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The Ultrasonic Assisted Extraction to Maximize Antioxidant and Tyrosinase Inhibition Activities from *Zingiber Officinale* Roscoe

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Abstract

Ultrasonic assisted extraction, a green extraction technology has been applied in process. The study of *Zingiber* officinale rhizome extract using the mixture solvent of EtOH and H_2O obtained various polarity of crude extract. The extraction of Z. officinale with 50% EtOH provided the highest yield around 37%. Crude extract of Z. *officinale* was examined antioxidant activity against DPPH scavenging. The 70% EtOH extract of Z. *officinale* showed the highest activity of free radical scavenging with IC_{50} value of 0.63 μg/mL. Furthermore, the crude 70% EtOH extract exhibited the highest tyrosinase inhibition activity with percent inhibition between 34.89-93.58%.

KEYWORDS: *Zingiber officinale*, ginger, antioxidant activity, tyrosinase activity

1 INTRODUCTION

Zingiber officnale Roscoe, commonly known as ginger is a member of *Zingiber*acae family. This plant has been found in the tropic region. The rhizome of ginger has been used as traditional medicine to treat cold, asthma and stomachache (Kanadea and Bhatkhandeb, 2016). In the literatures, there are more than 400 compounds found in ginger. The major components were consisted of starch (40-70%), protein (6-20%), water (8-10%), ash (8-10%), fixed oil (2-11%) and essential oil (1.5-3%) (Jan et al., 2022). Some groups of secondary metabolites have been produced especially phenolic compounds and terpenoids (Pawin et al., 2022). The biological activity of ginger extract showed antioxidant activity, anticancer, anti-inflammatory and antibacterial activities.

Ultrasonic assisted extraction (UAE) has been developed to support the green extraction technology. The bioactive compounds in plant were extracted using the ultrasonic wave to attack cell wall then the compounds were eluted. There are two types of ultrasonic extraction, ultrasonic bath and probe ultrasonic equipment. First, ultrasonic bath type is commonly

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known in the experiment using stainless still bath with ultrasonic transducers. This type of extraction is controlled frequency around 40 kHz and temperature of extraction process. In contrast, probe type is directly delivered ultrasonic wave to extraction media and operated with frequency at 20 kHz and extract in the reactor (Sukona et al., 2018)

Antioxidants are recognized for their potential in promoting health and lowering the risk for cancer, hypertension and heart disease (Wolfe and Liu, 2003 and Valko et al., 2007). The uses of natural antioxidants from plant extracts have experience growing interest due to some human health professionals and consumers concern about the safety of synthetic antioxidants in foods (Sun and Ho, 2005 and Suhaj, 2006).

Several investigations have been done for identification of many naturally occurring substances in higher plants with antioxidant and other protective biochemical functions. Tyrosinases are responsible for melanin formation in all life domains. Tyrosinase inhibitors are used for the prevention of severe skin diseases. Disorder in melanin formation has been found to cause a variety of skin diseases in humans such as hyperpigmentation, lentigo, vitiligo and skin cancer. The inhibition of tyrosinase has an important role to prevent melanin accumulation in skin.

Herein, we reported the investigation of extraction condition of Z. *officinale* rhizome and evaluated the antioxidant and anti-tyrosinase activities.

2 RESEARCH OBJECTIVES

To search for the highest yield of extraction condition under ultrasonic treatment and evaluated for antioxidant and tyrosinase inhibition activities.

3 LITERATURE REVIEW

The literature reported the chemical compositions of ginger are the group of phenolic compounds especially 6-gingerol which was the major component. This compound was derivatised to 6-shogaol via dehydration then further hydrogenation to be 6-paradol. The derivative compounds from 6-gingerol exhibited various activities such as anti-inflammatory, antioxidant, anti-diabetic, anti-allergy and anticancer. Previous study, ginger has been extracted using conventional method including soxhlet and maceration which provided low extraction rate and long extraction time. Furthermore, the green extraction technology has been applied in extraction process. The popular method in ginger extraction is microwave assisted extraction (MAE) and ultrasonic assisted extraction (UAE). This alternative method exhibited the high content of bioactive compound contents such as total phenolic compounds and yield (Kamaruddin et al., 2023).

4 RESEARCH METHODOLOGY

4.1 Instrumental and reagents

The ultrasonic extraction ultrasonic probe was 10 mm diameter of hon, power 10KW and frequency 20KHz. 1,1-diphenyl-2-picrylhydrazyl (DPPH) was purchased from TCI, Japan.

Trolox and Kojic acid were obtained from Sigma, USA. Ethanol and methanol were analytical grade.

4.2 Ultrasonic assisted extraction, UAE (Probe-type)

Fresh gingers were purchased from Chiang Rai, Thailand. The rhizomes were washed and sliced to small pieces then dried in the oven at 50°C for 2 days. The dried gingers were blended and extracted using the mixture of EtOH and water with ultrasonic treatment for 30 minutes. The extract was filtrated and removed solvent under reduced pressure. The crude ginger was obtained as brown viscous.

4.3 DPPH radical scavenging activity

A solution of 200μMDPPH in MeOH was prepared, and 180μL of this solution was mixed with 180μL of samples at different concentrations $(1.25 - 25\text{mg/mL})$. The reaction mixture was vortexed thoroughly and incubate in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Trolox was used as reference. Percentage DPPH radical scavenging activity was calculated by the following equation:

$$\%\text{DPPH radical scavenging activity} = \{(A_0 - A_1)/A_2\} \times 100$$

4.4 Tyrosinase inhibition assay

Tyrosinase inhibitory activity was determined spectrophotometrically in 96 -well plates with a final volume of 100μL. First, added 10μL of 20% ethanol and 45μL of 0.02M PBS buffer were mixed with 10μL of purified enzyme into the well plate. Ten microliters of sample were added to the wells. Then, various 10μL of tyrosinase inhibitor were mixed with 25μL of L-dopa and were added to the well plate and incubated on shaker at 37°C for 10 minutes. Kojic acid was used as a positive control $(0.001 - 1\text{mg/mL})$. The reaction mixture was measured the absorbance at 475 nm. (Deri et al., 2016)

5 RESULTS

5.1 Extraction of gingers rhizome The ginger powder was extracted by ultrasonic assisted extraction as showed in Table 1. The ginger extracted with EtOH obtained the highest yield from the amplitude of ultrasonic wave at 85% around 16%. The extraction of ginger with 70%EtOH, 50%EtOH and H_2O provided yield as 30,37 and 32%, respectively.

Table 1: *The extraction condition and yield of crude ginger extract*

Condition	Solvent	%Amplitude	% Yield
1	100% EtOH	25	11
2	100% EtOH	50	13
3	100% EtOH	85	16
4	70% EtOH	85	30
5	50% EtOH	85	37
6	H_2O	85	32

5.2 DPPH radical scavenging activity

Crude gingers extracted with 100%EtOH, 70%EtOH, 50%EtOH and H_2O were evaluated for DPPH radical scavenging activity. The highest activity of free radical scavenging was 70% EtOH extract. The DPPH activities of ginger extracts increased in a concentration dependent manner (Figure 1). At a concentration of 50mg/mL, the DPPH scavenging activity of crude gingers extracted with H_2O, 50% EtOH, 70%EtOH, and 100%EtOH were $68.94 \pm 0.71, 75.42 \pm 0.82, 87.21 \pm 0.38$ and 64.65 ± 0.84%, respectively. Furthermore, the IC_{s0} of crude gingers extracted with H_2O, 50%EtOH, 70%EtOH, and 100% EtOH were $12.95 \pm 0.06, 3.08 \pm 0.39, 0.63 \pm 0.14$ and 7.91 ± 0.47mg/ml, respectively. Trolox showed inhibitory activity with $IC_{ss} 8.96 \pm 0.09 \mu g/mL$ (Figure 1).

5.3 Tyrosinase inhibition assay

Tyrosinase inhibition activity of ginger extract compared with kojic acid as a positive control at 10 mM. The tyrosinase inhibition ranges of concentration at $0.78 - 50$mg/mL of H_2O, 50%EtOH, 70% EtOH, and 100% EtOH were $30.65 - 62.03\%, 38.08 - 87.71\%, 34.89 - 93.58\%$ and $9.8 - 27.98\%$, respectively while tyrosinase inhibition ranges of positive control was 34.89-93.58%. (Figure 2).

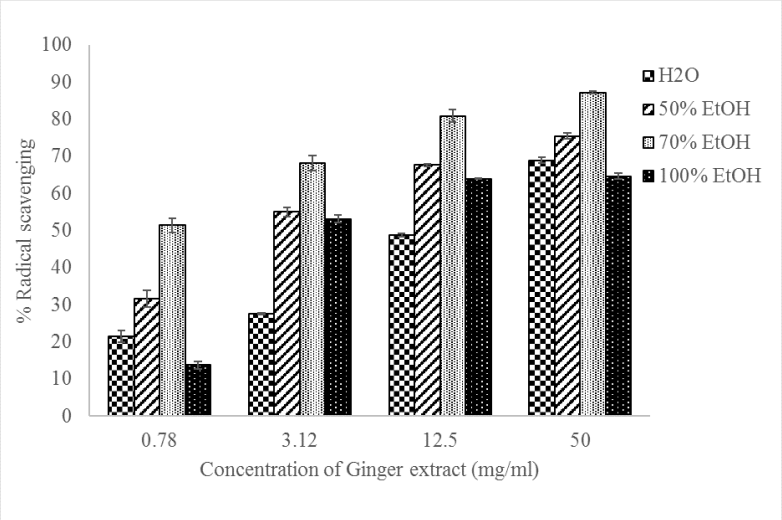

Figure 1: *The percent of radical scavenging of DPPH assay from ginger extracted with different solvent extraction systems (H_2O, 50 % EtOH, 70% EtOH, and 100% EtOH).*

6 DISCUSSION AND CONCLUSIONS

The ultrasonic assisted extraction of ginger showed the highest extraction yield of 100% EtOH as a solvent with different amplitudes was obtained from amplitude 85% thus this amplitude has been applied for other conditions. The variation of solvent including 70% EtOH, 50% EtOH and H_2O were further extracted using the optimized amplitude for 30 minutes. The crude extract with 50% EtOH provided the highest extraction yield due to the phytochemicals in ginger. The literature reported main components in ginger were phenylpropanoids and essential oils which mixture of polar and non-polar compounds (Rampogu et al., 2018) which was related to report of Cha et al., 2020. The optimization condition from reflux extraction using respond surface analysis of ginger extract showed the percent EtOH

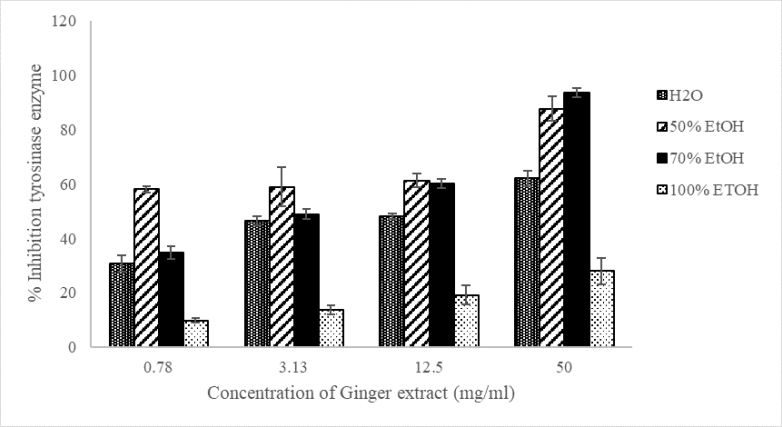

Figure 2: *Inhibition of tyrosinase activity of ginger extracted with H_2O, 50 % EtOH, 70% EtOH, and 100% EtOH*

content in the optimum condition about 41.38% and 14.94% of yield for 90 minutes (Cha et al., 2020). Thus, this method reduced time and increased yield of extraction process due to the ultrasonic wave directly attacked to cell wall of plant materials.

Antioxidant of ginger extract showed the DPPH radical scavenging with IC_{s0} values between 0.63− 12.95¯g/mL. The 70% EtOH extract exhibited the highest DPPH radical scavenging capacity according to the phenolic compounds can be eluted more than other condition. Total phenolic of EtOH and H_2O in ginger extract have been report which the 100%EtOH contained more phenolic content than H_2O extract around 95.67 and 10.79mg GAE/g dryweight (Roekruangrit et al., 2018). Furthermore, solvents used for phenolic compounds extraction had significant effects on DPPH scavenging capacity determination of ginger extracts. Recently, DPPH scavenging method has been widely used in antioxidant activity studies of herb extracts (Chatha et al., 2006 and Canadanovic-Brunet et al., 2005). In fact, free radical scavenging method (DPPH) show the reduction of alcoholic DPPH solutions in the presence of a hydrogen donating antioxidant (Koleva et al., 2002) and phenolic compound have been reported and provided to be potent hydrogen donators to the DPPH radical because of their excellence structural chemistry (Rice-Evans et al., 1997).

The results indicated that the ethanolic extracts (70%EtOH) showed the best enzyme inhibition when compared with other extracts ($p < 0.05$). The ginger extraction with 70%EtOH showed a stronger tyrosinase inhibitory activity ($IC_{s0} = 7.78 \pm 0.28$mg/mL) than 50%EtOH ($IC_{s0} = 9.05 \pm 0.4$ followed by H_2O ($IC_{s0} = 23.08 \pm 1.139$mg/mL) and 100%EtOH ($IC_{s0} = 115.45 \pm 0.48$mg/mL). The tyrosinase inhibitory effects of the extract may have depended on the antioxidant properties and the phenolic compounds acting as active sites of tyrosinase and inducing steric or conformational changes, thereby resulting in lower enzymatic activity (Chang et al., 2011 and Prasad et al., 2009).

In conclusion, the optimization of ginger extract condition, the high efficiency condition was 50% EtOH with UAE for 30 minutes that obtained 37% yield when compared with reflux extraction. The crude extract of 70% EtOH showed the best activity including antioxidant with the IC_{si} value of 0.63 ± 0.14 μg/mL and tyrosinase inhibition between $34.89 - 93.58\%$. Therefore, ginger extract is interesting to further study toxicity then can be applied in cosmetic

and supplement products.

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