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Water-based extraction methods of Moringa oleifera leaves for xanthine oxidase inhibitory activity

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Abstract

The purpose of this study is to investigate the medicinal properties of Moringa oleifera (M. oleifera) leaves towards xanthine oxidase inhibitory activity by using water-based extraction methods such as boiling extraction, subcritical water extraction (SWE) and ultrasonic-assisted extraction (UAE). Boiling extraction is chosen as the control method in this study due to its commonly used in the industry. Water based extraction is investigated to determine if it can replace the conventional extraction method which uses organic solvent and time consuming. Temperature and time were investigated using central composite design (CCD) for maximum xanthine oxidase inhibitory activity in SWE and UAE. The boiling extraction is operated at fixed temperature of 100 °C while SWE is utilised at temperature of 100 to 180 °C and UAE at 30 to 60 °C with same time within 10 to 30 min. These variables are used to determine optimum condition on each method for xanthine oxidase inhibitory activity of M. oleifera leaves, then compared with the control method to identify ideal method of extraction. Thus, SWE (69.65 ± 0.1 %) at 180 °C and 20 min is the ideal method compared to UAE (48.89 ± 0.3 %) at 60 °C and 30 min and boiling extraction (41.88 ± 0.1 %) at 100 °C and 20 min due to higher percentage of xanthine oxidase inhibitory activity obtained on M. oleifera leaves. The findings of this study show that SWE offer better alternative for M. Oleifera extraction towards xanthine oxidase inhibitory compares to other methods.

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1.0 Introduction

Water-based extraction methods is a green technique that has become the reference method for the researchers to obtain therapeutic value from the medical plants (Zagula et al., 2017). It is due to the used of water as a solvent which is low cost, safe and abundantly available compared to alcohol solvents. The extraction methods are divided into two categories which are conventional and non-conventional. Boiling extraction is the conventional technique which has been traditionally used to extract the phytochemical compounds from plant materials. Up to date, the boiling extraction method is still widely utilised in the industry compared to subcritical water extraction (SWE) and ultrasonic-assisted extraction (UAE), hence boiling extraction was chosen as the control method in this study. SWE is a method that operate in subcritical condition at temperature within 100 to 374 °C and pressure high enough to maintain the liquid state while UAE was assist by the ultrasonic waves.

M. oleifera or locally known as '*rembungai*' is a small deciduous tree native to tropical Asia but also naturalized in Africa. These *M. oleifera* leaves are proved to obtain valuable phytochemical compounds like alkaloids, flavonoids, saponins, terpenoids, phenolics and tannins that are useful in therapeutic activity especially xanthine oxidase inhibitor (Leone et al., 2015; Nouman et al., 2016). For instance, numerous research has discussed on medicinal properties in *M. oleifera* that possess as antioxidant, antimicrobial, anti-cancer, anti-diabetic and anti-diarrheal activity (Hossain et al., 2015; Tiloke et al., 2016; Tshabalala et

al., 2019). Therefore, the extracted phytochemical compounds on *M. oleifera* leaves is one of the safest alternatives in the pharmaceutical field instead of commercial extract that use chemical which may give various side effect.

As for the xanthine oxidase inhibitory activity, it is related to anti-gout activity where occurs due to the excess production of uric acid from purine bases in human body. Xanthine oxidase is an enzyme that enhances the production of uric acid that leads to gout disease. However, there is limited study on *M. oleifera* for xanthine oxidase inhibitory activity compared to the other medicinal plants as *Euphorbia hirta, Dioscorea hispida, Plantago major* and *Symphytum officinale* (Tjitraresmi et al., 2018; Mehmood et al., 2019). Hence, the research on xanthine oxidase inhibitory activity for *M. oleifera* remains inadequate.

In general, the objective of this study is to extract, analyse and compare extracted *M. oleifera* leaves on each water-based extraction methods at optimum condition to obtain the maximum percentage of xanthine oxidase inhibitory activity. This research is focusing on the effect of extraction parameter such as temperature and time on xanthine oxidase inhibitory activity from *M. oleifera* leaves using CCD in Response Surface Methodology (RSM) as suggested by Stamenković et al. (2018). Finally, the optimum condition from the control method which is boiling extraction is compared with SWE and UAE to identify ideal method of extraction on *M. oleifera* leaves for xanthine oxidase inhibitory activity.

2.0 Methodology

2.1 Chemicals and reagents

Potassium di-hydrogen phosphate (KH_2PO_4), di-potassium hydrogen phosphate (K_2HPO_4), xanthine and dimethylsulphoxide (DMSO) were purchased from Evergreen Engineering & Resources, Selangor. Xanthine oxidase (buttermilk) was purchased from Merck Sdn Bhd, Selangor.

2.2 Plant materials and sample preparation

M. oleifera dried leaves was acquired from MR Moringa Sdn Bhd, Kuala Terengganu, Terengganu, Malaysia. The sample was grinded into powder form by using blender and was sieved into 1.18 mm and 0.6 mm size of particles using sieved shaker. The moisture content was measured to be 7%.

2.3 Extraction

In extraction process, the sample was extracted using boiling, SWE and UAE. The SWE and UAE was run for 13 times on each method. The solid to liquid ratio was fixed at 1:20 (sample to solvent ratio) for all extraction method. The temperature and time have been selected as the extraction parameter due its major influence on the results obtained. As for boiling extraction, the heating plate is used in extraction process. The extraction parameter of boiling extraction as temperature was fixed at 100 °C and time at 10, 20, and 30 min (Henriques et al., 2018). Temperature was reported to be the major factor in most of the extraction of bioactive compound from plants.

Then, for SWE, the subcritical water extractor was used in extraction process. The pressure was fixed at 10 MPa. Temperature which was the first parameter for SWE extraction is range from 100 to 180 °C while the second parameter; time is range from 10 to 30 min (Zhang et al., 2018; Nile et al., 2019). As for UAE, the ultrasonic bath was used in the extraction process. The power and frequency were fixed at 100% and 28 kHz, respectively. The extraction parameter for UAE was set for temperature between 30 to 60 °C and time within 10 to 30 min (Moorthy et al., 2017; Dadi et la., 2019). It is important to note that the temperatures used were generally below 70 °C to avoid reduction in cavitation effects that could further affect the degradation of the thermolabile compounds (Chemat et al., 2017; Roohinejad et al., 2017). The extracted samples were stored without further purification at −18 °C for further analyses.

Table 1: Factors and levels in CCD for SWE.

Factors	Levels		
	−1	0	1
Temperature (°C)	100	140	180
Time (min)	10	20	30

Table 2: Factors and levels in CCD for UAE.

Factors	Levels		
	−1	0	1
Temperature (°C)	30	45	60
Time (min)	10	20	30

2.4 Design of experimental (DoE)

An experimental design was performed to investigate the effect of two factors: temperature and extraction time on the xanthine oxidase inhibitory activity of *M. oleifera* leaves.

RSM was used to identify the combination of two factor on one response which has been optimised by SWE and UAE extraction process. Centralised Composite Design (CCD) was utilised to perform this experiment by 13 runs with 5 repetitions at the centre point. Several outliers detected from the analysis was repeated and the average was calculated. The factors and respective levels applied for SWE and UAE method are shown in Table 1 and Table 2.

2.5 Xanthine oxidase assay

Anti-gout activity from *M. oleifera* was analysed based on the xanthine oxidase inhibitory activity which is indicated by the higher percentage of inhibition. The extract was first retrieved from the freezer and was filtered before analysed. Next, the assays activity was done by mixing all the chemicals such as potassium phosphate (buffer pH 7.5), extracted sample, enzyme solution [0.2 units/ml of xanthine oxidase enzyme (degradation temperature at 135 °C) in phosphate buffer] and distilled water to pre-incubate for 15 min at 37 °C. Then, the substrate solution (0.15 mM of xanthine) was added and incubated for another 15 min at 37 °C.

The sample was analysed using UV-vis spectrophotometer at 295 nm to obtain the absorbance of sample, A_{sample}. Furthermore, the procedure was repeated by replacing the extracted sample with DMSO for the absorbance of control, $A_{control}$. The DMSO was used to maximize the production of uric acid and compared with the sample. The percentage of xanthine oxidase inhibitory activity is calculated using Eq. (1)

$$\% \text{ inhibition } = \frac{A_{control} - A_{sample}}{A_{sample}} \times 100\% \qquad (1)$$

where: $A_{control}$ is absorbance of control and A_{sample} is absorbance of sample.

2.6 Comparison

The percentage of xanthine oxidase inhibitory activity at optimum condition obtained from boiling extraction was compared with SWE and UAE. This was done to determine the ideal method of extraction on *M. oleifera* leaves for xanthine oxidase inhibitory activity.

3.0 Results and discussion

3.1 Xanthine oxidase inhibitory activity for boiling extraction

The result shows the percentage of xanthine oxidase inhibitory activity for boiling extraction was varied from 20.12 ± 0.4% to 41.88 ± 0.1%. Fig. 1 exhibited percentage of inhibition against the extraction time at 100 °C which is boiling point of water.

The percentage of inhibition increased steadily from 20.12 ± 0.4% to 41.88 ± 0.1% within 10 to 20 min but was slightly reduced to 36.59 ± 0.6% after 30 min. This was due to the *M. oleifera* leaves long exposure to the water boiling point which caused a degradation of required bioactive compounds for xanthine oxidase inhibitory activity (Suo et al., 2016). Therefore, the optimum condition of boiling extraction was acquired at 100 °C and 20 min. with percentage of xanthine oxidase inhibitory activity of 41.88 ± 0.1%

3.2 Xanthine oxidase inhibitory activity for SWE

On the other hand, the percentage of xanthine oxidase inhibitory activity for SWE was determined within 25.88 ± 0.1% to 69.65 ± 0.1%. By referring to Fig. 2, 3D surface graph for interaction between temperature and time against percentage inhibition was highlighted.

The percentage of inhibition was gradually increased at higher extraction temperature and time. Theoretically, it was related to the dielectric constant of water where the values were reduced like organic solvent under subcritical condition (Manousi et al., 2019).

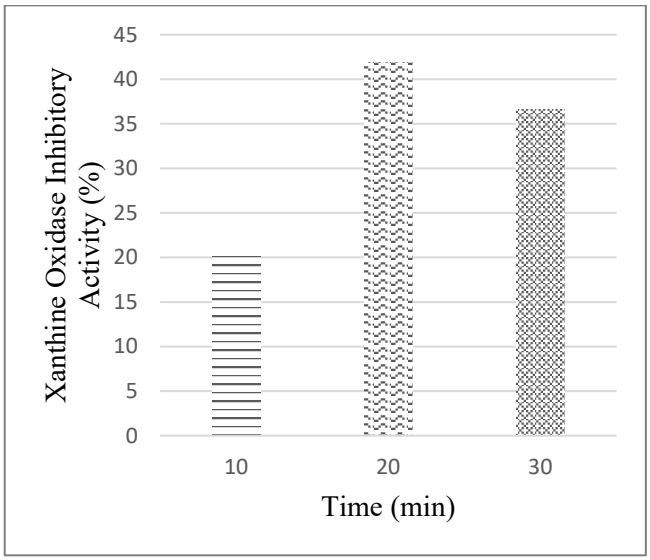

Fig. 1: Percentage of inhibition of xanthine oxidase activity against time for boiling extraction.

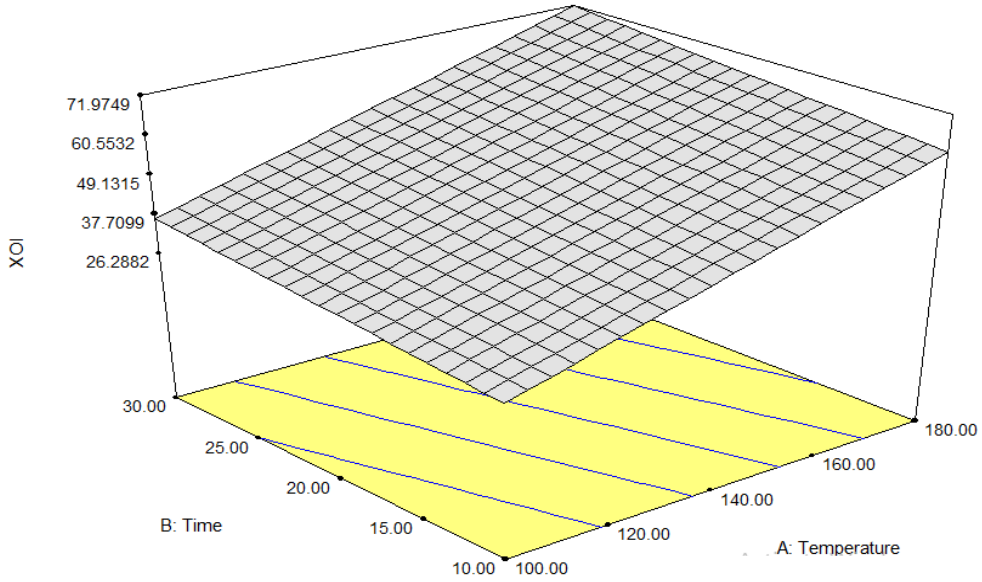

Fig. 2: 3D surface graph of interaction between temperature and time against percentage of inhibition for SWE.

In this condition, the use of water as the solvent was an advantage since it able to induce more phytochemical compounds needed as xanthine oxidase inhibitor efficient extraction process.

However, inhibition percentage was moderately decreased from 69.65 ± 0.1% to 63.58 ± 0.6% when the extraction temperature and time achieved its maximum condition which is at 180 °C and 30 min. It is because of longer extraction time utilised might attributed to the possible reduce in the concentration of bioactive compounds because of solute degradation molecular transformation (Munir et al., 2018). These degradation of analytes or formation of new compounds are unavoidable at very high temperature (Plaza & Turner, 2015). Thus, it could be assumed that the optimum condition for SWE is at 180 °C and 20 min with percentage of xanthine oxidase inhibitory activity of 69.65 ± 0.1%.

The results were further supported by the analysis of variance (ANOVA) where it indicated that the 3D surface graph in Fig. 2 was in linear form with the R^2 obtained is 87.6%. Then, the outcome shows that both factors which temperature and time significance towards the response since were ($p < 0.05$) as shown details in Table 3.

Table 3: Details on ANOVA for SWE method.

Source	Value		
	Mean square	F-value	p-value
A (temperature)	1873.37	65.10	< 0.0001
B (time)	160.58	5.58	0.0398
Residual	28.78	-	-
Lack of fit	31.85	1.32	0.4120
R^2 (%)		87.6	

3.3 Xanthine oxidase inhibitory activity for UAE

For UAE, the percentage of xanthine oxidase inhibitory activity was acquired in range of 23.19 ± 0.5% to 48.89 ± 0.3%. As shown in Fig. 3, it represents 3D surface graph for interaction between temperature and time against percentage inhibition.

In general, the percentage inhibition was constantly increase at higher extraction temperature and time which is up to 48.89 ± 0.3%. The higher percentage inhibition is due to the rise in mass transport in ultrasonic assisted extraction process that caused a more cavitation effect of ultrasonic waves and disrupted plant cell walls (Tiwari, 2015). Thus, it able to induce more solvent to penetrate the cellular materials and more phytochemical compounds from the plant material were extracted. Hence. The optimum condition for UAE was at 60 °C and 30 min with the percentage of xanthine oxidase inhibitory activity of 48.89 ± 0.3%.

In addition, the results were further supported by the analysis of variance (ANOVA) where it indicated that the 3D surface graph in Fig. 3 is in linear form with the R^2 obtained is 75.5%. Then, the outcome shows that the temperature factor is significance towards the response as while the time factor is not significance towards the response since ($p > 0.05$) as shown details in Table 4.

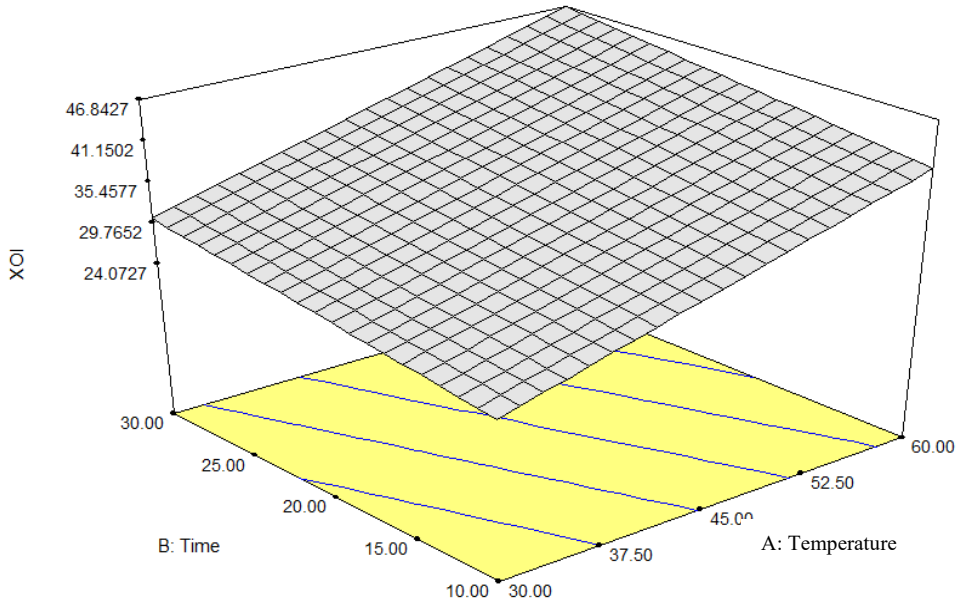

Fig. 3: 3D surface graph of interaction between temperature and time against percentage of inhibition for UAE

Table 4: Details on ANOVA for UAE method.

Source	Value		
	Mean square	F-value	p-value
A (temperature)	398.37	26.63	0.0004
B (time)	62.86	4.20	0.0675
Residual	14.96	-	-
Lack of fit	22.25	5.52	0.0601
R^2 (%)		75.5	

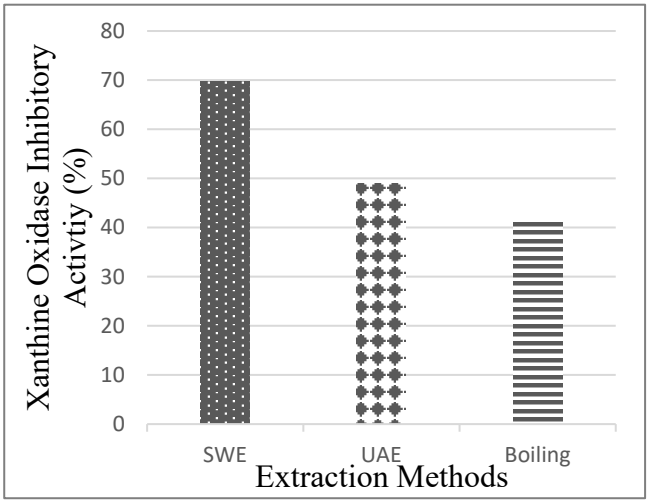

Fig. 4: Graph comparison of xanthine oxidase inhibitory activity against extraction methods.

3.4 Comparison

In comparison, the percentage of xanthine oxidase inhibitory activity at optimum condition obtained from boiling extraction is compared with SWE and UAE method as represent in Fig. 4.

From the results, the inhibition percentage of xanthine oxidase activity for boiling extraction or control method at optimum condition is the lowest which is 41.88 ± 0.1% compared to SWE and UAE at 69.65 ± 0.1% and 48.89 ± 0.3%. It is due to this modern method of extraction was assist by the other factor such as SWE is related to the application of subcritical condition while UAE was influence by the ultrasonic waves.

However, boiling extraction is just the traditional method used by local folk with the application of heat. In summary, these results indicated that the extraction of *M. oleifera* for xanthine oxidase inhibitory activity using SWE is more efficient due to higher percentage of xanthine oxidase inhibitory activity obtained at 69.65 ± 0.1%. Finally, SWE method also able to induce the phytochemical compounds that act as xanthine oxidase inhibitor for anti-gout activity

4.0 Conclusions

As the conclusion, the ideal method of extraction of *M. oleifera* leaves for xanthine oxidase inhibitory activity is SWE method at optimum condition of 180 °C and 20 min. Thus, the results also prove that temperature and time are highly significant with the process in SWE of *M. oleifera* due to higher inhibition percentage of 69.65 ± 0.1% obtained. The validation of the optimum condition showed a good correlation between optimised and experimental data which is indicated by the R^2 value and p-value. Therefore, the

findings from this study shows great potential for the extraction of *M. Oliefera* bioactive compound using water-based extraction which is not only safe but also requires shorter extraction time.

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