ULTRASONIC ASSISTED EXTRACTION POLYPHENOLS AND ANTIOXIDANT FROM NIGELLA SATIVA SEED

J. Gimbun[1,2*], N. F. Ishak[1], N. I. S. Muhammad[3], S. F. Pang[1], M. A. A. Kadir[1], H. Ramli[1], M. F. Abdullah[1], Z. Giman[1], and Z. Khadisah[1]

[1]Faculty of Chemical & Natural Resources Engineering, Universiti Malaysia Pahang, 26300 Gambang, Malaysia

[2]Center for Advanced Research in Fluid Flow (CARIFF), Universiti Malaysia Pahang, 26300 Gambang, Malaysia

[3]Faculty of Technology, Universiti Malaysia Pahang, 26300 Gambang, Malaysia

ABSTRACT

This paper presents an ultrasonic assisted extraction study of essential oil from Nigella sativa seeds. It was found that the extraction of phenolic, flavonoid and antioxidant favours pure solvent, i.e. ethanol and methanol. Extraction of phenoilc, flavonoid and antioxidant, increases with increasing temperature until 50°C but reduced thereafter. Increasing the sonication power from 112 W to 224 W improved extraction of phenolic, flavonoid and antioxidant markedly, although a further increase to 277 W is not an improvement. A lower sonication frequency (35 kHz) yielded higher polyphenols and antioxidant content as opposed to the higher frequency (53 kHz). The highest total phenolic content (0.66 mg GAE/g sample), total flavonoid content (1.84 µg QE/g sample) and antioxidant activities (63.15%) using ethanol at sonication power of 224 W, extraction time of 30 minutes and temperature of 50°C.

KEYWORDS*: Nigella sativa; ultrasonic assisted extraction; flavonoid; phenolic; antioxidant*

1.0 INTRODUCTION

Nigella sativa is commonly known as habbatus sauda belongs to a botanical family of Rununculaceae. The N. sativa seed is rich with medicinal value and has been used as a natural remedy since ancient time, especially by people in the Mediterranean region. Previous research revealed that it contained an abundance of active ingredients useful for anticancer and anti-inflammatory, anti-dermatophyte,

* Corresponding author email: j.gimbun@gmail.com

asthma, hypertension, diabetes, cough, bronchitis, fever, dizziness and gastrointestinal disturbances.

The first step to recover and purify the essential oil from plant materials involves an extraction process. The yield of essential oil is dependent on the solvent used, extraction method and condition (Ncube et al., 2008). However, extraction of polyphenols from N.0 sativa is often limited to conventional extraction method such as maceration (Bourgou et al., 2008) and soxhlet extraction (Kaleem et al., 2006). Conventional extractions such as soxhlet extraction and maceration (ME) are normally performed at high temperatures for several hours, which may induce thermal degradation due to long period exposure to heat and also may cause the oxidation of the active component during extraction. Supercritical extraction is less favourable owing to its energy consumption and higher capital cost. The localize superheating in microwave-assisted extraction induces a rapid temperature rise, thus possesses challenge in temperature control to reduce the degradation cause by heat and prolonged period of extraction, an alternative extraction method is needed. Ultrasonic Assisted Extraction (UAE) technique reduces the inner and external mass transfer limitation and hence increases the yield of extraction. Furthermore, ultrasonic wave can break the cell membranes reducing control of inner mass transport. Several researchers (Zhang et al., 2009; Tabaraki and Nateghi, 2011; Chen et al., 2012; Pan et al., 2012) studied the influence of solvent type, extraction temperature, extraction time, ultrasound power on the extraction yield obtained from UAE. However, there is no study of UAE on N. sativa seed extraction. Therefore, the UAE method was employed in this work. Solvent type plays an important role in essential oil extraction. A combined effect of the different extraction methods (ME, and UAE) and varying solvent polarity to the polyphenol extraction from N. sativa has never been studied previously, and hence this is one of the objectives of this work.

2.0 MATERIALS AND METHODS

2.1 Chemicals

The chromatography grade solvents such as the n-hexane, 2-propanol and methanol were purchased from Merck (Darmstadt, Germany). The dimethyl sulfoxide (DMSO), Thymoquinone, 1,1-diphenyl-2-icrylhydrazyl (DPPH), aluminium chloride ($AlCl_3$), sodium hydroxide (NaOH), sodium nitrate ($NaNO_3$), Folin-Ciocalteu reagent, gallic acid and quecertin were obtained from Sigma Aldrich (St. Louis, MO).

Solvent for extraction such as 95% ethanol (Copens Scientific (M) Sdn Bhd), 99.8% methanol and 40-50% n–hexane (KOFA Chemical (M) Sdn Bhd) was obtained from a local supplier. The *N. sativa seed* was obtained from Hikmah Herbal Tech Sdn. Bhd.

2.2 Essential Oil Extraction

The volume weighted mean diameter of the milled powdered plant material was 0.19 mm was weighed (25wt. %) and mixed with solvent in a 250 ml sealed erlenmeyer flask. UAE was carried out in an ultrasonic bath (JK-DUCH-6210LHC, China) at either 35 or 53 kHz for time ranged from 15 to 120 minutes and the temperature was set at a range from 30°C to 60°C. Maceration was performed at 50°C in a stirred vessel using a similar plant material to solvent ratio. The temperature of 50°C was chosen based on the result from UAE extraction which shows the highest extraction yield of essential oil at 50°C. The result of the effect of extraction temperature is presented in section 3.4. The supernatant was then separated from the residue by vacuum filtration through 0.45 μm nylon membrane.

2.3 Total Phenolic Content (TPC)

Total phenolic contents were assayed using Folin-Ciocalteu reagent, following the Singleton's method. An aliquot (0.125 ml) of a suitable diluted extract was added to 0.5 ml of ultrapure water and 0.125 ml of the Folin-Ciocalteu reagent. Serial dilution was performed to ensure the UV-Vis reading fall into the linear range of the calibration curve. The exact concentration of the phenolic content was obtained by multiplying by the dilution factor used. The mixture was vortex for 3 minutes, before adding 1.25 ml of 7% Na_2CO_3 solution. The solution was then adjusted with ultrapure water to a final volume of 3 ml and mixed thoroughly. After leaving in the dark for 90 minutes at 25°C, the absorbance versus prepared blank was read at 760 nm using a calibrated ultraviolet–visible spectroscopy (Hitachi U-1800, Japan). Total phenolic contents of seeds (two replicates per treatment) were expressed as mg gallic acid equivalent per gram (mg GAE/100g sample) through a calibration curve with gallic acid. The calibration curve ranged from 50 to 400 μg/ml (R_2 = 0.99). All samples were performed in two replicates.

2.4 Total Flavonoid Content (TFC)

Total flavonoid content was measured according to aluminium chloride colorimetric assay correspond to the ones by Sameh and Gamal (2008). A 250 μl diluted extract was mixed with 75 μl $NaNO_2$ (5%). After 6 min vortex, 150 μl of 10% $AlCl_3$ and 500 μl of NaOH (1M) were added to

the mixture. Finally, the mixture was adjusted to 2.5 ml with ultrapure water. The absorbance versus prepared blank was read at λ = 510 nm using a calibrated ultraviolet–visible spectroscopy (Hitachi U-1800, Japan). Total flavonoid contents of seeds (two replicate per treatment) were expressed as mg quercetin equivalents per gram (mg QE/100g sample) through the calibration curve quercetin. The calibration curve range was 50-500 μg/ml.

2.5 DPPH Assay

The electron donation ability of the obtained extracts was measured by bleaching of the purple coloured solution of 1,1-diphenyl-2-picryhydrazyl radical (DPPH) according to the method of Hanato et al. (1988). Diluted essential oil prepared in methanol were added to 0.5 ml of a 0.2 mmol/l DPPH methanolic solution. The mixture was shaken vigorously and left standing in dark at room temperature for 30 minutes. The absorbance of the resulting solution was then measured at λ = 517 nm after 30 minutes using a calibrated ultraviolet–visible spectroscopy (Hitachi U-1800, Japan). The ability to scavenge the DPPH radical was calculated using Equation 1:

$$\text{DPPH scavenging effect (\%)} = \frac{(A_0 - A_1)}{A_0} \times 100 \quad (1)$$

where A_o is the absorbance of the control at 30 minutes, and A_1 is the absorbance of the sample at 30 minute.

2.6 Statistical Analysis

Each experiment was repeated in triplicates. Analysis of variance (ANOVA) was performed by using the data analysis tools in Microsoft Excel 2010, and a least significant difference (LSD) test was used to compare the means with a confidence interval of 95%.

3.0 RESULTS AND DISCUSSION

3.1 Comparison Between Maceration And Ultrasonic Assisted Extraction

The effect of extraction method was studied using pure ethanol solvent at 50°C. The maceration was performed in a water bath for 4 hours, whereas the ultrasonic assisted extraction was performed in an ultrasonic bath for 30 minutes at 35 kHz. The result of the test is shown in Table 1. The UAE has a much higher yield on antioxidant and phenolic

content, although maceration has a higher yield of flavonoid content. Nevertheless, the difference in flavonoid yield is outweighed by the higher yield of both antioxidant and phenolic content. Moreover, the UAE is a much faster method at 30 minutes compared to 240 minutes for maceration. Thus, UAE was employed for the rest of this work.

Table 1. Comparison between maceration (ME) and ultrasonic assisted extraction (UAE).

Extraction method	**Time** (min)	**TFC** (μg QE/g DW)	**TPC** (mg GAE/g DW)	**Antioxidant** (% inhibition of DPPH)
ME	240	2.48	0.23	60.30
UAE	30	1.83	0.58	68.69

3.2 Effect of Solvent Type and Extraction Method

The effect of solvent type to the yield of polyphenols and antioxidant extracts from *Nigella sativa* seeds was studied to find a solvent that can give the highest yield. Six types of solvent were tested, namely methanol, ethanol, hexane, 70% aqueous methanol, 50% aqueous methanol and 50% aqueous ethanol. The effect of solvent was studied using the maceration technique using at 50°C for 4 hours. The result in Figure 1 shows methanol has excellent yield of phenolic, flavonoid and antioxidant activity, but lower yield of flavonoid content. Meanwhile, ethanol shows the excellent simultaneous extraction of phenolic, flavonoid and antioxidant content. Hexane provides excellent extraction of flavonoid, but give a relatively lower yield on phenolic and antioxidant content. Aqueous methanol is not an improvement compared to the pure methanol. Nevertheless, the aqueous ethanol has a slightly better phenolic, but has a very low flavonoid yield. Extraction using ethanol shows the highest simultaneous extraction of TFC (2.47 μg QE/g DW), TPC (0.23 mg GAE/g DW) and antioxidant activities (60%). Methanol has a higher yield of TPC (0.38 mg GAE/g DW) and antioxidant activities (67%) but very low TFC (0.25 μg QE/g DW). The extraction yield from methanolic extract is comparable to that of Sen et al. (2010) who studied antioxidant activities of methanol extracts from N. sativa cultivated in Turkey. Meanwhile, the TPC and TFC yield are comparable to that of Meziti et al. (2012) who studied the methanolic extraction of N. sativa seed. Extraction using n-hexane, 50% aqueous methanol and 50% aqueous ethanol is not notable, thus, ethanol was employed for the remainder of this work.

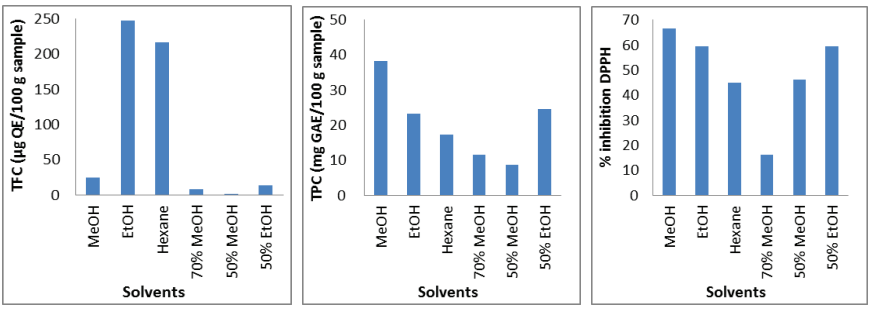

Figure 1. Effect of solvent to TFC, TPC and antioxidant extraction from N. sativa.

3.3 Effect of the Sonication Frequency and Extraction Time

The effect of sonication frequency was studied using a 50% aqueous ethanol at 50°C with sonication power of 224 W. The sonicator has a dual frequency feature (i.e., 35 and 53 kHz) that enable studies on the effect of frequency to extraction yield and quality. Figure 2 shows the effect of sonication frequency on total phenolic, total flavonoid and antioxidant of the essential oil extracted from N. sativa. It was found that lower sonication frequency (35 kHz) is more effective in extracting the phenolic, flavonoid and antioxidant content from N. sativa seeds. Similar finding was reported by Kulkarni and Rathod (2014) whereby the extraction yield is much higher at 25 kHz compared to 40 kHz. They mentioned that higher yield at lower frequency is due to the less scattering and attenuation of sound waves at lower frequencies. Furthermore, the cavitation is easily achievable at lower frequency as compared to higher frequency. It was found that there is no significant improvement on the effect of residence time after 30 minutes for TFC, TPC and antioxidant content when the sonication frequency of 35 kHz is employed. However, at higher frequency (53 kHz) a slightly different result for TPC was obtained which showed the optimum time at 60 minutes, although the TFC yield reduced slightly. Hence, 30 minutes and 35 kHz were employed for UAE throughout this work. In fact phenolic compounds and antioxidant reduced after 30 minutes, as a result of sonication induced degradation over prolonged time exposure.

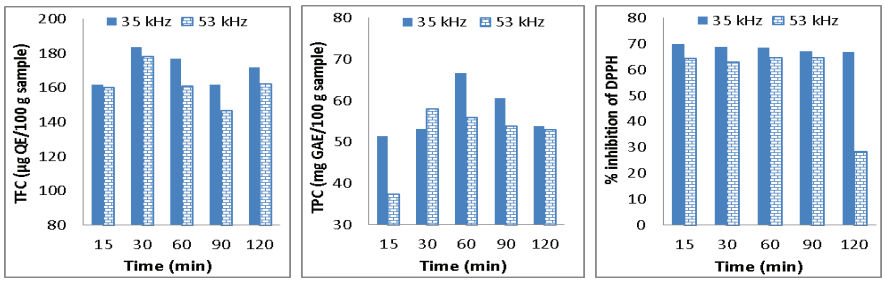

Figure 2. Effect of sonication frequency to TPC, TFC and antioxidant extraction from N.sativa

3.4 Effect of Sonication Power and Temperature

The effect of sonication power was studied at 50°C using 50% aqueous ethanol as solvent at sonication frequency of 35 kHz. Whereas, the effect of temperature was studied at similar condition except the sonication power was fixed at 224W. The highest TPC, TFC and antioxidant activities were obtained at sonication power 224W and a temperature of 50°C (Fig. 3). Extraction at the higher temperature (60°C) is not an improvement due to thermal degradation of phenolic and flavonoid content. It was found that UAE is better than ME as it can provide a much higher extraction of TFC, TPC and antioxidant within 30 minutes compared to 4 hours for ME.

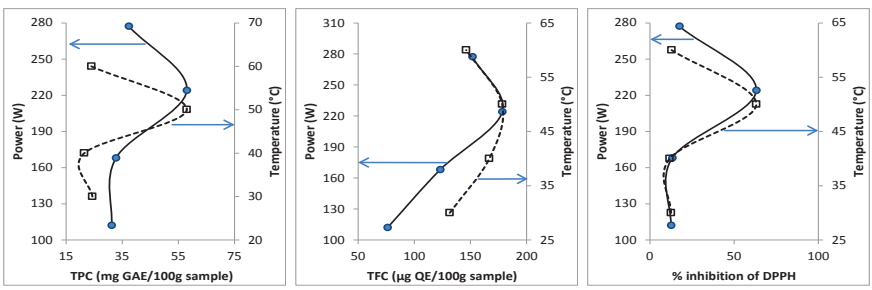

Figure 3. Effect of power and temperature to TFC, TPC and antioxidant extraction from N. sativa.

4.0 CONCLUSIONS

The ultrasonic assisted extraction of polyphenols and antioxidant extraction from N. sativa seeds was successfully elucidated. It was found that the extraction of polyphenols and antioxidant favours pure solvent such as ethanol and methanol. Extraction of phenoilc, flavonoids and antioxidant, increases with temperature until 50°C but reduced thereafter. Increasing the sonication power from 112 W to

224 W improved extraction of polyphenols and antioxidant markedly, although a further increase to 277 W is not an improvement. A lower sonication frequency (35 kHz) yielded higher polyphenols and antioxidant content as opposed to the higher frequency (53 kHz). The highest total phenolic content (0.66 mg GAE/g sample), total flavonoid content (1.84 μg QE/g sample) and antioxidant activities (63.15%) using 50% ethanol at sonication power of 224 W, extraction time of 30 minutes and temperature of 50°C.

ACKNOWLEDGEMENT

Funding from the Universiti Malaysia Pahang (RDU120366) is gratefully acknowledged.

REFERENCES

Bourgou, S., Ksouri, R., Bellila, A., Skandrani, I., Falleh, H., & Marzouk, B. (2008). Phenolic composition and biological activities of Tunisian Nigella sativa L. shoots and roots. *Comptes Rendus Biologie, 331*(1), 48-55.

Chen, R., Li, Y., Dong, H., Liu, Z., Li, S., Yang, S., & Li, X. (2012). Ultrasonics Sonochemistry Optimization of ultrasonic extraction process of polysaccharides from Ornithogalum Caudatum Ait and evaluation of its biological activities. *Ultrasonics Sonochemistry, 19*(6), 1160–1168.

Hanato, T., Kagawa, H., Yasuhara, T., & Okuda, T. (1988). Two new flavonoids and other constituents in licorice root: their relative astringency and radical scavenging effects. *Chemical & Pharmaceutical Bulletin, 36*(6), 2090-2097.

Kaleem, M., Kirmani, D., Asif, M., Ahmed, Q., & Bano, B. (2006). Biochemical effects of Nigella sativa L seeds in diabetic rats. *Indian journal of experimental biology, 44*(9), 745-748.

Kulkarni, V. M., & Rathod, V. K. (2014). Mapping of an ultrasonic bath for ultrasound assisted extraction of mangiferin from Mangifera indica leaves. *Ultrasonics sonochemistry, 21*(2), 606-611.

Meziti, A., Meziti, H., Boudiaf, K., Mustapha, B., & Bouriche, H. (2012). Polyphenolic profile and antioxidant activities of Nigella sativa seed extracts in vitro and in vivo. *World Academy of Science, Engineering and Technology, 64*(6), 24-32.

Ncube, N. S., Afolayan, A. J., & Okoh, A. I. (2008). Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. *African journal of biotechnology, 7*(12), 1797-1806.

Pan, G., Yu, G., Zhu, C., & Qiao, J. (2012). Ultrasonics Sonochemistry Optimization of ultrasound-assisted extraction (UAE) of flavonoids compounds (FC) from hawthorn seed (HS). *Ultrasonics Sonochemistry, 19*(3), 486–490.

Sameh, F. A., & Gamal, M. E. (2008). Increase in flavonoids content in red onion peel by mechanical shredding. *Journal of Medicinal Plants Research, 2*(9), 258-260.

Şen, N., Kar, Y., & Tekeli, Y. (2010). Antioxidant activities of black cumin (Nigella sativa l.) Seeds cultivating in different regions of Turkey. *Journal of Food Biochemistry, 34*, 105–119.

Tabaraki, R., & Nateghi, A. (2011). Ultrasonics Sonochemistry Optimization of ultrasonic-assisted extraction of natural antioxidants from rice bran using response surface methodology. *Ultrasonics Sonochemistry, 18*(6), 1279–1286.

Zhang, H. F., Yang, X. H., Zhao, L. D., & Wang, Y. (2009). Ultrasonic-Assisted Extraction of epimedin C from fresh leaves of Epimedium and extraction mechanism. *Innovative Food Science and Emerging Technologies, 10*(1): 54–60.