



# **The Effects of Extraction Conditions on Extraction Yield and Syringin Content in Producing Standardized *Tinospora crispa* Aqueous Extract with High Antioxidant Activity**

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## **Authors' contributions**

*This work was carried out in collaboration among all authors. Authors ZMdS and ZA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MKNH and KKAK managed the analyses of the study. Author MSMA managed the literature searches. All authors read and approved the final manuscript.*

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## **ABSTRACT**

The herbal plant known as *Tinospora crispa* is reported to have many beneficial effects on health and has great potential in future to be developed as a health product either in the form of traditional medicine, food supplements or in pharmaceutical preparations. However, so far knowledge on processing procedures to produce quality standardized extracts of this plant to be used in product development has still not widely reported. Therefore, the objective of this study was to determine the optimal extraction conditions for producing a standardized *T. crispa* aqueous extract (STCAE) with high extraction yield and high syringin content. Experiments were conducted to determine the

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effects of various extraction conditions involving temperature (25–100 ° C), extraction time (0.5–6 hours) and liquid (water) to solid ratio (5: 1–25: 1 ml of solvent per g stem dry). Using optimized conditions obtained, the extract was standardized based on syringin and was further investigated on its antioxidant activity through DPPH, FRAP and TBA bioassays. Results revealed that the optimum extraction conditions were found to be 1 h extraction time and 15:1 ml g<sup>-1</sup> liquid-to-solid ratio. For the extraction temperature, 60°C was found to be the best. STCAE was produced on the basis of the extract to contain with at least 0.4 wt% of syringin. STCAE was found to possess high antioxidant activities through DPPH, FRAP and TBA bioassays.

**Keywords:** *Tinospora crispa*; solid-liquid extraction; antioxidant activities.

## 1. INTRODUCTION

*Tinospora crispa* (L.) Hook. f. & Thomson, is a climber that can be found in primary rainforest of South East Asia including Malaysia, Indonesia, Thailand and Vietnam. This tropical liana (woody) with shiny green leaf has several local names including “Patawali” in Malaysia [1], “Andawali” in Indonesia [2], “Makabuhay” in Philippines [3] and “Boraphet” in Thailand [4]. This plant has been traditionally used to reduce hypertension, glucose level in the blood, remedy for various ailments such as fever, asthma, intestinal worms, and skin infections [1,5,6,7].

Scientific studies have shown that crude extracts and isolated compounds of *T. crispa* have various pharmacological activities. It was reported that the crude extracts and isolated compounds of *T. crispa* possessed a broad range of pharmacological activities such as cardioprotective, anti-diabetic, anti-inflammatory, antioxidant, immunomodulatory, cytotoxic and antimalarial activities [8]. Moreover, it was discovered that *T. crispa* possessed an anti-hypercholesterolemic activity and is beneficial in preventing the heart-related diseases [9,10]. However, it is noted that those above-mentioned studies do not indicate what active ingredient which responsible for the desired effects. Most pharmacological studies were based on crude extracts of the plant and the bioactive compounds responsible for the bioactivities have not been well identified. Further investigations are required to transform the experience based claims on the use of *T. crispa* in traditional medicine practices into evidence-based information.

Nowadays, there are many traditional health products on the market that use herbal extracts as its main ingredient and it is formulated either into capsules, tablets, liquids, pills, etc. [11]. However, to ensure its quality and safety is always guaranteed the products should be standardized based on certain standard of

requirements [12]. One of the important methods to standardize herbal products is through phytochemical based standardization. It helps in adjusting the herbal products to a defined content of a constituent/s which have therapeutic activity thus guarantees the quality of the product produced.

Syringin, isolated from stem parts of *T. crispa* has been reported to possess remarkable biological activities such as anti-hypertension [13,14,15], anti-diabetic [7] and anti-inflammatory activities [10]. Due to its ability and effects to the abovementioned therapeutic claims, it is essential to use syringin as standard or referral marker in this study for the standardization and future product quality assessment purposes.

Optimization can be referred as an improvement of performance of a system, process, or product to obtain the optimum benefit from it [16]. The yield of extract material, concentration of referral markers and antioxidant activity were strongly dependent on the best extraction condition [17]. However until to date, information regarding the best extraction conditions of *T. crispa* remains limited. Thus, this study was aimed to investigate the effect of different extraction conditions on extraction yield, syringin content and antioxidant activity of standardized *T. crispa* aqueous extract.

## 2. OBJECTIVES

To investigate the effect of extraction conditions on extraction yield, syringin content and antioxidant activity of standardised *T. crispa* aqueous extract.

## 3. MATERIALS AND METHODS

### 3.1 Collection of Raw Material

About 10 kg of fresh stem of *Tinospora crispa* were collected from Forest Research Institute

Malaysia (FRIM) at Kepong, Selangor. The plant was authenticated by FRIM botanist (Voucher number: SBID009/15). The stems were cleaned, washed, cut and dried using an oven dryer with operating temperatures of around 55°C. The weight of the sample was monitored daily until a constant weight was obtained. Subsequently, the dried stems of the plant were ground to a particle size of about 1 to 4 mm using a 20 hp pilot scale grinder. The ground sample was kept at room temperature in a closed environment before the extraction process was performed.

### 3.2 Optimization of Extraction Parameter of *Tinospora crispa*

Optimization of the aqueous extraction process was carried out by evaluating the effects of different extraction parameters including ratio of solvent to solid, temperature and duration on the extraction yield and concentration of target compound of the extract. Yield was defined as total weight of extract produced per weight dried raw material used. Generally, higher yield is preferred as it means the extraction parameters used is the most cost effective. Whereas syringin was selected as the referral marker due to its ability to reduce cholesterol.

#### 3.2.1 Effect of solvent to solid ratio on extraction yield and Syringin content of *Tinospora crispa* Aqueous Extract (TCAE)

About 2 g dried *T. crispa* stem was placed into three 250ml round bottle flasks and labeled as 1, 2 and 3 which each number represents different ratio of solvent to solid (ml/g) as shown in Table 1:

**Table 1. Different ratio of solvent to solid of extraction process**

No	Ratio	Weight of sample and quantity of water required
1	1:5	2 g <i>T. crispa</i> + 50ml *RO water
2	1:10	2 g <i>T. crispa</i> + 100ml RO water
3	1:15	2 g <i>T. crispa</i> + 150ml RO water
4	1:20	2 g <i>T. crispa</i> + 200ml RO water
5	1:25	2 g <i>T. crispa</i> + 250ml RO water

\*Reverse osmosis water

The temperature used for this extraction process was 60°C and the duration of extraction was at 6 hours based on the findings reported by previous study [18]. The extraction was carried out using water bath (Memmert WNB 45 Germany). Hot water bath was used to provide uniform heating during extraction. The extraction process was conducted triplicate. The extracted materials were filtered using filter paper (Whatman No. 1). The filtrates were then freeze-dried to remove water. The freeze-drying process was carried out using laboratory Freeze Dryer (85XL, Millrock, USA). The yield of *Tinospora crispa* aqueous extract (TCAE) was calculated using the following equation [18]:

$$\text{Yield (Wt. \%)} = \frac{W_d}{V_e} \times R_{ss} \times 100$$

Where,  $W_d$  is weight of the dried plant (g),  $V_e$  is volume of the aqueous extract used for freeze drying (ml) and  $R_{ss}$  is solvent to solid ratio (ml/g). Meanwhile, the syringin concentration of the extract was quantified using High Performance Liquid Chromatography (HPLC) analysis. The concentrations of syringin yield were reported in Wt. % by using formula [19]:

$$(i) \text{Syringin yield (Wt. \%)} = \frac{\text{Conc (mg/L)} \times \text{Volumn (L)}}{\text{Weight (g)}} \times 100$$

#### 3.2.2 Effect of temperature on extraction yield and Syringin content of TCAE

Twenty grams of dried samples were placed into five 250ml round bottle flasks and labeled as 1, 2, 3, 4 and 5 where each number represents room temperature (25°C), 40, 60, 80 and 100°C. Two hundred milliliters of reverse osmosis (RO) water (1:10 g/ml) were added onto each flask and the extraction period was set at 6 h. The temperature was monitored using a thermometer until the targeted temperature was obtained. Extraction was performed using a water bath (Memmert WNB 45 Germany). The extraction process was conducted triplicates. The extracted material was filtered using filter paper (Whatman No. 1) and freeze-dried to remove water. The yield of TCAE syringin content was calculated following the equations in section 3.2.1.

#### 3.2.3 Effect of duration on extraction yield and Syringin content of TCAE

Twenty grams of dried samples were weighted into seven 250 ml round bottle flasks and labeled

as 1, 2, 3, 4, 5, 6 and 7 where each number indicates the extraction period at 30 minutes, 1, 2, 3, 4, 5 & 6 hours, respectively. RO water was used as a solvent for extraction with a solvent to solid ratio at 1:10 (w/v) and the temperature was set at 60°C. Extraction was performed using water bath (Memmert WNB 45 Germany). The extraction process was conducted triplicates. The extracted material was filtered using filter paper (Whatman No. 1) and freeze-dried. The yield of TCAE syringin content was calculated following the equations in section 3.2.1.

### 3.3 Determination of Syringin Content from TCAE Using HPLC

After the freeze-drying process, TCAE (20 mg) was diluted with 1 ml of water and sonicated using a sonicator (Hwashin Power Sonic Model 405, Korea) for 10 minutes. Then, the samples were filtered using a syringe filter (Whatman 0.45µm PVDF) before being injected into the HPLC system. The HPLC system consists of a Waters 600 System Controller, Waters 2996 Ultraviolet (UV) detector and was equipped with a Waters 717 Autosampler. The UV Waters 2996 detector detects chemical compounds that pass through the HPLC column and sends the data to the computer for analysis. A Column oven was used to maintain the column temperature during analysis. A Symmetry Waters 5 30 µm C18 column with dimension 250 x 4.6 mm was used as the stationary phase.

The mobile phase was in gradient mode and comprised of 0.1% Orthophosphoric acid, H<sub>3</sub>PO<sub>4</sub> and 100% HPLC grade acetonitrile. The mobile phase combinations were selected through optimization for better separation of compounds and shorter time (35 minutes). The injection volume was 10 µl and flow rate was adjusted to 1.0 ml/min. Maximum number of peak presence was observed at wavelength 220 nm. The presence of standard compound syringin in TCAE was identified by comparing the retention times and UV spectra with the standard. The experiments were conducted in triplicates and the results were presented in ppm (mg/L).

#### 3.3.1 Quantification of syringin content

100 µg/ml of standard syringin stock solutions was prepared by dissolving 1 mg of standard in 10 ml of methanol: water (70:30). The solution

were stored in a dark glass bottles at a temperature of 4°C. A working standard solutions, covering concentrations from 5 µg/mL to 200µg/ml were prepared. The quantity of TCAE syringin content was measured based on the calibration curve obtained and converted to wt. % (w/w) follows the equations in section 3.2.1.

#### 3.3.2 Preparation of standardized *Tinospora Crispa* Aqueous Extract (STCAE)

The *T. crispa* aqueous extract (TCAE) was standardized based on a standard compound, syringin. A prototype of STCAE was prepared based on designated optimal extraction parameter obtained. The production process involves extraction, concentration and freeze drying. Evaporation of liquid extract was performed under vacuum below 60°C. The concentrated liquid extract was freeze dried using a laboratory Freeze Dryer (85XL, Millrock, USA) stored at -20°C until used.

#### 3.4 Determination of Antioxidant Activity of STCAE

The quality of STCAE was further verified through its antioxidant activity. The 1,2- diphenyl-2-picrylhydrazyl (DPPH) assay, Thiobarbituric acid (TBA) Test and Ferric Reducing Antioxidant Power (FRAP) assay were performed, in which Vitamin C and butylated hydroxytoluene (BHT) were acted as standard.

##### 3.4.1 DPPH radical avenging

The antioxidant activity of STCAE was measured based on its scavenging activity against a stable free radical 1,1- diphenyl-2-picrylhydrazyl (DPPH), as previously described [20]. The activity was compared against the standard antioxidants, namely, BHT and vitamin C. Briefly, for the control, 1 ml of 0.45 mM DPPH was added to 0.5 ml of absolute ethanol. For the sample solution, 1 ml of 0.45 mM DPPH was added to 0.5 ml of extract (5 mg/ml). The step was repeated by replacing the extract with BHT (5 mg/ml) and vitamin C (5 mg/ml). Each sample, was incubated for 30 min and after incubation, absorbance was recorded at 517 nm. The percentage of inhibition representing the sampling activity against DPPH was calculated based on the following equation:

(ii) Inhibition (%) =

$$\frac{\text{Absorbance of control} - \text{Absorbance} \times 100\% \text{ of test sample}}{\text{Absorbance of control}}$$

### 3.4.2 Thiobarbituric Acid Test (TBA)

The TBA values of STCAE were determined using the method from previous researcher [21]. One ml of sample from Ferric Thiocyanate method (FTC) was added to two ml of Trichloroacetic acid (TCA) and two ml of Thiobarbituric acid (TBA). The mixture was then placed in a boiling water bath at 100°C for 10 minutes. After cooling, it was then centrifuged at 3000 rpm for 20 minutes. The absorbance of the supernatant was measured spectrophotometrically at 532 nm and was quantified using the following formulation:

$$(iii) \text{Percentage of inhibition (\%)} = \frac{\text{OD control} - \text{OD sample} \times 100\%}{\text{OD control}}$$

### 3.4.3 Ferric reducing antioxidant power

The ferric reducing ability of STCAE was evaluated following the method described by previous report [22]. The reagent was freshly prepared by mixing 10 mM 2, 4, 6-tripyridyl triazine (TPTZ) and 20 mM ferric chloride in 0.25M acetate buffer (pH 3.6). Then, 100 µl of extract was added to 300 µl of distilled water, followed by 3 ml of FRAP reagent. The absorbance was recorded at 593 nm spectrophotometrically after 4 min of incubation at room temperature. The reducing ability of the extracts was compared with BHT. The results were expressed as the concentration of antioxidants having ferric reducing ability equivalent to that of 1 mM FeSO<sub>4</sub>, expressed in millimolar per litre.

### 3.5 Statistical Analysis

All data were analyzed using the computer software Statistical Package for Social Sciences (SPSS) version 20.0 and were expressed as mean ± standard deviation. Comparisons of group means was done by one-way analysis of variance (ANOVA) with a probability less than .05 (p < 0.05) taken as indicative of significant difference. The mean value (x) and standard deviation (SD) were calculated for each variable measured. Turkey's pos hoc test was used for multiple group comparison. P < 0.05 is considered significant.

## 4. RESULTS

### 4.1 Optimization of Extraction Parameter of *Tinospora crispa*

#### 4.1.1 Effect of solid to solvent ratio on extraction yield and concentration of syringin

Fig. 1 shows the effect of solid to solvent ratio on the extraction yield of TCAE. The results showed that the extraction yield increased from 9.69% to 16.22% as the solid to solvent ratio increased from 1:10 to 1:25 (g/ml). The highest yield was showed in a ratio of 1:25 (16.25 ± 0.38%), followed by a ratio of 1:20 (16.10 ± 0.23%) and a ratio of 1:15 (15.86 ± 0.55%), respectively. However, no significant changes were observed in the yield of TCAE between them. Meanwhile, the solid to solvent ratio at 1:10 was found to be significantly lower (p<0.05) compared to the other groups (11.03 ± 0.40%).

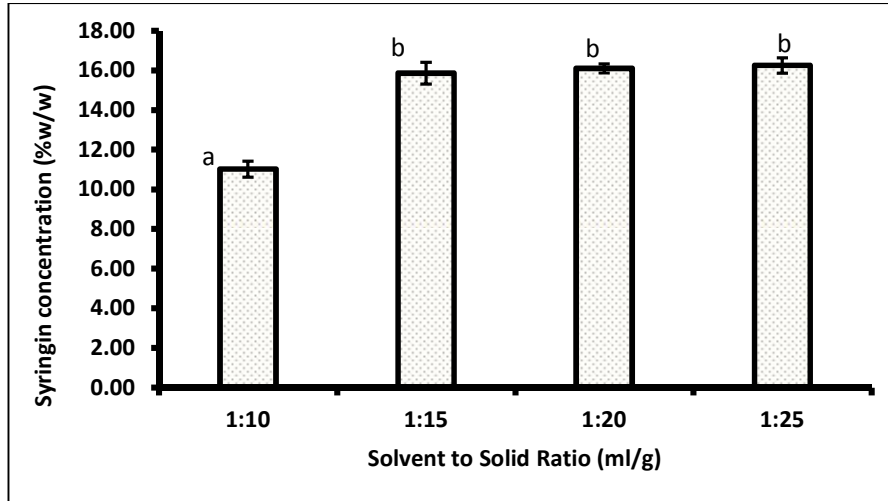
Fig. 2 shows the effect of solid to solvent ratio on the concentration of syringin. The results showed that the syringin concentration increased from 0.44 ± 0.01% to 0.49 ± 0.04 % as the solid to solvent ratio increased from 1:10 to 1:25 (g/ml). The highest syringin concentration was shown in the ratio 1:25 (0.49 ± 0.04%), followed by the ratio 1:20 (0.47 ± 0.02%), the ratio 1:15 (0.46 ± 0.01%) and a ratio of 1:10 (0.44 ± 0.01%), respectively. However, there was no significant change observed on syringin concentrations between all ratios tested. The results indicated that the most suitable and most cost effective solvent to solid ratio to obtain optimal extraction yield and high syringin concentration was at a ratio of 1:15 (g/ml).

#### 4.1.2 Effects of temperature on extraction yield and concentration of syringin

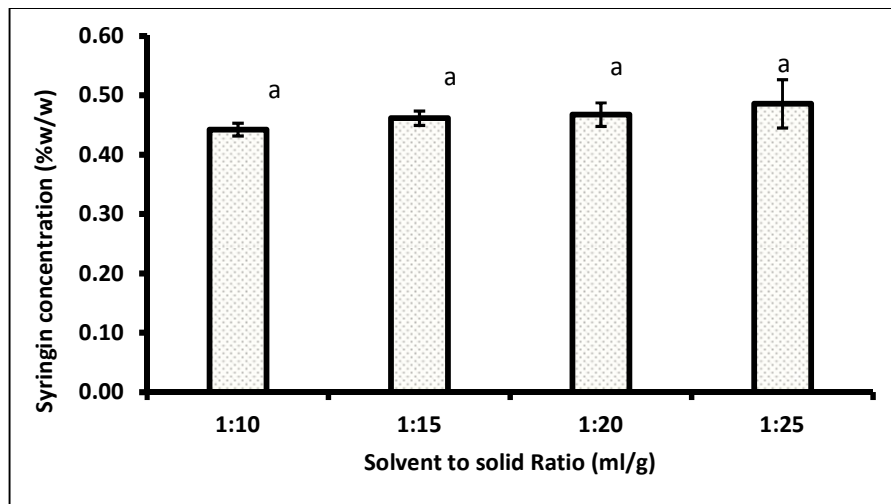
Fig. 3 shows the effect of different temperature on the yield of TCAE. The result showed that the extraction yield of TCAE was found to increase with the increase on extraction temperature. Result indicates the most suitable temperature for extraction of *T. crispa* was at 60°C since there is no significant change on the yield observed at temperature 60°C (15.95±0.21%), 80°C (15.87±0.29%) and 100°C (16.02 ± 0.07%) respectively. The extraction yield of TCAE was significantly lower (p<0.05) at 40°C (14.18 ± 0.32%) followed by 25 °C (7.98 ± 0.63%) when compared to the other groups.

Fig. 4 shows the effect of different temperature on the syringin concentration. The result showed that the syringin concentration was found to increase from a temperature of 25 to 60°C and began to drop dramatically at temperature 80 and 100°C. The results indicated that the most suitable temperature for extraction of *T. crispa* was at 60°C since it has produced significantly ( $p < 0.05$ ) the highest concentration of

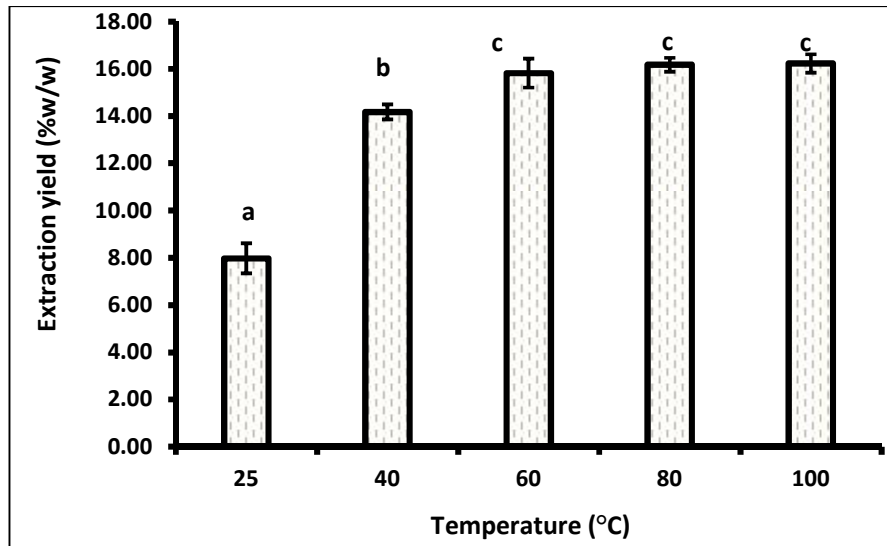
syringin compared to the other groups. Syringin concentrations at 25, 40, and 60°C were increased by 0.37, 0.38 and 0.45% dry wt and decrease to 0.18 and 0.07% dry wt, at temperature 80 and 100°C respectively. The results indicate the most suitable temperature and the most cost effective to obtain optimal extraction yield and syringin concentration of TCAE was at 60°C.



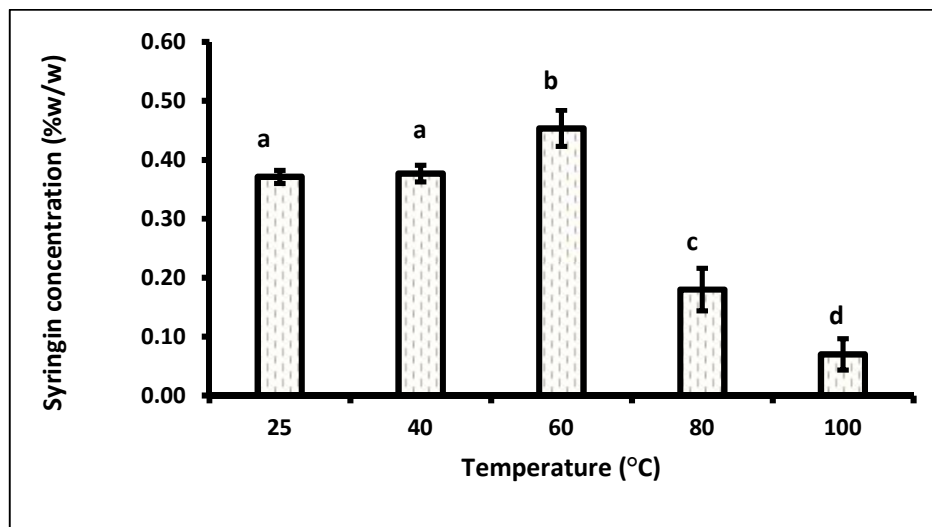
**Fig. 1. Effect of Different Solvent to Solid Ratio (ml/g) on Extraction Yield of TCAE.** Notes: Each value represents the mean  $\pm$  SD. Bars with different alphabet are significantly different ( $p < 0.05$ ). No significant change observed on the yield of the solvent to solid ratio at 1:15 mg/ml and above



**Fig. 2. Effect of Different Solvent to Solid Ratio (ml/g) on Syringin Concentration of TCAE.** Notes: Each value represents the mean  $\pm$  SD. Bars with different alphabet are significantly different ( $p < 0.05$ ). Results indicate the most suitable solvent to solid ratio and the most cost effective to obtain optimal extraction yield and syringin concentration of TCAE was at ratio 1:15 (g/ml)



**Fig. 3. Effect of Varying Temperature (°C) on Extraction Yield of TCAE.** Notes: Each value represents the mean  $\pm$  SD. Bars with different alphabet are significantly different ( $p < 0.05$ ). Result indicates the most suitable temperature for extraction of *T. crispa* was at 60°C since there is no significant change on the yield of TCAE observed at higher temperature

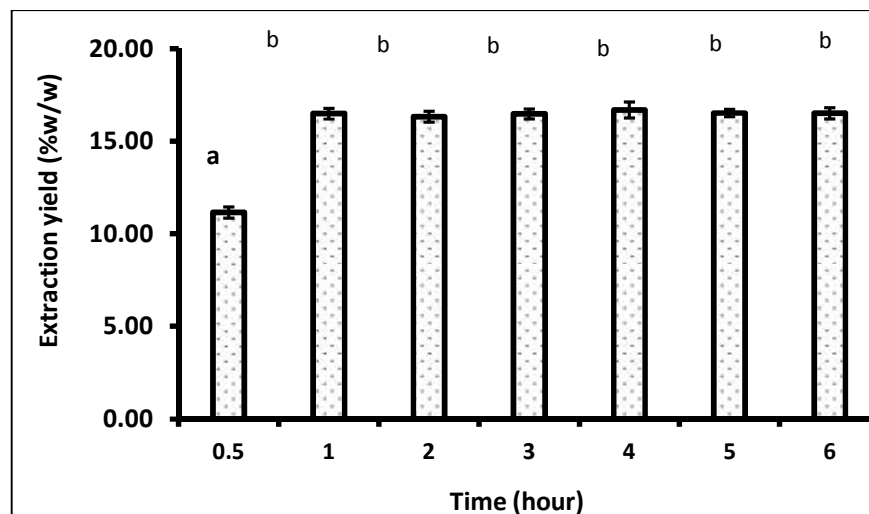


**Fig. 4. Effect of Varying Temperature (°C) on the Concentration of Syringin.** Notes: Each value represents the mean  $\pm$  SD. Bars with different alphabet are significantly different ( $p < 0.05$ ). Temperature at 60 °C produced significantly ( $p < 0.05$ ) the highest concentration of syringin compared to the other groups

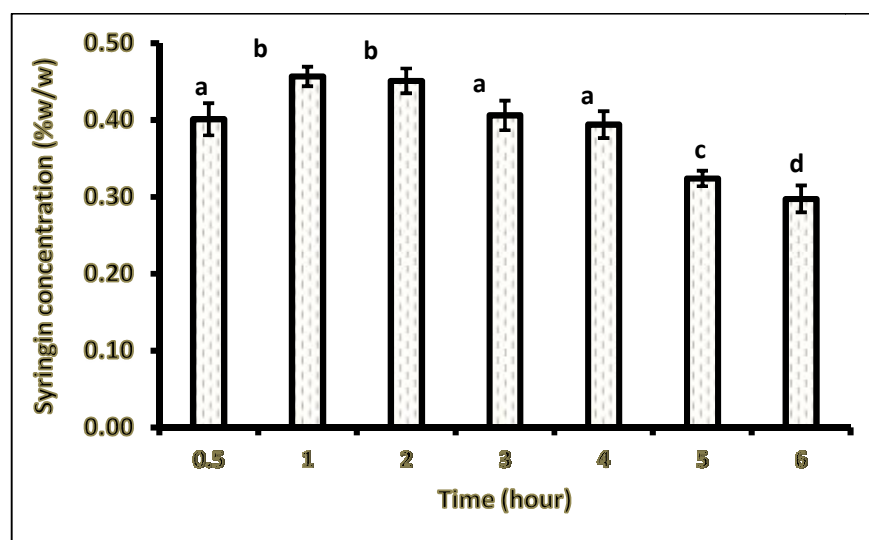
#### 4.1.3 Effect of extraction time on extraction yield and concentration of Syringin

Fig. 5 shows the effect of different extraction time on TCAE yield. The results found that there was no significant change in the TCAE yield after the first hour of extraction. This indicates that the

extraction process achieves equilibrium in about 1 hour. The yields of TCAE obtained were found to be 11.10 $\pm$ 0.08% (30 minutes), 16.49 $\pm$ 0.10% (1 hour), 16.33 $\pm$ 0.0.29% (2 hours), 16.48 $\pm$ 0.27% (3 hours), 17.02 $\pm$ 0.14% (4 hours), 16.52 $\pm$ 0.20% (5 hours) and 16.51 $\pm$ 0.30% (6 hours), respectively.



**Fig. 5. Effect of Different Extraction Time on Extraction Yield of TCAE.** Notes: Each value represents the mean  $\pm$  SD. Bars with different alphabet are significantly different ( $p < 0.05$ ). The result showed no significant change in the extraction yield of TCAE after the first hour of extraction process



**Fig. 6. Effect of Varying Extraction Time on the Concentration of Syringin.** Notes: Each value represents the mean  $\pm$  SD. Bars with different alphabet are significantly different ( $p < 0.05$ ). Concentration of syringin increased from 30 minutes to 1 hour of the extraction process and begins to decline after 1 hour

Fig. 6 shows the effect of different temperature on syringin concentration. The results showed that the syringin concentration increased from 30 minutes to 1 hour and began to decrease after 1 hour of the extraction process. The degradation could be occurred as a result of heat exposure. The results indicate the most suitable extraction time to obtain the optimal extraction yield and high syringin concentration was at 1 hour.

#### 4.2 HPLC Profile of TCAE Containing Syringin

Fig. 7 shows the HPLC chromatogram of the syringin referral standard while Fig. 8 represents the HPLC chromatogram of TCAE containing syringin at 25°C. The reversed-phase HPLC chromatogram of aqueous extract of TCAE exhibited peaks of syringin corresponding to



retention times at  $16.22 \pm 0.057$  minutes. Syringin was determined by comparing the HPLC chromatograms of the extracts as well as by spiking the extracts with the syringin standard. The same approach was performed to determine the contents of syringin at subsequent temperatures.

#### 4.2.1 Standardization of TCAE

The TCAE was standardized based on the referral compound syringin. The quantitative determination of syringin by HPLC indicated that syringin corresponding to retention times at  $16.22 \pm 0.057$  minutes. The calibration curve

plotted for the standard solution of syringin over the concentration range of 20-1000  $\mu\text{g/mL}$  showed a correlation coefficient ( $r^2$ ) of 0.98. From the calculation, it was found that STCAE is defined to contain at least 0.4 wt% of syringin of total extract.

#### 4.3 Antioxidant Activity of STCAE *in vitro*

Using the optimal extraction parameters obtained, *T. crispa* standardized extract was prepared and evaluated on its antioxidant activity through DPPH, TBA and FRAP bioassays and the results were shown in Figs. 9, 10 and 11.

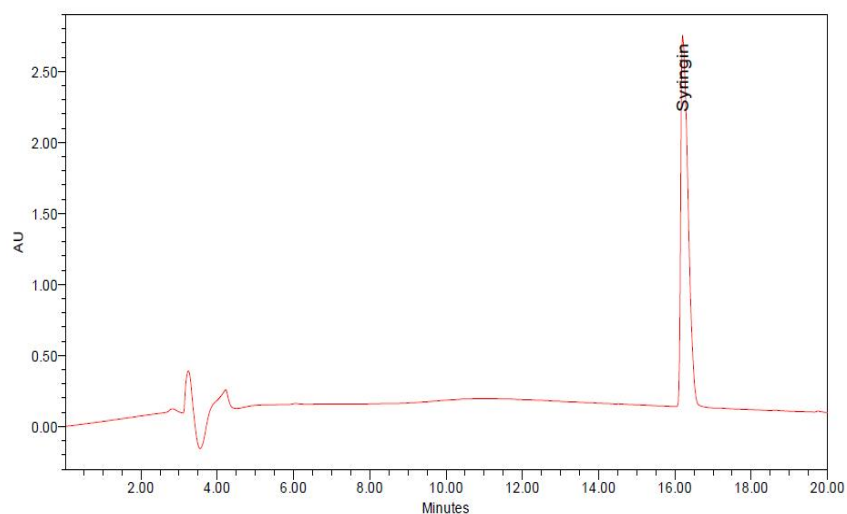


Fig. 7. HPLC chromatogram of syringin standard compound

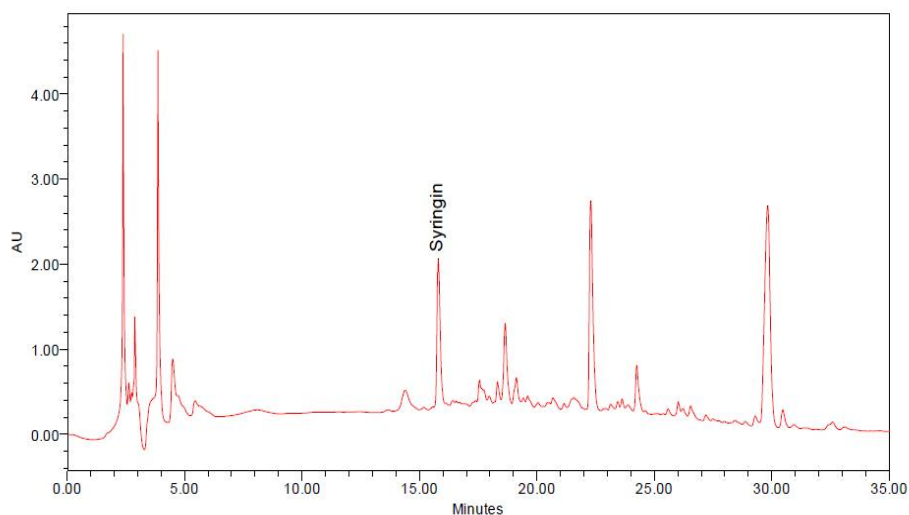
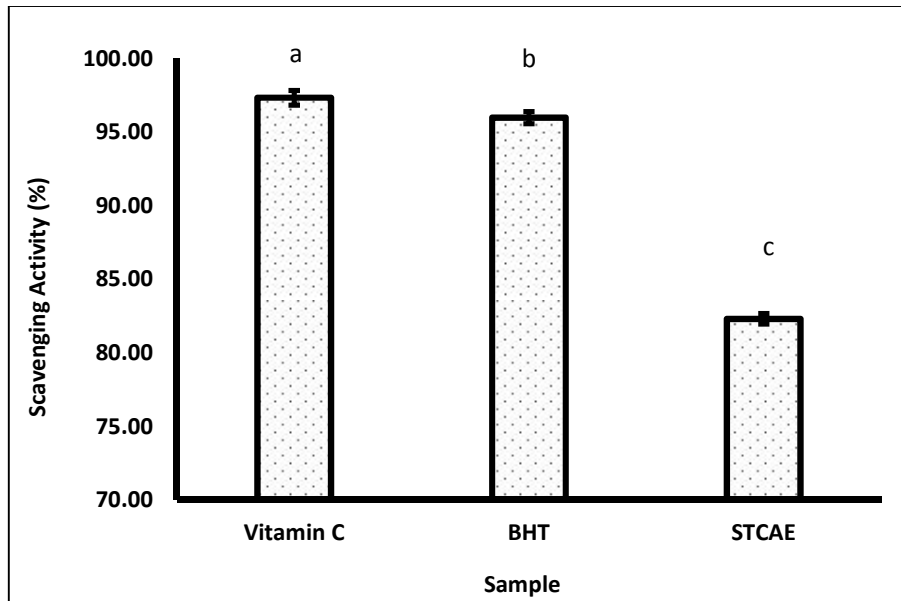


Fig. 8. HPLC chromatogram of TCAE containing syringin at 25°C

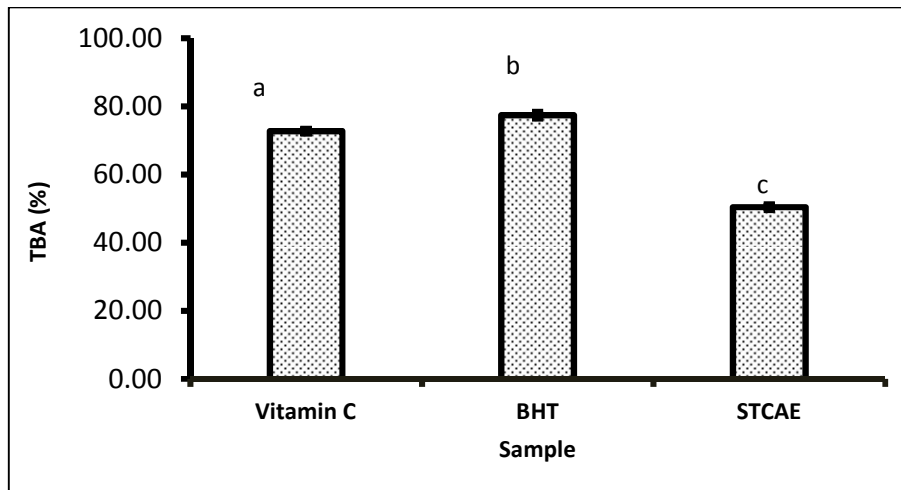
### 4.3.1 DPPH radical scavenging

The DPPH assay was utilized to evaluate the ability of antioxidants to scavenge free radicals. The scavenging activities of STCAE, vitamin C and Butylated hydroxytoluene (BHT) against DPPH radicals were compared and shown in Fig. 9. Results showed that STCAE exhibited high

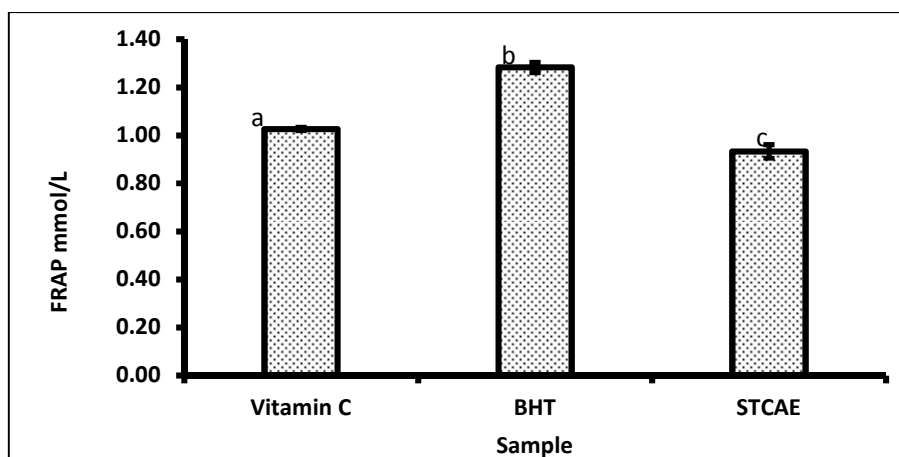
scavenging activity with the percentage of inhibition of  $82.31 \pm 0.37\%$ . However, result showed that the inhibition power of STCAE is significantly lower ( $p < 0.05$ ) when compared to vitamin C and BHT. Vitamin C exhibited the highest inhibition activity with  $97.33 \pm 0.50\%$  followed by BHT  $95.98 \pm 0.41\%$  respectively.



**Fig. 9.** DPPH free radical scavenging of standardized *Tinospora crispa* aqueous extract compared to vitamin C and butylated hydroxytoluene (BHT). Data expressed as mean ± SD. Bars with different alphabets are significantly different ( $p > 0.05$ )



**Fig. 10.** Thiobarbituric Acid Test (TBA) of standardized *Tinospora crispa* aqueous extract compared to vitamin C and butylated hydroxytoluene (BHT). Data expressed as mean ± SD. Bars with different alphabets are significantly different ( $p > 0.05$ )



**Fig. 11. Ferric reducing antioxidant power (FRAP) assay of standardized *Tinospora crispa* aqueous extract compared to vitamin C and butylated hydroxytoluene (BHT). Data expressed as mean  $\pm$  SD. Bars with different alphabets are significantly different ( $p > 0.05$ )**

#### 4.3.2 Thiobarbituric Acid Test (TBA)

The percentage of inhibition of STCAE evaluated in TBA test is shown in Fig. 10. The result showed that, STCAE exhibited moderate lipid peroxidation inhibition activity with MDA value  $50.46 \pm 0.25\%$ . Result indicates that the percentage of inhibition of STCAE was significantly lower ( $p < 0.05$ ) when compared to vitamin C and Butylated hydroxytoluene (BHT). BHT exhibited the strongest antioxidant activity with MDA value  $77.41 \pm 0.34\%$  followed by vitamin C with MDA value  $72.74 \pm 0.25\%$  respectively.

#### 4.3.3 Ferric Reducing Antioxidant Power

The reducing ability of STCAE against the ferric ion which act as oxidant agent is shown in Fig. 11. The results showed that, STCAE exhibited high antioxidant activity with FRAP value of  $0.89 \pm 0.07$  mmol/L. However, the FRAP value of STCAE is not comparable with both Butylated hydroxytoluene (BHT) and vitamin C which again exhibited the strongest antioxidant activity with the value of  $1.28 \pm 0.02$  mmol/L and  $1.03 \pm 0.01$  mmol/L respectively.

## 5. DISCUSSION

Optimization of extraction process is essentially required to obtain the optimum concentration of phytochemical constituents and to maintain their activities [23]. Optimization is also referred as an improvement of performance of a system, process or product to obtain the optimum benefit

from it i.e., high yield of extraction [20]. Higher extraction yield is preferred as the extraction parameters applied are the most cost effective and the most preferable by the industry players. The aim of an extraction process should be to provide for the maximum yield of substances and of the highest quality which consist of high concentration of target compounds and therapeutic effect of the extracts [24].

There are two most commonly methods used in optimization studies, namely the classical single factor experiments and the response-surface methodology (RSM). The classical single factor experiments are a one-factor-at-a-time approach, in which only one factor is varying at a time while all others are kept constant. Present study used the single-factor experiments, despite being having some drawbacks, such as time-consuming, expensive, possible interaction effects between variables cannot be evaluated and misleading conclusions may be drawn [25,21]. However, single factor experiments can provide fundamental information on the ranges for significant extraction parameters on the extraction of targeted compounds from plant materials.

The quantity of solvent during the extraction process is one of the important factors to obtain high extraction yield [26]. Result from present study revealed that, the extraction yield of TCAE was found to increase with the increase in ratio of solvent to solid. These results were consistent with the mass transfer principle where the driving force for mass transfer is according to the

concentration gradient between solid and solvent. A high solid-to-solvent ratio could promote an increasing concentration gradient, resulting in an increase of diffusion rate that allows for greater solid extraction by the solvent [27,28].

The results also showed a marked increase in syringin yield when the ratio of solvent to solid increased proportionally from 10 to 25 ml/g. However, there was no obvious change in syringin yield, as the ratio continued to increase. Therefore, to avoid wastage of solvent use, 15 ml/g was selected as the optimal ratio of solvent to solid. Moreover, the 15 ml/g ratio also showed the highest syringin concentration compared to other ratios studied.

Temperature plays an important role in the extraction of bioactive compounds from plant material. The Results of this study have shown that, the most suitable temperature for obtaining high extraction yield and high concentration of syringin were at 60°C. The similar trend was observed from previous finding who reported that optimum condition to obtain higher yield of syringin from the bark of *Ilex rotunda* was 50°C in which the yield gradually decreased when extraction temperatures increased up to 80°C [29].

Higher temperature increased the solubility and diffusion coefficient of the solute, allowing higher yield and extraction rate [30]. However, elevating the temperatures up to a certain level might affect decomposition of antioxidants which were already mobilized at lower temperatures [31]. Other than that, denaturation of membranes as well as degradation of the polyphenolic compounds may influence quantification of the bioactive compounds [32]. Moreover, extraction costs are expected to increase with increasing of the extraction temperature.

Meanwhile, the result also suggested that, the most suitable extraction time and the most cost effective for obtaining high yield of TCAE was at 1 hour. Result obtained was dissimilar to previous studies who mentioned that the optimum extraction time was at 6 hours [24]. These phenomena could be explained by the Fick's second law of diffusion, predicting that a final equilibrium between the solute concentrations in the solid matrix (plant matrix) and in the bulk solution (solvent) might be reached after a certain time, leading to deceleration in the extraction yield [33].

Moreover, prolonged extraction time increases the chance of decomposition and oxidation of phenolics due to their long exposure to unfavorable environmental factors like temperature, light and oxygen [34]. On the other hand, the increased extraction time is uneconomical and time consuming from the industrialization point of view.

Beside extraction conditions, there are several factors that might affect the differences in percentage of secondary metabolite present in herbal plants. First, different geographical locations of the plant species as sampling locations of the plants were varied from island population, coastal population, and hill forest population [35,36].

The standardized *T. crispa* aqueous extract obtained by following the optimized extraction procedures was further evaluated for its antioxidant properties. The free radical scavenging ability of STCAE was assessed by the discoloration of DPPH solution and was compared to vitamin C and BHT. Results showed that STCAE able to scavenge DPPH radical with the percentage of inhibition of 87.06±0.23%. which in accordance with the previous reports who mentioned that crude aqueous extract of *T. crispa* extracted at 60°C for 6 hours exhibited 85.95±0.52% of scavenging activity against DPPH radical [24].

The result also indicates that, STCAE exhibits significantly lower ( $p<0.05$ ) antioxidant activity (50.46±0.25%) when compared to controls. Interestingly, antioxidant activity showed in this study was higher when compared to previous study [18] who found that crude water *T. crispa* extract obtained from 60°C and 6 hours of extraction parameters exhibited 39.20±2.97% antioxidant activity. It was reported that, prolonged extraction time increases the chance of decomposition and oxidation of phenolics due to their long exposure to unfavorable environmental factors like temperature, light and oxygen [37].

In the FRAP assay, the difference of FRAP value between STCAE with vitamin C and BHT were about 0.09 mmol/L and 0.35 mmol/L respectively. The result clearly showed that STCAE has high antioxidant activity due to the small difference of FRAP value between STCAE and controls. Antioxidant compounds in some herbs are likely to be heat labile [38]. The processes of steaming, flaking, and boiling of plants have been reported

to decrease their biological compounds [39]. Short extraction period and low extraction temperature conducted in present study might preserved the constituents of biological compounds thus contribute to a high antioxidant reading.

High antioxidant activity of STCAE is most probably due to the presence of syringin. It was reported that, syringin isolated from *F. rhynchophylla*, exhibited a strong radical scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) and a potent inhibitory effect against nitric oxide (NO) synthesis, respectively. Syringin also demonstrated the peroxy radical scavenging capacity comparable to that of glutathione [18].

## 6. CONCLUSION

Based on the findings of this study, it can be concluded that to obtain high extraction yields and high syringin concentrations of *T. crispera*, 60°C was selected as the appropriate temperature. The best extraction time was at 1 hour while the optimum solvent to solid ratio was 1:15 g/ml. STCAE contains at least 0.4 wt% of syringin. STCAE was found to possess high antioxidant activity through DPPH, FRAP and TBA bioassays. The results indicate that *T. crispera* can be used as an easily accessible source of natural antioxidants and can be used further as a possible health supplement in the pharmaceutical industry.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

- Najib NAR, Furuta T, Kojima S, Takane K, Ali MM. Antimalarial activity of extracts of Malaysian medicinal plants. *J. Ethnopharmacol.* 1999;64:249-254.
- Koay YC, Amir F. A review of the secondary metabolites and biological activities of *Tinospora crispera* (Menispermaceae). *Trop. J. Pharm. Res.* 2013;12:641–649. DOI: 10.4314/tjpr.v12i4.30
- Quisumbing E. Medicinal plants of the Philippines. Quezon: Katha Publishing Co Inc. Rackova, L., Majekova, M., Kostalova, D. & Stefek, M. (2004). Antiradical and antioxidant activities of alkaloids isolated from *Mahonia aquifolium*. *Structural aspects. Bioorganic & Medicinal Chemistry.* 1951;12(17):4709-4715.
- Li S, Long C, Liu F, Lee S, Guo Q, Li R. Herbs for medicinal baths among the traditional Yao communities of China. *J. Ethnopharmacol.* 2006;108:59–67. DOI: 10.1016/j.jep.2006.04.014.
- Zakaria ZA, Mat Jais AM, Somchit MN, Sulaiman MR, Faizal FO. The *in vitro* antibacterial activity of *Tinospora crispera* extracts. *J. Biol. Sci.* 2006;6(2):398-401.
- Kongkathip N, Dhumma-upakorn P, Kongkathip B, Chawanoraset K, Sangchomkao P, Hatthakitpanichakul S. Study on cardiac contractility of cycloeucalenol and cycloeucalenone isolated from *Tinospora crispera*. *J. Ethnopharmacol.* 2002;83:95-99.
- Noor H, Ashcroft SJH. Antidiabetic effects of *Tinospora crispera* in rats. *J. Ethnopharmacol.* 1989; 27:149-161.
- Ahmad W, Jantan I, Bukhari SNA. *Tinospora crispera* (L.) Hook.f. & Thomson: A review of its ethnobotanical, phytochemical, and pharmacological aspects. *Frontiers in Pharmacology.* 2016; 7(59):1-19.
- Zulhairi A, Hasnah B, Sakinah I, Nur Amalina I, Zamree MS, Mohd Shahidan A. Nutritional composition, antioxidant ability and flavonoid content of *Tinospora crispera* stem. *Adv. Nat. Appl. Sci.* 2009;3:88–94.
- Kamarazaman IS, Amom Z, Ali RM. Inhibitory properties of *Tinospora crispera* extracts on TNF- $\alpha$  induced inflammation on human umbilical vein endothelial cells (HUVECS). *Int. J. Trop. Med.* 2012a;7:24–29. DOI: 10.3923/ijtm.2012.24
- Harwoko & Choironi NA. Quality standardization; 2016.
- Hariyati S. Standardization extracts of Indonesian medicinal plant, one of the important stages in the development of

- Indonesia traditional medicine. Info POM. 2005;6(4):1-5.
13. Rao USM, Zin T, Sundaram CS. The effect of syringin on the expression of TNF- $\alpha$ , iNOS, ICAM-1 and its' mRNA in the heart, brain and kidneys of spontaneously hypertensive rats. *Der Pharmacia Lettre*. 2016;8(3):53-61.
  14. Kim NY, Pae HO, Ko YS, Yoo JC, Choi BM, Jun CD. *In vitro* inducible nitric oxide synthesis inhibitory active constituents from *Fraxinus rhynchophylla*. *Planta Med*. 1999;65:656-658.
  15. Kim HC, An RB, Jeong GS, Oh SH, Kim YC. 1,1-Diphenyl-2-picrylhydrazyl radical scavenging compounds of Fraxini Cortex. *Nat. Prod. Sci*. 2005;11:150-154.
  16. Araujo AL, Mota Soares CM, Moreira De Freitas MJ. Characterization of material parameters of composite plate specimens using optimization and experimental vibrational data. *Composite Part B: Engineering*. 1996;27:185-191.
  17. Bezerra MA, Santelli RE, Oliveira EP, Villar LS, Escalera LA. Response surface methodology (RSM) as a tool for optimization in analytical chemistry. *Talanta*. 2008;76:965-977.
  18. Zulkhairi A, Abdah MA, Kamal NHM, Nursakinah I, Moklas MAM, Hasnah B, et al. Biological Properties of *Tinospora crispa* (Akar Patawali) and its antiproliferative activities on selected human cancer cell lines. *Mal. J. Nutr*. 2008;14:173-187.
  19. Pin KY, Chuah TG, Abdul Rashid A, Law CL, Rasadah MA, Choong TSY. Drying of Betel Leaves (*Piper betle* L.): Quality and drying kinetics. *Drying Technology*. 2009;27(1):149-155. DOI: 10.1080/07373930802566077
  20. Yen GC, Hsieh CL. Antioxidant activity of extracts from Du-zhong (*Eucommia ulmoides*) toward various lipid peroxidation models *in Vitro*. *Journal of Agricultural and Food Chemistry*. 1998;46(10):3952-3957. DOI: 10.1021/jf9800458
  21. Otto C, Geiss HC, Empen K, Parhofer KG. Long-term reduction of C-reactive protein concentration by regular LDL apheresis. *Atherosclerosis*. 2004;174(1):151-156.
  22. Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Analytical Biochemistry*. 1996;239:70-76.
  23. Aziz RA, Sarmidi MR, Kumaresan S. Phytochemical processing: The next emerging field in chemical engineering aspects and opportunities. *J. Kejurut. Kim. Malay*. 2003;3:45-60.
  24. Spigno G, De FDM. Microwave -assisted extraction of tea phenols: A phenomenological study. *J. Food Eng*. 2009;93:210-217.
  25. Bas D, Boyaci IH. Modeling and optimization 1: Usability of response surface methodology. *Journal of Food Engineering*. 2007;78:836-845.
  26. Virot M, Tomao V, Le Bourvellec C, Renard CM, Chemat F. Ultrason Sonochem. 2010;17: 1066-107.
  27. Al-Farsi MA, Chang YL. Optimization of phenolics and dietary fibre extraction from date seeds. *Food Chemistry*. 2007;108(3):977-985.
  28. Tan PW, Tan CP, Ho CW. Antioxidant properties: Effect of solid-to-solvent ratio on antioxidant compounds and capacities of Pegaga (*Centella asiatica*). *International Food Research Journal*. 2011;18:553-558.
  29. Rao USM, Zin T, Abdurrazak M, Ahmad BA. Chemistry and pharmacology of syringin, a novel bioglycoside: A review. *Asian J Pharm Clin Res*. 2015;8(3):20-25.
  30. Zhao LC, He Y, Deng X, Xia XH, Liang J, Yang GL, et al. Ultrasound-assisted extraction of Syringin from the bark of *Ilex rotunda* thumb using response surface methodology. *Int. J. Mol. Sci*. 2012;13(6):7607-7616. DOI: 10.3390/ijms13067607
  31. Silva EM, Souza JNS, Rogez H, Rees JF, Larondelle Y. Antioxidant activities and polyphenolic contents of fifteen selected plant species from the Amazonian region. *Food Chemistry*. 2007; 101:1012-1018.
  32. Liyana-Pathirana CM, Shahidi F. Optimization of extraction of phenolic compounds from wheat using response surface methodology. *Food Chemistry*. 2005;93:47-56.
  33. Abad-Garcia B, Berrueta LA, Marquez DML, Ferrer IC, Gallo B, Vicente F. Optimization and validation of methodology based on solvent extraction and liquid chromatography for the simultaneous determination of several polyphenolic families in fruit juices. *Journal of Chromatography A*. 2007;1154:87-96.
  34. Naczek M, Shahidi F. Extraction and analysis of phenolics in food. *Journal of Chromatography A*. 2004;1054:95-111.
  35. Ullah N, Khurram M, Amin MU, Khan TA, Khayyam SU, Khan FA, et al. Impact of

- geographical locations on *Mentha spicata* antibacterial activities. *Journal of Medicinal Plants Research*. 2012;6(7): 1201-1206,
36. Dong J, Ma X, Wei Q, Peng S, Zhang S. Effects of growing location on the contents of secondary metabolites in the leaves of four selected superior clones of *Eucommia ulmoides*. *Industrial Crops and Products*. 2011;34:1607-1614.
37. David GS, George EI, Diejun C, Atanu B, Fred JE, Roque LE. Phenolic content and antioxidant capacity of supercritical carbon dioxide-treated and air- classified oat bran concentrate microwave-irradiated in water or ethanol at varying temperature. *J Food Chem*. 2007;108:23-30.
38. Bryngelsson S, Mannerstedt-Fogelfors B, Kamal-Eldin A, Anderson R, Dimbergs LH. Lipids and antioxidant in groats and hulls of Swedish oats (*Avena sativa* L). *J Sci Food Agric*. 2002;82:606-614.
39. Rasheed A, Roja C. Formulation, standardization and pharmacological evaluation of a poly herbal traditional remedy- Ashwagandharishtam. *Oriental Pharmacy and Experimental Medicine*. 2012;51-58.

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