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Effect of extraction conditions on physical and antioxidant properties of Yanang (*Tiliacora triandra*) leaf extract

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Abstract

The purpose of this study was to investigate the effect of extraction conditions on the physical and antioxidant properties of Yanang (*Tiliacora triandra*) leaf extract. The studied extraction conditions were leaves to water ratio (1:6 and 1:9), blending speed (22,000 and 32,000 rpm), heating temperature (60, 70, and 80 °C), and heating time (1, 3, 6, 9, 12, and 15 min). The extracts were examined for physical (color and stability) and antioxidant total phenolic content (TPC), 2,2-diphyneyl-2-picryl-hydrazyl (DPPH) radical scavenging activity, and Xanthine oxidase inhibitory activity) properties. Delta E (ΔE^*) decreased with an increase in ratio but it increased with increase blending speed. Increases in ratio, blending speed, and time lowered the stability, however, the temperature did not affect the stability. The antioxidant properties improved when the ratio and blending speed increased. Higher temperature and longer time declined the antioxidant properties of the extract. Results showed that the extraction conditions much affected antioxidant activity than physical properties. The degradation of TPC followed the zero-order reaction (R^2 0.676-0.938) while the degradations of DPPH radical scavenging (R^2 0.738-0.969) and Xanthine oxidase inhibitory (XOI) activities (R^2 0.790-0.986) followed the second-order reaction. The most suitable extraction condition was the extraction using a 1:9 ratio, 32,000 rpm, 60°C, and 1 min. This study proposed the Arrhenius equation of TPC, DPPH radical scavenging activity, and XOI activity with a correlation coefficient of 0.792, 0.938, and 0.735, respectively.

Keywords: *Tiliacora triandra*, Antioxidant, Xanthine oxidase, Extraction condition

1. Introduction

Yanang (*Tiliacora triandra)* belongs to the family of *Menispermaceae*. In Southeast Asian countries, Yanang has been used as traditional medicine and in cuisines especially in traditional bamboo shoot soup [1]. Yanang root is known as antipyretic and antimalarial agents [2] while its leaves have been used for anticancer and immune modulator [3]. It has been revealed that the water extract of Yanang leaf does not cause acute or subchronic toxicities in either male or female rats [4].

Yanang leaves own an antioxidative property since they contain phenolic compounds namely ferulic acid, *p*-coumaric acid, sinapic acid, and syringic acid [5,6]. Yanang leaves are also well-known as a remedy for gout (gouty arthritis) which is the condition that the body accumulates excess uric acid. Gout treatment involves the use of therapeutic agents such as xanthine oxidase inhibitors [7]. Xanthine oxidase is the enzyme that converts hypoxanthine into xanthine and then into uric acid. Xanthine oxidase inhibitor can block this biosynthesis of uric acid. It is believed that xanthine oxidase inhibitor augments the excretion of uric acid and reduces the uric acid formation, thus, consequently reducing the risk of gout [8].

Recently, canned bamboo shoots in Yanang leaf extract is a new product introduced to the food market. The production of canned bamboo shoots in Yanang leaf extract requires an extraction process of Yanang leaves. This process is previously done to obtain Yanang leaf extract which is further hot-filled into an aluminum can

contained with bamboo shoots before sterilization. The Yanang leaf extraction involves an extraction process, itself, and a pre-heat treatment process. Several extraction conditions should be considered such as leaves to water ratio, blending speed including heating temperature, and heating time. Impaired physicochemical properties of food products during processing have been reported such as an increased color difference Delta E (ΔE^*) with heating temperature and time [9-11], a decrease of physical stability in the walnut beverage with a rise of heating temperature and time [12]. Other studies demonstrate that thermal process decreases 72-78% of total phenolic content (TPC) and 61-74% of 2, 2-diphyneyl-2-picryl-hydrazyl (DPPH) radical scavenging activity [13,14]. However, few studies have been investigated on the physical and antioxidant properties of Yanang leaf extract during processing, especially on Xanthine oxidase inhibitory (XOI) activity. Therefore, the objectives of this study were to investigate the effect of extraction conditions on the physical property (ΔE^* and physical stability) and antioxidant property (TPC, DPPH radical scavenging activity, and XOI activity) of Yanang leaf extract.

2. Materials and methods

2.1 Plant material

Yanang (*Tiliacora triandra*) leaves were purchased from a local market in Prachinburi province, Thailand. After washing with excess tap water, only green-colored leaves with more than 3 cm width x 5 cm length were collected, naturally air-dried, and kept at 6 °C until used within a week.

2.2 Effect of extraction conditions on properties of Yanang leaf extract

Yanang leaf extract was freshly prepared by blending with leaves to water ratios of 1:6 or 1:9 using a blender (Buono-17778P, Taiwan) at blending speeds (22,000 and 32,000 rpm) for 1 min. The extract was then filtered through 2 layer-muslin cloth, adjusted to initial volume with water, and heated at different temperatures (60, 70, and 80 °C). The samples were collected at 1, 3, 6, 9, 12, and 15 min after heating to investigate the physical (color and physical stability) and antioxidant (TPC, DPPH radical scavenging activity, and XOI activity) properties.

2.3 Color measurement

The CIE color parameters (L^*, a^*, and b^*) of the extract were measured with the Hunter colorimeter (HunterLab color Flex 4510, USA.) using illuminant D and 65° standard observer. The total color difference (ΔE^*) was calculated [15].

2.4 Measurement of physical stability

The physical stability of the Yanang leaf extract was performed applying the method described by Granato et al. [16]. One hundred milliliters of the extract were placed into a 100 mL cylinder and kept at room temperature for 3 days. The extract will separate into 2 parts; the aqueous part and the precipitated-particle part. The physical stability can be calculated by the following formula

$$Physical\ stability\ (\%) = \frac{Height\ of\ the\ aqueous\ part\ (cm.)}{Height\ of\ the\ whole\ extract\ (cm.)} \times 100$$

2.5 Determination of TPC

TPC of the Yanang leaf extract was determined using a spectrophotometer (Optima SP-300, Japan). The mixture of 20 µL of the extract, 1.58 mL water, and 100 µL Folin-Ciocalteu reagent was incubated at room temperature for 5 min. Then, 300 µL of 2% (w/v) Na_2CO_3 was added. After incubation in the dark for 2 h, the absorbance of the mixture was measured at 765 nm. TPC was expressed as mg gallic acid equivalent per 100 mg dry basis (mg gallic acid equivalent (GAE)/ 100 mg db) [5].

2.6 Determination of DPPH radical scavenging activity

DPPH radical scavenging activity of the Yanang leaf extract was determined as the method described by De Ancos et al. [17]. The extract was diluted with water 10 times before being mixed with 3.9 mL of 25 mM DPPH. After placing in the dark for 30 min, the absorbance of the mixture was measured at 515 nm. The DPPH

radical scavenging activity was expressed as µg butylated hydroxytoluene (BHT)/mL since BHT was used as a standard.

2.7 XOI activity assay

XOI activity of the Yanang leaf extract was determined following the method described by Azmi et al. [7]. Xanthine was used as a substrate. The assay mixture consisted of 300 µL of 50 mM sodium phosphate buffer (pH 7.5), 100 µL of Yanang leaf extract sample, 100 µL of 0.2 U/mL freshly prepared xanthine oxidase in phosphate buffer, and 100 µL of distilled water. After pre-incubation at 37 °C for 15 min, 200 µL of 0.15 mM xanthine was added to the mixture. The mixture was then incubated at 37 °C for 30 min. The reaction was stopped with the addition of 200 µL of 0.5 M HCl. The absorbance was measured at 295 nm against a blank which was prepared in the same way but the enzyme solution was replaced with phosphate buffer. An assay of the control sample, prepared by using 100 µL DMSO instead of the extracted sample, was also performed to have a maximum uric acid formation. Allopurinol, a well-known xanthine oxidase inhibitor, was used as a positive control at a concentration of 100 µg/mL. Percentage of XOI activity was calculated as followings

$$XOI\ activity\ (\%) = \frac{(Abs_{control} - Abs_{extract\ sampl})}{Abs_{control}} \times 100$$

where Abs $_{control}$ is the absorbance of control sample when DMSO was used and Abs $_{sample}$ the absorbance of Yanang leaf extract sample

2.8 Statistical analysis

Experiments were carried out in triplicate. The data were expressed as mean ± standard deviation. The means of all parameters were examined using analysis of variance (One-way ANOVA). Duncan's New Multiple Range Test (DMRT) was used to determine the multiple comparisons of mean values at a level of $p<0.05$. Correlations were established by regression analysis. An SPSS statistical program version 16 was used to carry out the calculation.

3. Results and discussion

3.1 Physical properties of Yanang leaf extract

3.1.1 Color

Change in color of Yanang leaf extract compared with fresh Yanang leaf extract was expressed as ΔE^* (Table 1). After extraction using 22,000 rpm, ΔE^* of Yanang leaf extract ranged from 2.07 to 8.85. The least ΔE^* was obtained from the condition using a 1:9 ratio at 60 °C for 1 min. During 15 min heating at 60 °C, ΔE^* of the extract with 1:6 and 1:9 ratio was 3.73 - 4.92 and 2.07 - 2.96, respectively. It was 4.93 - 5.79 and 3.69 - 4.13 at 70 °C and was 7.74 - 8.85 and 2.91 - 3.72 at 80 °C, respectively. At 32,000 rpm, ΔE^* ranged from 6.41 to 16.43. The least ΔE^* was obtained from the condition using a 1:6 ratio at 60 °C for 1 min. During 15 min heating at 60 °C, ΔE^* of the extract with 1:6 and 1:9 ratio was 6.41 - 10.18 and 7.56 - 8.98, respectively. It was 7.73 - 11.36 and 7.26 - 8.52 at 70 °C and was 11.61 - 16.05 and 9.28 - 10.27 at 80 °C, respectively.

The results showed that an increase in heating temperature and time increased ΔE^*. This indicated that the change in color of Yanang leaf extract was more likely due to higher temperature and time. This finding was in accordance with previous studies which reported that an increase in heating temperature raised ΔE^* in pear puree [10] and tomato paste [11]. In addition, it has been also reported that changes in the color of chicken breast meat during thermal treatment increased with heating time while the rate of change increased with heating temperature [9].

The change in color of Yanang leaf extract during the extraction process was due to the fact that Yanang leaf contains phenolic compounds which can be oxidized and relates to browning reaction. Phenolic compounds and pigments presented in Yanang leaf are namely ρ-hydroxybenzoic acid, minecoside, flavone glycoside cinnamic acids derivative, and monoepoxybetacarotene [18].

The results showed that an increase in ratio decreased ΔE^*. The extract prepared with a 1:9 ratio had a lower ΔE^* than that prepared with a 1:6 ratio. This might be due to the extract prepared with a 1:9 ratio being more dilute resulting in Yanang leaf particles receiving a smaller amount of heat and consequently having a lower ΔE^*. However, an increase in blending speed from 22,000 rpm to 32,000 rpm increased ΔE^*. The higher the blending speed used, the smaller Yanang leaf particles obtained. These small particles possessed a large surface area which was more susceptible to heat. Therefore, the extract with higher blending speed showed more color change resulting in higher ΔE^*.

Table 1 Changes in ΔE^* and stability of Yanang leaf extract during different extraction conditions.

Blending speed (rpm)	Temp (°C)	Ratio (w/v)	Time (min) 0	1	3	6	9	12	15
		ΔE^*							
22,000	60	1:6	0	3.73 ± 0.11^{a}	3.91 ± 0.21^{a}	4.45 ± 0.48^{b}	4.23 ± 0.25^{b}	4.92 ± 0.32^{c}	4.79 ± 0.28^{c}
		1:9	0	2.07 ± 0.18^{a}	2.39 ± 0.08^{b}	2.52 ± 0.14^{c}	2.73 ± 0.15^{d}	2.78 ± 0.10^{d}	2.96 ± 0.28^{e}
	70	1:6	0	4.93 ± 0.12^{a}	5.46 ± 0.19^{b}	5.70 ± 0.11^{c}	5.66 ± 0.12^{c}	5.73 ± 0.13^{c}	5.79 ± 0.17^{c}
		1:9	0	4.13 ± 0.19^{b}	3.69 ± 0.21^{a}	3.70 ± 0.27^{a}	3.78 ± 0.24^{bc}	3.99 ± 0.83^{bc}	3.75 ± 0.16^{bc}
	80	1:6	0	7.74 ± 0.45^{a}	8.59 ± 0.62^{bc}	8.17 ± 0.45^{ab}	8.85 ± 0.48^{c}	8.56 ± 0.50^{bc}	8.74 ± 0.55^{c}
		1:9	0	3.72 ± 0.70^{b}	3.24 ± 0.51^{a}	3.08 ± 0.41^{a}	2.95 ± 0.29^{a}	2.91 ± 0.25^{a}	2.97 ± 0.20^{a}
32,000	60	1:6	0	6.41 ± 0.88^{a}	9.63 ± 3.12^{b}	9.07 ± 0.84^{b}	9.60 ± 2.45^{b}	10.18 ± 1.94^{b}	10.06 ± 1.20^{b}
		1:9 ns	0	7.56 ± 1.23	8.94 ± 1.87	8.10 ± 2.61	8.89 ± 3.28	8.79 ± 2.03	8.98 ± 1.75
	70	1:6	0	7.73 ± 0.85^{a}	9.92 ± 2.36^{ab}	8.95 ± 1.64^{ab}	10.36 ± 3.47^{ab}	10.09 ± 2.24^{ab}	11.36 ± 4.47^{b}
		1:9 ns	0	7.79 ± 2.47	7.26 ± 1.42	7.66 ± 1.10	7.78 ± 1.84	8.52 ± 1.94	7.61 ± 1.01
	80	1:6	0	11.61 ± 3.26^{a}	15.99 ± 3.05^{b}	12.43 ± 2.79^{a}	11.79 ± 1.79^{a}	16.43 ± 4.08^{b}	16.05 ± 5.05^{b}
		1:9 ns	0	10.27 ± 2.52	9.45 ± 1.10	9.86 ± 1.45	9.28 ± 0.83	9.85 ± 1.31	9.54 ± 0.77
		Stability (%)							
22,000	60	1:6	86.2 ± 0.9^{a}	72.5 ± 1.8^{c}	76.7 ± 0.9^{b}	70.9 ± 2.4^{c}	76.2 ± 1.6^{b}	72.5 ± 2.4^{c}	74.1 ± 0.9^{bc}
		1:9	91.2 ± 2.4^{ab}	89.9 ± 2.4^{abc}	87.3 ± 1.6^{c}	88.9 ± 1.6^{abc}	92.1 ± 1.0^{a}	90.5 ± 1.2^{abc}	88.4 ± 1.8^{bc}
	70	1:6	86.2 ± 0.9^{a}	73.0 ± 1.6^{c}	75.7 ± 0.9^{b}	73.5 ± 0.9^{bc}	75.1 ± 0.9^{bc}	74.6 ± 1.6^{bc}	73.0 ± 1.6^{c}
		1:9	91.2 ± 2.4^{a}	88.4 ± 0.9^{b}	89.4 ± 0.9^{ab}	90.0 ± 0.9^{ab}	91.0 ± 1.8^{ab}	90.0 ± 0.9^{ab}	90.0 ± 0.9^{ab}
	80	1:6	86.2 ± 0.9^{a}	73.5 ± 1.8^{b}	75.7 ± 0.9^{b}	74.1 ± 2.4^{b}	75.1 ± 0.9^{b}	75.1 ± 0.9^{b}	75.1 ± 0.9^{b}
		1:9	91.2 ± 2.4	88.9 ± 1.6	89.4 ± 0.9	90.0 ± 2.4	89.4 ± 0.9	91.0 ± 0.9	90.0 ± 0.8
32,000	60	1:6	78.0 ± 1.8^{a}	71.4 ± 1.6^{b}	77.9 ± 0.92^{b}	70.4 ± 1.8^{b}	71.4 ± 1.6^{b}	72.0 ± 0.9^{b}	69.3 ± 0.9^{b}
		1:9	82.8 ± 1.6^{a}	75.1 ± 4.0^{b}	76.7 ± 0.92^{b}	76.2 ± 4.2^{b}	76.2 ± 1.6^{b}	77.8 ± 3.2^{ab}	77.2 ± 4.6^{ab}
	70	1:6	78.0 ± 1.8^{a}	71.4 ± 1.6^{bc}	73.0 ± 1.6^{b}	70.9 ± 0.9^{bc}	72.0 ± 0.9^{bc}	70.9 ± 0.9^{bc}	70.4 ± 0.9^{c}
		1:9	82.8 ± 1.6^{a}	76.2 ± 4.2^{b}	77.8 ± 1.6^{ab}	76.7 ± 3.3^{b}	76.2 ± 1.6^{b}	77.8 ± 3.2^{ab}	77.8 ± 4.2^{ab}
	80	1:6	78.0 ± 1.8^{a}	71.4 ± 1.6^{c}	72.0 ± 0.9^{bc}	70.4 ± 0.9^{bc}	69.3 ± 0.9^{bc}	70.9 ± 0.9^{bc}	71.4 ± 1.6^{b}
		1:9	82.8 ± 1.6^{a}	73.5 ± 1.8^{c}	77.2 ± 0.9^{bc}	75.7 ± 4.0^{bc}	75.1 ± 0.9^{bc}	76.7 ± 1.8^{bc}	78.3 ± 3.3^{b}

Means in the same line indicated by different superscripts are significantly different at 95% confidence level (p-value ≤ 0.05).
ns Not statistically significant different p-value >0.05).

3.1.2 Physical stability

At 22,000 rpm, the physical stability of fresh Yanang leaf extract with 1:6 and 1:9 ratios was 86.2 and 91.2%, respectively (Table 1). After extraction, it was 70.9 - 76.7% and 87.3 - 92.1%, for 1:6 and 1:9 ratio, respectively. At 32,000 rpm, the fresh extracts with1:6 and 1:9 ratios presented 78.0 and 82.8%, respectively. The physical stability of the prepared extract with a 1:6 ratio was 69.3 - 77.9 % and that with a 1:9 ratio were 73.5 - 78.3%.

Both fresh and prepared extracts obtained from 32,000 rpm had lower physical stability than those obtained from 22,000 rpm. Increases in ratio and heating time lowered the physical stability at any blending speed.

3.2 Antioxidant properties of Yanang leaf extract

3.2.1 TPC

TPC of Yanang leaf extract using 22,000 rpm varied from 405.00 ± 32.89 to 806.67 ± 32.01 mg GAE/100 mg db. (Figure 1(A)). The extraction condition using 1:9 ratios at 80 °C for 15 min gave the least TPC. TPC of the extract with 1:6 ratio was 752.37 – 806.44, 713.94 - 806.67, and 509.26 – 641.11 mg GAE/100 mg db at 60, 70, and 80 °C, respectively. During 15 min heating at 60 °C, the TPC of the extract with a 1:9 ratio was 618.04 - 651.00 mg GAE/100 mg db. It was 518.79 – 571.82 mg GAE/100 mg db at 70 °C and 405.00 – 443.33 mg GAE/100 mg db at 80 °C.

At 32,000 rpm, TPC of Yanang leaf extract was between 733.94 ± 41.44 and 1,174.15 ± 37.28 mg GAE/100mg db (Figure 1(B)). The least TPC was obtained from the extraction condition using 1:9 ratio at 80 °C for 15 min. This condition also gave the least TPC at 22,000 rpm as well. TPC of the extract with 1:6 ratio was 1,111.93 - 1,174.15 mg GAE/100 mg db at 60 °C. It was 986.00 - 1,104.52 and 1,003.04 - 1,157.11 mg GAE/100 mg db at 70 and 80 °C, respectively. TPC of the extract with 1:9 ratio was 827.28 - 873.39, 755.61 – 800.94, and 733.94 – 805.61 mg GAE/100 mg db at 60, 70, and 80 °C, respectively.

The results revealed that an increase in ratio and blending speed enhanced the TPC of the Yanang leaf extract while an increase in heating temperature and time reduced TPC. The former has resulted from more extractability with a larger amount of water and smaller Yanang leaf particles. It was following higher ΔE^* since

the phenolic compounds can be oxidized and cause color alteration. The latter was due to the fact that phenolic compounds are heat-labile [19].

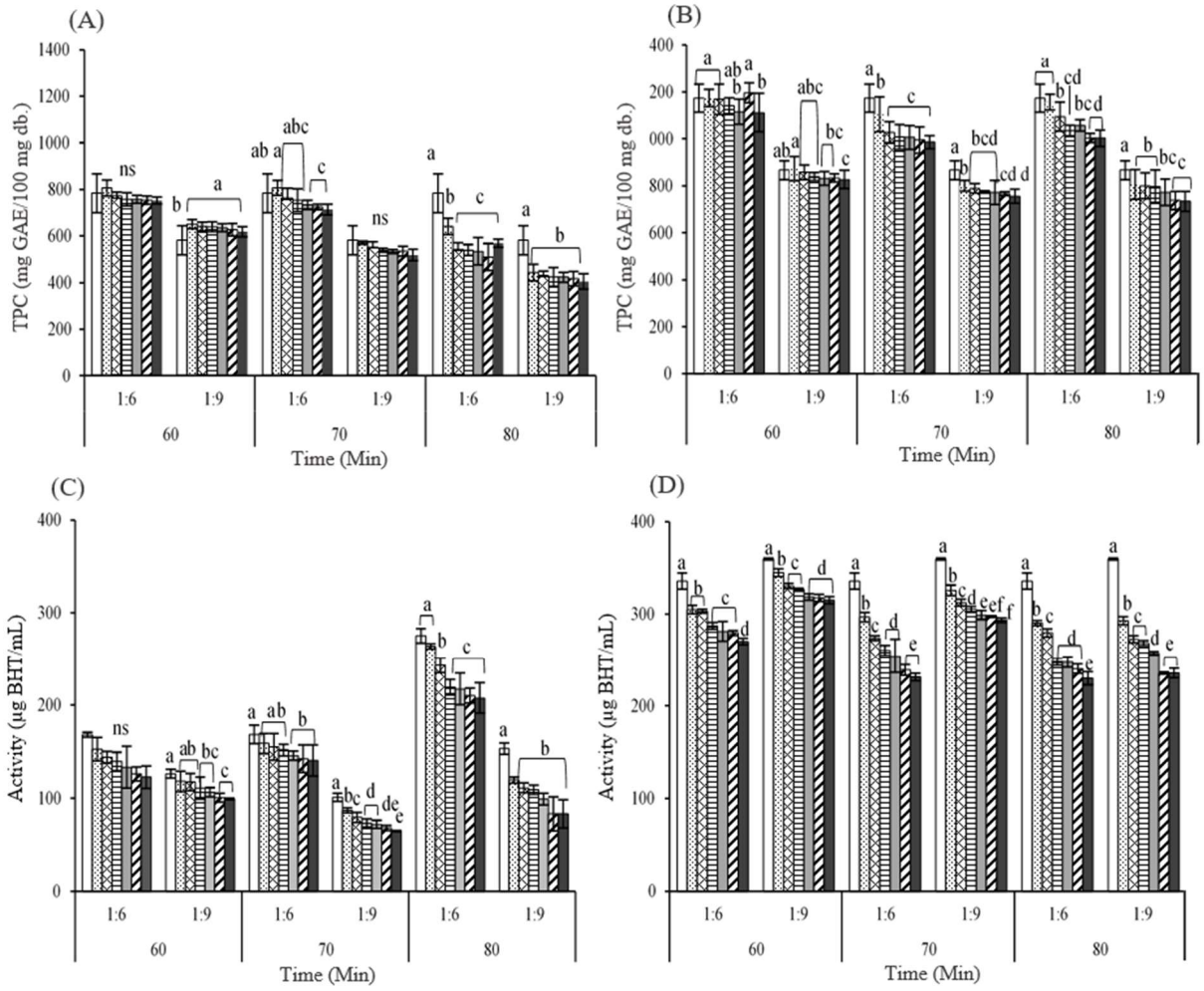

Figure 1 Changes in (A-B) TPC (mg GAE/100 mg db.) and (C-D). DPPH radical scavenging activity (µg BHT/mL) of Yanang leaf extract during different extraction conditions: Blending speed (A,C) 22,000 rpm, (B,D) 32,000 rpm; Time (☐) 0 min, (▨) 1 min, (▨) 3 min, (▬) 6 min, (▭) 9 min,(◪) 12 min, (■) 15 min.

A previous study reported that 80% ethanolic Yanang leaf extract using 1:10 ratio at 70 °C, 15 min had TPC at a level of 8.60 mg GAE/g db [20]. Another study prepared Yanang leaf powder by drying at 60 °C for 3 h and then separately extracted 100 mg powder with 12 mL water, ethanol or acetone at 25 °C for 15 min. Their results found that the extraction using water provided the highest TPC which was 97.90 mg GAE/g compared with those using ethanol (26.70 mg GAE/g) and acetone (16.46 mg GAE/g) [5]. Various TPC of Yanang leaf extract among researchers could be a result of different extraction methods including sample preparation, type of solvent, extraction temperature, and time.

3.2.2 DPPH radical scavenging activity

Determination of DPPH radical scavenging activity of Yanang leaf extract was performed to evaluate the antioxidant ability of the extract by donating a hydrogen atom to free radicals and scavenging radicals. As shown in (Figure 1(C)), Yanang leaf extract using 22,000 rpm possessed DPPH radical scavenging activity ranging from 64.73 ± 0.54 to 263.84 ± 2.79 µg BHT/mL. The least DPPH radical scavenging activity was obtained from the condition using a 1:9 ratio at 70 °C for 15 min. The DPPH radical scavenging activity of the extract with a 1:6 ratio was 122.65– 152.74, 140.51 - 158.80, and 207.55 -263.84 µg BHT/mL at 60, 70, and 80 °C, respectively. That of the extract with 1:9 ratio was 99.16 – 118.14, 64.73 – 86.92, and 83.41 – 119.35 µg BHT/mL at 60, 70, and 80 °C, respectively.

At 32,000 rpm, the DPPH radical scavenging activity of the extract was 229.79 ± 7.17 - 335.80 ± 8.82 µg BHT/mL (Figure 1 (D)). The least DPPH radical scavenging activity was acquired from the condition using a

1:6 ratio at 80 °C for 15 min. The extract with 1:6 ratio had 270.15 – 304.84, 231.13 – 296.76, and 229.79 – 290.39 μg BHT/mL at 60, 70, and 80 °C, respectively. The DPPH radical scavenging activity of the extract with a 1:9 ratio was 315.10 – 344.71, 293.22 – 325.67, and 235.53 – 292.79 μg BHT/mL at 60, 70, and 80 °C, respectively.

The results showed that an increase in blending speed enhanced not only TPC but also the DPPH radical scavenging activity. This was due to smaller Yanang leaf particles obtained from higher blending speed. These small particles are susceptible to extraction and cause higher TPC. Since phenolic compounds owned an antioxidant activity, the Yanang leaf extract with higher TPC consequently had a higher DPPH radical scavenging activity.

The results also revealed that the DPPH radical scavenging activity, as well as TPC of Yanang leaf extract, decreased with an increase in heating temperature and time. This was because of a decline in phenolic compounds, which are heat-labile and possess an antioxidant property, therefore, resulted in lowering DPPH radical scavenging activity. Moreover, a free radical formation from the oxidative reaction is accelerated by increase temperature and time [19]. Few pieces of research have been carried out to determine the DPPH radical scavenging activity in Yanang leaf. Varied IC_{50} values of DPPH radical scavenging activity in Yanang leaf extract have been reported. IC_{50} values of DPPH radical scavenging activity in Yanang leaf powder extracted with water, ethanol and acetone were 0.197, 0.333, and 0.419 mg/g, respectively [5]. Yanang leaf extracted with water possess the IC_{50} value of 16.19 ppm and those extracted with methanol possess the IC_{50} value of 9.63 ppm [3]. Another study reported the IC_{50} value of 68.83 μg/mL in Yanang leaf extracted with methanol [21]. The difference in DPPH radical scavenging activity among researchers was caused by diverse methodologies such as sample preparation and extraction method.

3.2.3 Xanthine oxidase inhibitory (XOI) activity assay

Xanthine oxidase involves in the uric acid biosynthesis from the purine base. It accelerates oxidation of purine base to xanthine and further to uric acid [22,23]. It is well known that an accumulation of uric acid in the body causes gout. Hence, inhibition of xanthine oxidase can reduce the synthesis of uric acid, consequently reducing the risk of gout. Change in XOI activity of Yanang leaf extract with different extraction conditions was shown in Figure 2.

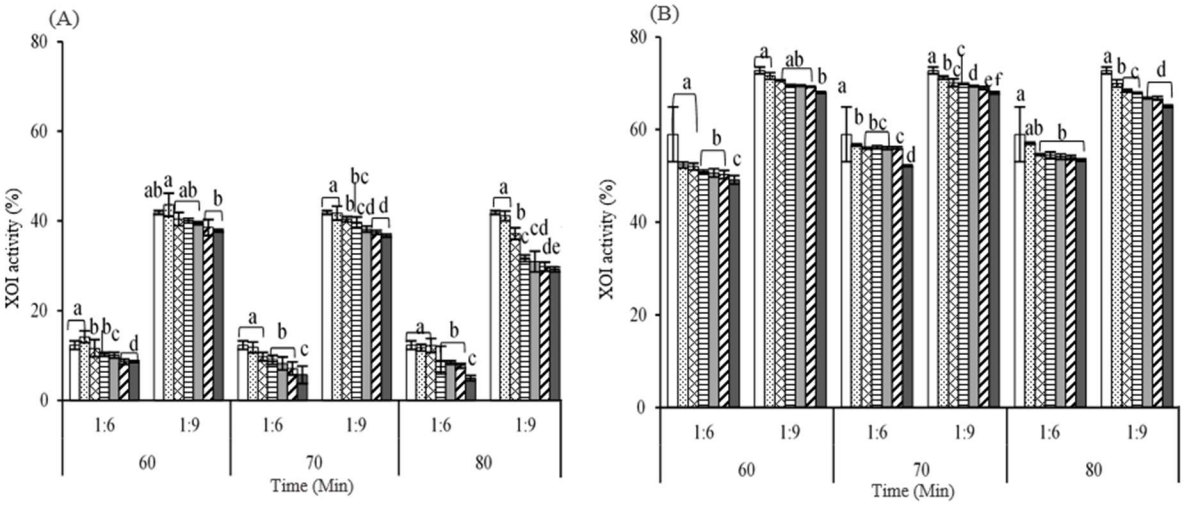

Figure 2 Change in XOI activity (%) of Yanang leaf extract during different extraction conditions: Blending speed (A) 22,000 rpm, (B) 32,000 rpm; Time (□) 0 min, (▨) 1 min, (▨) 3 min, (▬) 6 min, (▬) 9 min, (▨) 12 min, (■) 15 min.

At 22,000 rpm, the XOI activity of the extract ranged from 4.92 ± 0.56 to 43.60 ± 6.55%. The lowest activity was obtained from the condition using a 1:6 ratio at 80 °C for 15 min. The XOI activity of the extract with a 1:6 ratio was 8.57 - 14.16, 5.66 – 11.79, and 4.92 – 11.69% at 60, 70, and 80 °C, respectively. That of the extract with a 1:9 ratio was 37.84 - 43.60, 36.72 - 41.73, and 29.17 - 41.09%, respectively.

At 32,000 rpm, the Yanang leaf extract showed the XOI activity in the range of 49.16 ± 0.91 – 71.59 ± 0.70%. The lowest activity was obtained from the condition using a 1:6 ratio at 60 °C for 15 min. The XOI activity of the extract with a 1:6 ratio was 49.16 – 52.36, 52.18 – 56.68, and 53.43 – 57.06% at 60, 70, and 80 °C, respectively. That of the extract with a 1:9 ratio was 68.00 – 71.59, 67.96 – 71.21, and 65.00 – 69.99%, respectively.

The XOI activity of Yanang leaf extract improved with an increase in ratio and blending speed but it declined with increased heating temperature and time. The effect of extraction condition on XOI activity was in accordance with that on TPC (Figure 1). This might be due to the fact that phenolic acid exhibited an inhibitory effect of xanthine oxidase [24], thus a change in TPC consequently altered the XOI activity. Few studies have been conducted to determine the XOI activity of Yanang leaf extract. Previous research found that alcoholic Yanang leaf extracts had no XOI activity [21,25].

3.2.4 Effect of extraction conditions on physical and antioxidant properties of Yanang leaf extract

Effect of extraction conditions (2 levels of leaves to water ratio, 2 levels of blending speed, 3 levels of heating temperature, and 6 levels of heating time) on physical and antioxidant properties of Yanang leaf extract was examined following the analysis of variance. As shown in Table 2, extraction conditions significantly affected both physical and antioxidant properties of Yanang leaf extract except for the temperature on stability (p-value = 0.474). Results also showed that there is some interaction of factors that do affect certain property but some do not however it affects other properties. For example, an interaction of blending speed and ratio (AC) affected the stability of Yanang leaf extract (p-value < 0.001) while the interaction of blending speed and heating time (AD) had no such effect (p-value = 0.243), however it affected ΔE^* (p-value < 0.001). The interaction of three factors, blending speed with temperature and ratio (ABC) and that of blending speed with temperature and time (ABD) did not affect physical properties but had a significant effect on antioxidant properties. The interaction of four factors (ABCD) affected only DPPH radical scavenging and XOI activities.

Table 2 p-value and other parameters extracted from analysis of variance.

Source	p-value				
	Physical properties		Antioxidant properties		
	ΔE^*	Stability	TPC	DPPH radical scavenging activity	XOI activity
Model	<0.001	<0.001	<0.001	<0.001	<0.001
A: Blending speed	<0.001	<0.001	<0.001	<0.001	<0.001
B: Temperature	<0.001	0.474	<0.001	<0.001	<0.001
C: Ratio	<0.001	<0.001	<0.001	<0.001	<0.001
D: Heating time	<0.001	<0.001	<0.001	<0.001	<0.001
AB	<0.001	0.238	<0.001	<0.001	<0.001
AC	0.021	<0.001	<0.001	<0.001	<0.001
AD	<0.001	0.243	0.001	<0.001	<0.001
BC	<0.001	0.695	0.795	<0.001	<0.001
BD	<0.001	0.366	<0.001	<0.001	<0.001
CD	<0.001	<0.001	<0.001	0.011	0.014
ABC	0.065	0.992	<0.001	<0.001	<0.001
ABD	0.072	0.946	0.008	0.031	<0.001
ACD	<0.001	<0.001	0.005	0.603	<0.001
BCD	<0.001	0.990	<0.001	0.003	<0.001
ABCD	0.394	0.823	0.206	0.043	<0.001
R^2	0.881	0.956	0.970	0.992	0.998
Adjusted R^2	0.866	0.935	0.966	0.989	0.997

From the results, it was found that the extraction conditions mostly affected antioxidant activity; therefore, the rest of this study will be focused on the antioxidant properties of Yanang leaf extract. According to the antioxidant properties of the extract especially the DPPH radical scavenging and XOI activities, the most suitable extraction condition was the extraction using higher Yanang leaves to water ratio and higher blending speed but less heating temperature for the shortest period of heating time (Figure 1 and 2) which referred to the extraction with 1:9 ratio using 32,000 rpm and heat at 60 °C for 1 min.

To get a better understanding, a degradation of antioxidant properties was further analyzed and evaluated using zero-order (Equation 1), first-order (Equation 2) and second-order reactions (Equation 3). The most appropriate model was selected based on the coefficient of determination (R^2).

$$C = C_0 - kt \quad (1)$$

$$ln\,C = \ln C_0 - kt \quad (2)$$

$$\frac{1}{C} = +\frac{1}{C_0} + kt \quad (3)$$

where C is the antioxidant properties at time t, C_0 the antioxidant properties at time 0, k the reaction rate constant and t the time (min). The effect of temperature on the degradation rate constant was assumed to follow the Arrhenius equation (Equation 4)

$$k = k_0 e^{\frac{-E_a}{RT}} \quad (4)$$

where k is the rate constant, k_0 the pre-exponential factor, E_a the activation energy for the degradation (J/mol), R the gas constant (8.314 J/mol.K) and T the absolute temperature (K)

The analysis showed that degradation of TPC was a zero-order reaction with coefficient of determination (R^2) 0.676 - 0.938 (Table 3). The degradations of DPPH radical scavenging and XOI activities were both described as a second-order reaction. The R^2 of DPPH radical scavenging activity degradation was 0.738 - 0.969 while that of XOI activity degradation was 0.790-0.986. Based on the most suitable extraction ratio (1:9) and speed (32,000 rpm), the temperature dependence of the degradations was determined by using the Arrhenius equation. A $ln\,k - 1/T$ graph was plotted to express the effect of temperature on the reaction rate constant. According to the Arrhenius equation, the correlation coefficient (R^2) for TPC, DPPH radical scavenging activity, and XOI activity was 0.792, 0.938, and 0.735, respectively (Table 4). These equations can be used to predict the TPC, DPPH radical scavenging, and XOI activities of Yanang leaf extract obtained from 1:9 ratio and 32,000 rpm.

Table 3 Reaction rate constant (k) and coefficient of determination (R^2) of antioxidant properties in Yanang leaf extract during the extraction process.

Speed (rpm)	Temp (°C)	Ratio (w/v)	Zero-order		Second-order			
			TPC		DPPH scavenging activity		XOI activity	
			k (mg GAE /100mL.min)	R^2	k (mL/µg BHT.min)	R^2	k (1/%.min)	R^2
22,000	60	1:6	1.993	0.813	$2x10^{-4}$	0.873	$2x10^{-3}$	0.910
		1:9	2.371	0.938	$1x10^{-4}$	0.969	$2x10^{-4}$	0.975
	70	1:6	3.981	0.812	$9x10^{-5}$	0.898	$5x10^{-3}$	0.952
		1:9	2.562	0.876	$3x10^{-4}$	0.896	$2x10^{-4}$	0.986
	80	1:6	66.36	0.863	$1x10^{-4}$	0.926	$6x10^{-3}$	0.835
		1:9	2.560	0.881	$3x10^{-4}$	0.903	$7x10^{-4}$	0.905
32,000	60	1:6	4.834	0.890	$4x10^{-5}$	0.848	$9x10^{-5}$	0.930
		1:9	3.019	0.857	$2x10^{-5}$	0.816	$5x10^{-5}$	0.892
	70	1:6	10.100	0.676	$8x10^{-5}$	0.903	$1x10^{-4}$	0.832
		1:9	3.227	0.870	$3x10^{-5}$	0.738	$5x10^{-5}$	0.871
	80	1:6	11.290	0.848	$8x10^{-5}$	0.858	$1x10^{-4}$	0.790
		1:9	7.383	0.849	$8x10^{-5}$	0.820	$9x10^{-5}$	0.852

Table 4 Arrhenius equation of antioxidant properties degradation in Yanang leaf extract using 1:9 ratio and 32,000 rpm.

Antioxidant properties	R^2	Arrhenius Equation
TPC	0.792	k = exp [(-626.77/T)+16.626]
DPPH radical scavenging activity	0.938	k = exp [(-975.79/T)+13.443]
XOI activity	0.735	k = exp [(-411.37/T)+ 0.2694]

4. Conclusion

This study showed how different extraction conditions affect the physical and antioxidant properties of Yanang leaf extract. Results revealed that heating temperature did not affect the stability and the antioxidant properties of Yanang leaf extract were much more susceptible to extraction conditions than its physical properties. The degradation of TPC followed a zero-order reaction while that of DPPH radical scavenging and XOI activities followed a second-order reaction. The most suitable extraction condition was the extraction using a 1:9 ratio, 32,000 rpm, 60 °C, and 1 min. However, the extraction condition cloud is optimized by other techniques such as a response surface methodology (RSM). Results also proposed the Arrhenius equations for antioxidant properties of Yanang leaf extract using 1:9 ratio and 32,000 rpm which benefit to predicting the

temperature-dependent degradations of TPC, DPPH radical scavenging, and XOI activities of Yanang leaf extract.

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