matrix with the used of green extraction techniques. This method of extraction has possibilities of being used at industrial scale especially in nutraceuticals and pharmaceutical industry that can contribute to the increase of production on herbs based products.

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REFERENCES

Akowuah, A.G., I. Zhari, I. Norhayati and A. Sadikun, 2005. The Effects of Different Extraction Solvents of Varying Polarities on Polyphenols of *Orthosiphon Stamineus* and Evaluation of The Free Radical Scavenging Activity. Food Chem, 93: 311-317.

Akowuah, A.G., I. Zhari, I. Norhayati, A. Sadikun, S.M.K hamsah, 2004. Sinensetin, Eupatorin, 30 hydroxy-5,6,7,4-tetramethoxy- flavone and rosmarinic acid content and antioxidative effect of *Orthosiphon stamineus* from Malaysia. Food Chem., 87: 559-566.

Ahamed Basheer M., A. Abdul Majid, 2010. Medicinal Potentials Of Orthosiphon Stamineus Benth. *Webmed Central CANCER*, 1(12):WMC001361

Sharifi, A., S.A. Mortazavi, A. Maskooki, M. Niakousari, A.H. Elhamirad, 2013. Optimization of Subcritical Water Extraction of Bioactive Compounds from Barberry Fruit (Berberis vulgaris) by Using Response Surface Methodology. International Journal of Agriculture and Crop Sciences, 6(2): 89-96

Chemat, F., M.A. Vian and G. Cravotto, 2012. Green extraction of natural products: Concept and principles. International Journal of Molecular Sciences, 13(7): 8615-8627. http://doi.org/10.3390/ijms13078615

Chin Chye Teo, Swee Ngin Tan, Jean Wan Hong Yong, Choy Sin Hew, Eng Shi Ong, 2010. Pressurized hot water extraction (PHWE). *Journal of Chromatography A*, 1217: 2484-2494.

Mar´ın, F.R., M.J. Frutos, J.A. Pe´rez-A´ lvarez, F. Mart´ınez-Sa´nchez, J.A. Del R´ıo, 2002. in: Atta-ur-Rahman (Ed.), Studies in Natural Products Chemistry, 26. Elsevier Science Publishers, Amsterdam, p:741.

Herrero, M., A.C ifuentes and E. Ibañez, 2006. Sub-and supercritical fluid extraction of functional ingredients from different natural sources: Plants, food-by-products, algae and microalgae: A review. Food Chemistry, 98: 136-148.

Herrero, M., M. Plaza and A. Cifuentes, 2010. Green processes for the extraction of bioactives from Rosemary : Chemical and functional characterization via ultra-performance liquid chromatography tandem mass spectrometry and in-vitro assays, 1217: 2512-2520. http://doi.org/10.1016/j.chroma.2009.11.032

Hiba, N., Rajha, Walter Ziegler, Nicolas Louka, Zeina Hobaika, Eugene Vorobiev, Herbert G. Boechzelt, and Richard G. Maroun, 2014. Effect of the Drying Process on the Intensification of Phenolic Compounds Recovery from Grape Pomace Using Accelerated Solvent Extraction. Int. J. Mol. Sci., 15: 18640-18658; doi:10.3390/ijms151018640.

Jamia, A.J., 2006. Malay traditional medicine: an overview of scientific and technol- ogy advancement. Asia Pacific Tech Monitor, 23: 37-49.

Jaganath, I.B., M.C hinna and A.R. Rahman, 2000. Herbs the green pharmacy of malaysia. pp: 126. Malaysia.: Vinpress Sdn. Bhd.

Khamsah, S.M., G. Akowah and I. Zhari, 2006. Antioxidant activity and phenolic content of *orthosiphon stamineus* Benth from different geographical origin. Journal of Sustainability Science and Management, 1(2): 14-20.

Kahkonen, M.P., A.I. Hopia, H.J. Vuorela, J.P. Rauha, K. Pihlaja, T.S. Kujala, M. Heinonen, 1999. Antioxidant of plant extracts containing phenolic compounds. J Agri Food Chem, 47: 3954-3962.

Matshediso, P.G., E. Cukrowska and L. Chimuka, 2015. Development of pressurised hot water extraction (PHWE) for essential compounds from *Moringa oleifera* leaf extracts. FOOD CHEMISTRY, 172: 423-427.

Hossain, M.B., C. Barry-Ryan, A.B. Martin-Diana, N.P. Brunton, 2011. Optimisation of accelerated solvent extraction of antioxidant compounds from rosemary (Rosmarinus officinalis L.), marjoram (Origanum majorana L.) and oregano (Origanum vulgare L.) using response surface methodology. Food Chemistry, 126 (1): 339-346.

Morad, N.A., N. Mulop, A.N. Darus, 2011. Introduction to Thermodynamics for Engineering Students, Pearson Custom Publishing, Kuala Lumpur, Malaysia

Merichel Plaza, Charlotta Turner, 2015. Pressurized hot water extraction of bioactives. Journal of Chromatography A, 1217: 2484-2494.

Quideau, S., D. Deffieux, C. Douat-Casassus, and L. Pouysegu, 2011. Plant Polyphenols: Chemical Properties, Biological Activities, and Synthesis. Angew. *Chem. Int. Ed*. 50: 586-621.

Ong, E.S., J.S.H. Cheong and D.G oh, 2006. Pressurized hot water extraction of bioactive or marker compounds in botanicals and medicinal plant materials. Journal of Chromatography A, 1112(1-2): 92-102.

Rodríguez-Meizoso, I., F.R. Marin, M. Herrero, F.J.S eñorans, G. Reglero, A. Cifuentes and E. Ibáñez, 2006. Subcritical water extraction of nutraceuticals with antioxidant activity from oregano. Chemical and functional characterization. Journal of Pharmaceutical and Biomedical Analysis, 41(5): 1560-1565. http://doi.org/10.1016/j.jpba.2006.01.018

Saidan, N.H., M.S.R. Hamil, A.H. Memon, M.M. Abdelbari, M.R. Hamdan, K.S. Mohd, A.M.S. Abdul Majid, I. Zhari, 2015. Selected metabolites profiling of *Orthosiphon stamineus* Benth leaves extracts Combined with chemometrics analysis and correlation with biological activities. BMC Complementary and Alternative Medicine 15:350 DOI 10.1186/s12906- 015-0884-0.

Serrano, J., R. Puupponen-Pimiä, A. Dauer, A.M. Aura and F. Saura-Calixto, 2009. Tannins: Current knowledge of food sources, intake, bioavailability and biological effects. Mol. Nutr. Food Res., 53: 310-329.

Silva, E.M., J.N.S. Souza, H. Rogez, J.F. Rees and Y. Larondelle, 2007. Antioxidant activities and polyphenolic contents of fifteen selected plant species from the Amazonian region. Food Chemistry, 101: 1012-1018.

Sirinivas, K., J.W. King, J.K. Monrad, L.R. Howard and C.M. Hansen, 2009. Optimization of subcritical fluid extraction of bioactive compounds using hansen solubility parameters. Journal of Food Science., 74(6): E342−E354.

Spigno, G., L. Tramelli and D.M.D. Faveri, 2007. Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics. Journal of Food Engineering, 81(1): 200208.

Tezuka, Y., P. Stampoulis, H. Banskota, S. Awale, K.Q.T ran, I. Saiki and S. Kadota, 2000. Constituent of the Vietnamese medicinal plant Orthosiphon stamineus. Chemical & Pharmaceutical Bulletin, 48(11): 1711-1719. http://doi.org/10.1248/cpb.48.1711

Venugopal, R., R.H. Liu, 2012. Phytochemicals in diets for breast cancer prevention: The importance of resveratrol and ursolic acid. Food Science and Human Wellness, 1(1): 1-13.

Wang, T., F. He and G. Chen, 2014. Improving bioaccessibility and bioavailability of phenolic compounds in cereal grains through processing technologies: A concise review. Functional Foods, **7:** 101-111.