

Effect of Processing Condition on the Yield of *Oscimum Gratissimum* Extract

(Kesan Keadaan Pemprosesan terhadap Jumlah Ekstrak bagi *Oscimum Gratissimum*)

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ABSTRACT

Ocimum gratissimum (*O. gratissimum*) is a medicinal plant which native in Southeast Asia. Containing various compound with medicinal value, it acts as herb and spices in cooking, perfumery and used in traditional preparation. It is reported that *O. gratissimum* has many phenolic compounds which responsible for many biological activities such as eugenol, limonene, ocimene and rosmarinic acid. Scientific studies regarding *O. gratissimum* are widely known due to its beneficial therapeutic properties, such as antioxidant, antibacterial, anti-inflammatory and others. However, different extraction techniques approaches will give different effects on extraction yield of bioactive compounds according to the processing condition such as solvent polarity, concentration of solvent, temperature and solvent to sample ratio that applied. This study applied Soxhlet and Ultrasonic-assisted Extraction (UAE) techniques to determine the extraction yield of *O. gratissimum*. The results indicated Soxhlet techniques requires 2 hours, sample to solvent ratio; 1:10 and temperature at 60°C to obtained highest yield of *O. gratissimum* extract with percentage yield at 1.723% compared to others processing conditions. Meanwhile, UAE techniques showed highest yield of *O. gratissimum* extract at sample to solvent ratio at 1:10 with results 20.60% and 60% Methanol as the best solvent for extraction. Throughout analysis, UAE techniques was chosen as the methods of extraction for *O. gratissimum* with processing conditions that gives high yield of extraction.

Keywords: *Ocimum gratissimum*; Extraction Yield; One-factor-at-a-time (OFAT); Soxhlet; ultrasonic-assisted extraction (UAE)

ABSTRAK

Ocimum gratissimum (*O. gratissimum*) adalah sejenis tumbuhan tempatan yang berasal dari Asia Tenggara. Mengandungi pelbagai sebatian kimia dengan nilai perubatan, tumbuhan tersebut biasanya digunakan sebagai herba rempah ratus di dalam masakan dan juga digunakan sebagai persediaan tradisional. *O. gratissimum* telah dilaporkan mempunyai banyak sebatian fenolik yang bertanggungjawab dalam setiap aktiviti biologi seperti eugenol, limonene, ocimene dan asid rosmarinik. Kajian saintifik berkenaan *O. gratissimum* telah banyak dijalankan disebabkan ianya mempunyai khasiat penyembuhan yang sangat baik. Walau bagaimanapun, perbezaan kaedah ekstrak akan memberi kesan yang berbeza terhadap jumlah ekstrak bergantung kepada keadaan pemprosesan yang digunakan seperti kekutuban pelarut, kepekatan pelarut, suhu dan nisbah pelarut-sampel. Kajian ini menggunakan teknik Soxhlet dan pengekstrakan-bantuan ultrasonic (UAE) untuk menentukan jumlah ekstrak yang ada dalam pokok *O. gratissimum*. Data menunjukkan teknik Soxhlet memerlukan masa selama 2 jam, nisbah pelarut-sampel sebanyak 1:10 dan suhu pada 60°C untuk mendapat jumlah ekstrak yang banyak dengan peratusan 1.723% berbanding dengan keadaan pemprosesan yang lain. Manakala, UAE teknik menunjukkan jumlah ekstrak yang tinggi pada nisbah sampel-pelarut pada 1:10 dengan bacaan 20.60% dan 60% Metanol sebagai solvent yang sesuai untuk pengekstrakan. Selepas analisis, UAE teknik dipilih sebagai kaedah pengekstrakan *O. gratissimum* mengikut keadaan pemprosesan yang mempunyai bacaan jumlah ekstrak yang tinggi.

Kata Kunci: *Ocimum gratissimum*; Jumlah Pengekstrakan; Satu-faktor-satu-masa (OFAT); Soxhlet; Pengekstrakan-dibantu ultrasonic (UAE)

INTRODUCTION

Ocimum gratissimum from *Lamiaceae* (or *Labiatae*) family has many biological activities such as antibacterial, antifungal, antidiarrheal, anti-inflammatory activity. *O. gratissimum* can be considered as an excellent source for pharmacological properties such as anticancer, antitussive, antibacterial or antiviral (Mehta et al. 2017). Review articles has highlighted the pharmacological significance of ursolic acid, which is pentacyclic triterpenoid ursolic acid and oleanolic acid, known to serve as the biomarker for this plant including possessing diverse pharmacological actions such as antidiabetic, anti-HIV, anti-carcinogenic, antiulcer, hepatoprotective and so on. Aside from pharmacological properties, ursolic acid possess important therapeutic properties that makes it highly valued bioactive entity both commercially and therapeutically, hence it is commonly used in industry. It gives flavor in food, has nice scent, and good benefits to human health. *Ocimum gratissimum* are known as kemangi, ruku-ruku (Indonesia), selasih (Malaysia), nimma tulasi (Kannada), ram tulasi (Hindi), and vriddhutulsi (Sanskrit).

Major compound from *O. gratissimum* are eugenol, limonene, myrcene, ocimene, *p*-cymene, γ -terpinene (from essential oil) and methyl chavicol, methyl cinnamate, rosmarinic acid (from extract). From a study conducted by (Yamani et al. 2016) camphor, eucalyptol and eugenol are among the compounds out of 54 compounds identified in the leaves, flower spikes, or essential oil, which are responsible for antimicrobial activity against bacteria. The essential oils are rich in phenolic compounds and wide array of other natural products including polyphenols such as flavonoid and hydroxyl group that showed the antioxidant activities (Ijaz et al. 2017). Polyphenols are plants secondary metabolites that plays a very important role as it acts as natural antioxidants where it can be found abundant that possess interesting properties, such as free-radical scavenging and inhibition of various oxidative stress in the body (Maghsoudlou et al. 2016). However, among the active components, eugenol is reported as the the most dominant among various other chemotypes as marker constituents that have been identified and extracted, hence being largely responsible for therapeutic properties (Mohan et al. 2011; Raina et al. 2013). These results met the agreement with the research done by (Gulcin 2011) who confirms that eugenol had the most powerful antioxidant activity and radical scavenging activity as eugenol inhibited a very high lipid peroxidation percentage which is 96.7% of a linoleic acid emulsion at a 15- μ g/mL concentration.

The antioxidant activity and total phenolic assay in methanolic extract from the leaves of this species by using ascorbic acid as standard antioxidant has been proven in a research paper by (Ravi et al. 2012) while (Balaji 2011) supported based on the results of DPPH free radical scavenging assay shown from his research that the extracts are capable of scavenging free radicals via electron (or) hydrogen donating mechanisms.

In this modernization era, the world has been far advanced in innovations of food and medical technology such as machines for different extraction methods. These technologies claimed that the discovery will facilitate our daily routines (Abdul Rahim 2012). There are many extraction methods such as maceration, percolation, hydrodistillation, microwave-assisted, accelerated-solvent, Soxhlet, and ultrasound-assisted extraction. Two methods with different condition extraction will be highlighted throughout this paper. Soxhlet extraction will use a thimble (soft, porous filter paper) which dried plant material will leachate its crude extract when react with vaporized then condensed solvent from round-bottomed flask. Soxhlet extraction is employed when the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. The advantage of this method is that it can be allowed with less monitoring while the process keep recycling solvent until it dissolves the large amount of material (Rassem et al. 2016). Meanwhile, ultrasonic-assisted extraction uses ultrasound energy ranging from 20 kHz to 2000 kHz. The formation and implosive collapse of bubbles in a liquid irradiated which is called acoustic cavitations will grow and recompress the solvent and solute vapour thus make it well extracted. This way, the time needed and solvent consumption for extraction is reduced, high yield of extract, improved quality and prevention against any extract degradation (Ahmed Khalil et al. 2017). This paper aims to determine the effect of different extraction condition on *O. gratissimum* extraction yield.

METHODOLOGY

EXPERIMENT DESIGN AND PROCEDURE

The leaves of *O. gratissimum* (Figure 1) were dried at 40°C (Njoku et al. 2017) for overnight (Ukoroiije et al. 2018) and crushed prior extraction. For Soxhlet extraction, 200g of dried samples was extracted by varying ratio-sample-to-solvent (1:10, 1:40), extraction time (2 and 6 hours) and temperature at 60°C and 78°C. Solvent used in Soxhlet extraction was absolute ethanol (Merck, Germany). The Soxhlet apparatus was set as shown in Figure 2. The total extract was then filtered using Whatman No. 1 filter paper. Excess solvent were removed under reduced pressure at 40°C by using rotary evaporator. Parameter for this extraction is analyzed by using one-factor-at-a-time (OFAT) design.

For ultrasonic-assisted extraction (UAE), only two parameters assigned. The parameters were sample-to-solvent ratio (1:10, 1:20, 1:25) and methanol (Merck, Germany) concentration (100%, 80%, 60%, 40%, 20% and 0%). For each run, 10 g of powdered leaves is weighed and mixed with 50 mL volume of methanol. The sample is subjected to UAE for 20 minutes with 50 A amplitude using Ultrasonic probe. Once the extraction completed, the extract was filtered using Whatman No.1 filter paper. The filtrate was then subjected to dry using rotary evaporator. After that, it is dried in oven at 45°C for 2 days. Figure 3 illustrated a UAE apparatus set-up. Excess solvent was removed under reduced pressure at



FIGURE 1. *Ocimum gratissimum* plant



FIGURE 2. Soxhlet extractor

40°C. Parameter for this extraction is analyzed similar to Soxhlet extraction.

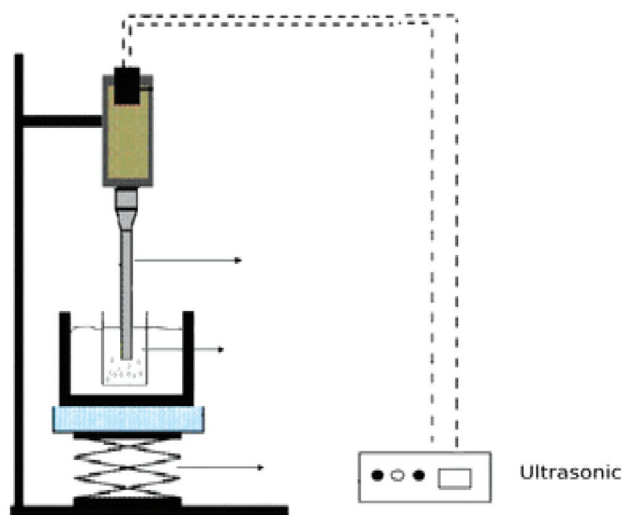


FIGURE 3. An ultrasonic-assisted extractor

DETERMINATION OF EXTRACT YIELD

Extraction yield is determined by the amount of crude extract after all the solvents removed through rotary evaporator. The dried extract should leave small clumps of solid attached on the walls of petri dish. The percentage of yield was weighed and calculated by using this equation.

Extraction yield (%w/w)

$$= \frac{\text{weight of dried material}}{\text{weight of raw material}} \times 100\% \quad (1)$$

RESULT AND DISCUSSION

EXTRACTION YIELD OF *O.GRATISSIMUM* LEAVES USING SOXHLET EXTRACTION

TABLE 1. Weight of yield according to parameters for Soxhlet extraction

Run	Factor A Time (hr)	Factor B sample- to-solvent (g/ml)	Factor C Temperature (°C)	Response I* Yield (g)	Mean percentage Yield (%)
1	2.00	1:10	60	3.4453	1.723
2	6.00	1:10	60	1.2034	0.602
3	2.00	1:40	60	1.2771	0.639
4	2.00	1:10	78	1.7201	0.860

*data is not repeated for three times

Percentage of yield from Soxhlet extraction

$$= (\Sigma X_{\text{weight of dried extract}} / \Sigma X_{\text{weight of raw material}}) \times 100\%$$

$$= \frac{3.4453 \text{ g}}{200.00 \text{ g}} \times 100\% = 1.723\%$$

Extraction Yield of *O. gratissimum* leaves by using UAE techniques

TABLE 2a. Weigh of yield according to sample-to-solvent ratio for UAE

Sample-to-solvent ratio	Mass of extraction (g)			Mean of yield (g)	Mean percentage
	1	2	3		
1:10	2.07	2.05	2.06	2.060	20.60
1:20	0.68	0.67	0.67	0.673	6.73
1:25	1.60	1.60	1.61	1.603	16.03

TABLE 2b. Weigh of yield according to methanol percentage for UAE

Methanol percentage	Mass of extraction (g)			Mean of yield (g)	Mean percentage
	1	2	3		
20%	0.69	0.67	0.68	0.680	6.80
40%	1.36	1.35	1.35	1.353	13.53
60%	1.71	1.72	1.73	1.720	17.20
80%	1.45	1.47	1.47	1.463	14.63
100 %	1.48	1.49	1.49	1.487	14.87

Percentage of yield from according to sample-to-solvent ratio for UAE (2a)

$$= (\Sigma X_{\text{mean of yield}} / \Sigma X_{\text{weight of raw material}}) \times 100\%$$

$$= \frac{2.06 \text{ g}}{10.00 \text{ g}} \times 100\% = 20.60\%$$

Percentage of yield from according to methanol percentage for UAE (2b)

$$= (\Sigma X_{\text{mean of yield}} / \Sigma X_{\text{weight of raw material}}) \times 100\%$$

$$= \frac{0.680 \text{ g}}{10.00 \text{ g}} \times 100\% = 6.80\%$$

In this study, there is a similar result between Soxhlet and UAE techniques in terms of sample to solvent ratio as shown in Figure 4b and Figure 5a, respectively. Soxhlet extraction revealed almost 1.72% of extraction yield vice versa to UAE techniques, which gave 20.60% higher for respective parameter. This is maybe due to the longer time extraction, 2 hours used in Soxhlet techniques causing increase the temperature of solvent compared to UAE techniques need

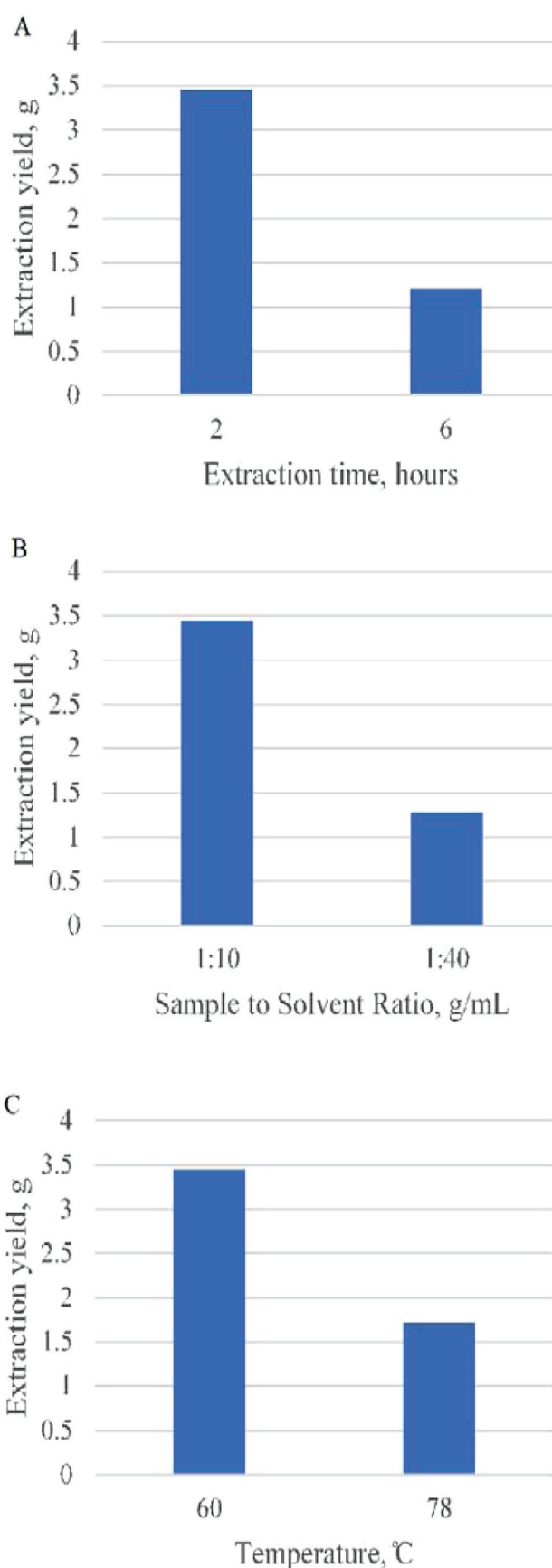


FIGURE 4. Effects of extraction time (a), sample to solvent ratio (b) and temperature (c) on the *O. gratissimum* yield using soxhlet extraction technique

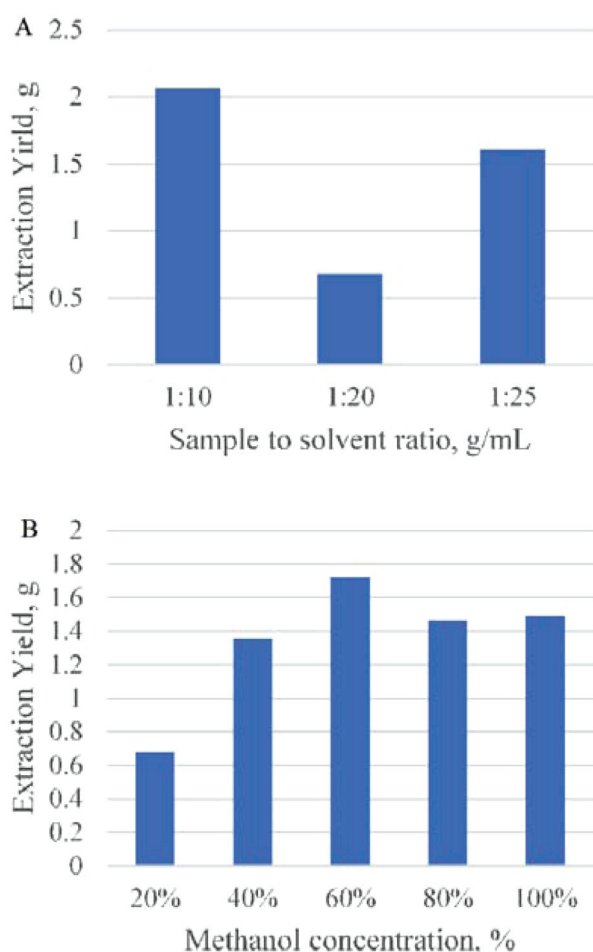


FIGURE 5. Effects of sample to solvent ratio (a) and methanol concentration (b) on the *O. gratissimum* yield using UAE extraction technique

a few minutes, 20 minutes to extract bioactive compounds (Porevsky et al. 2014; Amirah & Khan 2012). Therefore, the degradation of phytochemicals content occurred in Soxhlet techniques (de Lima Silva et al. 2015). The results for sample to solvent ratio at 1:20 for UAE techniques decrease due to the solvent retained or solvent not completely removed before weighing. Thus, the readings of extraction need to take until reached constant readings to ensure solvent in extract completely dry or removed (Zarei et al. 2017; Baba et al. 2018).

Throughout this study, we can see Soxhlet techniques obtained high extraction yield at low temperature, 60°C with percentage extraction yield at 1.723%. But, compared to extraction yield at 78°C only obtained 0.860% as shown in Figure 4c. This is maybe due to the boiling point of ethanol solvent which is 78.37°C affect the yield of extract. Thus, it can be concluding not only temperature influencing the yield of extract but also type of solvent (Efthymiopoulos et al. 2018).

In Figure 5b, showed that 60% Methanol as the best solvent to extract bioactive compounds by using UAE techniques with high percentage of extraction yield at

17.20%. According to Mohamed et al. (2018) study has showed the co-solvent more effective compared to mono-solvent in terms of extraction yield and increase the polarity of bioactive compounds to be extracted. Similar results were obtained in study of Kamarudin et al. (2016) which varied extraction methods and solvent concentration. Thus, choice of solvent is important according to the targeted polarity of bioactive compounds

Results from this study suggested that temperature of the solvent gave negligible effect towards extract yield. Higher temperature may affect the yield of extraction of bioactive compounds like showed in Soxhlet techniques. It is due to prolonged heating and certain phytochemical compounds are thermolabile, which destroyed when exposed to higher temperature during extraction. Thus, choice of solvent such as hexane, chloroform and toluene or mixture with other solvents may prevent degradation of bioactive compounds especially thermolabile compounds (Khoddami, Wilkes & Roberts 2013). Sample to solvent ratio for both techniques was chosen at 1:10. Meanwhile, extraction time for Soxhlet techniques at 2 hours are maintained temperature at 60°C to obtained high extraction yield. UAE techniques is extract high yield at 20 minutes using 60% methanol solvent.

CONCLUSION

Based on the results, UAE techniques is suitable to obtained wide polarity of compounds with high recovery yield of extract (20.60%) compared to Soxhlet techniques. This is due to the less extraction time and less consumption solvent by controlling temperature accordingly to obtained high yield of extract.

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