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Green technology extraction of *Dictyophora indusiata* at the egg stage: Unveiling the antioxidant potential and bioactive components for cosmetic applications

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ABSTRACT

Mushrooms have been introduced as potential sources of natural antioxidants. Different bioactive components and antioxidant activity have been reported for different extraction methods. Therefore, this study aimed to examine the bioactive components and antioxidant activity of *Dictyophora indusiata* in eggs extracted with water using microwave-assisted extraction (MAE), ultrasonic-assisted extraction (UAE), and hot water extraction (HWE). The results indicated that the extract yields ranged from 12.33 ± 0.42% to 15.60 ± 1.44%. UAE exhibited the strongest antioxidant activity in the 2,2'-azinobis (3-ethylbenzthiazoline-6-acid) assay (IC_{50} of 5.68 ± 0.09 mg/mL), whereas HWE demonstrated the best antioxidant activity in both the 2,2-diphenyl-1-picrylhydrazyl assay and the ferric reducing antioxidant power assay. Furthermore, the MAE yielded the highest total polysaccharide content (746.75 ± 3.04 mg glucose/g extract). The highest total phenolic content (9.73 ± 0.28 mg gallic acid/g extract) was observed in UAE. The total flavonoid content, a subtype of phenolic compounds, was enriched in HWE and UAE (3.96 ± 0.03 and 3.96 ± 0.12 mg quercetin/g extract, respectively). In summary, UAE was shown to be a promising method for extracting bioactive components with antioxidant activity, offering advantages in reducing extraction time and temperature, which is appropriate for cosmeceutical applications.

Keywords: *Dictyophora indusiata*; antiaging; antioxidant; green technology

1. INTRODUCTION

Currently, one in eight people in the world are aged 60 and over (Ismail et al., 2021). The Thai population is aging rapidly and is expected to become the second-most aged society in ASEAN countries after Singapore (Ong, 2022). Aging involves a gradual deterioration in physiological function and is related to several diseases, such as

cardiovascular diseases, musculoskeletal disorders, neurodegenerative diseases, and skin disorders (Costa et al., 2022). Several recent studies have prioritized health promotion through averting and delaying the aging phenomenon. Skin changes, including wrinkles, dryness, sagging, and roughness, are clear signs of aging in humans (Poomanee et al., 2023). These changes may originate from intrinsic or extrinsic factors (Sundaram et al., 2018). The intrinsic factors of aging correlate with the endogenous damage caused by reactive oxygen species (ROS), whereas extrinsic factors can develop through external influences such as exposure to ultraviolet light, liquor intake, pollutants, and physical stress (Shon et al., 2014). It has been reported that excess ROS can lead to oxidative damage to biomolecules (Liu et al., 2018). Some studies have revealed that skin aging caused by ROS might be due to disrupted antioxidant defences. In addition, ROS stimulate wrinkle formation and melanogenesis (Kim et al., 2011). Therefore, the utilization of antioxidants can be an effective approach to treat skin aging and age-related problems.

Edible mushrooms are fungi that are rich in nutrients and bioactive compounds. They are consumed as foods and dietary supplements and are incorporated into cosmeceutical products for antiaging, moisturizing, and skin lightening (Nazir et al., 2021). *Dictyophora indusiata* is an edible mushroom that is commonly used as a food in Asian countries because of its attractive appearance and great taste (Wang et al., 2019). It is in the family *Phallaceae* and phylum Basidiomycota (Nguyen et al., 2013). The development period of *D. indusiata* can be divided into two categories: an immature development period known as the egg (Figure 1) and a mature development period known as the fruiting body (Srisuk & Jirasatid, 2020). *D. indusiata* contains a variety of bioactive components, among which polysaccharides are the major components (Pan et al., 2023). Previous studies have reported that *D. indusiata* fruiting bodies have various biological activities, such as anti-inflammatory, antitumor, and immunomodulatory effects (Deng et al., 2013; Fu et al., 2015; Ruksiriwanich et al., 2022). Furthermore, the bioactive compounds of *D. indusiata* are also suitable for cosmeceutical applications. A study by Oyetayo and colleagues revealed that their hot water extract obtained from fruiting bodies of *D. indusiata* at a concentration of 2 mg/mL had a strong DPPH radical scavenging capacity and moderate radical scavenging capacities for both hydroxyl and superoxide anions (Oyetayo et al., 2009). Some studies also found 5-hydroxymethyl-2-furfural (HMF) in the methanol extract from the fruiting body of *D. indusiata*. As a result, HMF inhibits tyrosinase activity, which plays an important role in preventing the abnormal accumulation of melanin pigments (Sharma et al., 2004). However, there is limited information available regarding the bioactivities of *D. indusiata*, particularly its cosmeceutical potential during the egg stage. Thus, conducting further research on this stage is essential.

Generally, polysaccharides are prepared through water extraction. This method is easy to perform, eco-friendly, and suitable for large-scale extraction. Nevertheless, this process is time-consuming, and heat is required (Kang et al., 2019). The dried fruiting body can subsequently undergo organic solvent extraction, such as reflux with methanol (Habtemariam, 2019), but it negatively affects the sugar components within polysaccharides (Zhang et al., 2023) and is not considered environmentally friendly. This limitation restricts the use of these techniques (Liu et al., 2017). Green technologies such as microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE) have been incorporated into the extraction process to promote eco-friendly practices and often improve extraction efficiency (Wu et al., 2021). Both techniques may reduce the time and solvent requirements.

The objectives of this study were to determine the bioactive components of *D. indusiata* extracted with water using MAE and UAE during the egg stage compared with those extracted via the hot water extraction (HWE) method. *In vitro* antioxidant activities were also investigated. The selection of a suitable extraction method may be considered in the extraction of bioactive components for potential antioxidant sources in cosmeceutical applications.

Figure 1. The egg stage of *D. indusiata*

2. MATERIALS AND METHODS

2.1 Materials

During the egg stage, *D. indusiata* that originated from Krabi province, Thailand, were subjected to lyophilization by the Thailand Institute of Scientific and Technological Research (TISTR). Gallic acid (Sigma-Aldrich, China), quercetin (Sigma-Aldrich, India), D-glucose (CARLO ERBA Reagents GmbH, Italy), and Trolox (Sigma-Aldrich, Switzerland) served as standards in this study. All other reagents and solvents employed in this study were of analytical grade.

2.2 Extraction

2.2.1 HWE

HWE was prepared by boiling *D. indusiata* powder in water at a ratio of 1:20 at 95 ± 2°C for 2 h, as demonstrated in Figure 2. After centrifugation, the supernatant was deproteinized with Sevag reagent (chloroform:n-butanol = 4:1), and the deproteinized supernatant was then precipitated with cold ethanol overnight. The precipitate was subsequently collected, centrifuged, and lyophilized to obtain bioactive components (Hua et al., 2012).

2.2.2 MAE

D. indusiata powder was combined with water at a ratio of 1:20 and placed in a microwave, as shown in Figure 2. The extraction was conducted using a microwave (GE86NMD, Samsung, Malaysia) for 20 min at a power of 300 W and a temperature of 75 ± 2°C. After the reaction was terminated, the supernatant was separated by centrifugation. Next, the supernatant was deproteinized with Sevag reagent

(chloroform:n-butanol = 4:1) and precipitated with cold ethanol overnight. Finally, the precipitate was collected, centrifuged, and lyophilized to obtain bioactive components.

2.2.3 UAE

D. indusiata powder was mixed with water at a ratio of 1:20 and subjected to extraction using an ultrasonic bath (CPX series, Branson, USA) for 15 min, as illustrated in Figure 2. The extraction conditions included an ultrasonic power of 234 W, an ultrasonic frequency of 47 ± 6 kHz, and a temperature of 40 ± 2°C. Following extraction, the supernatant was obtained through centrifugation. This mixture was subsequently deproteinized with Sevag reagent (chloroform:n-butanol = 4:1), followed by precipitation with cold ethanol overnight. The precipitate was then collected, centrifuged, and lyophilized to obtain bioactive components.

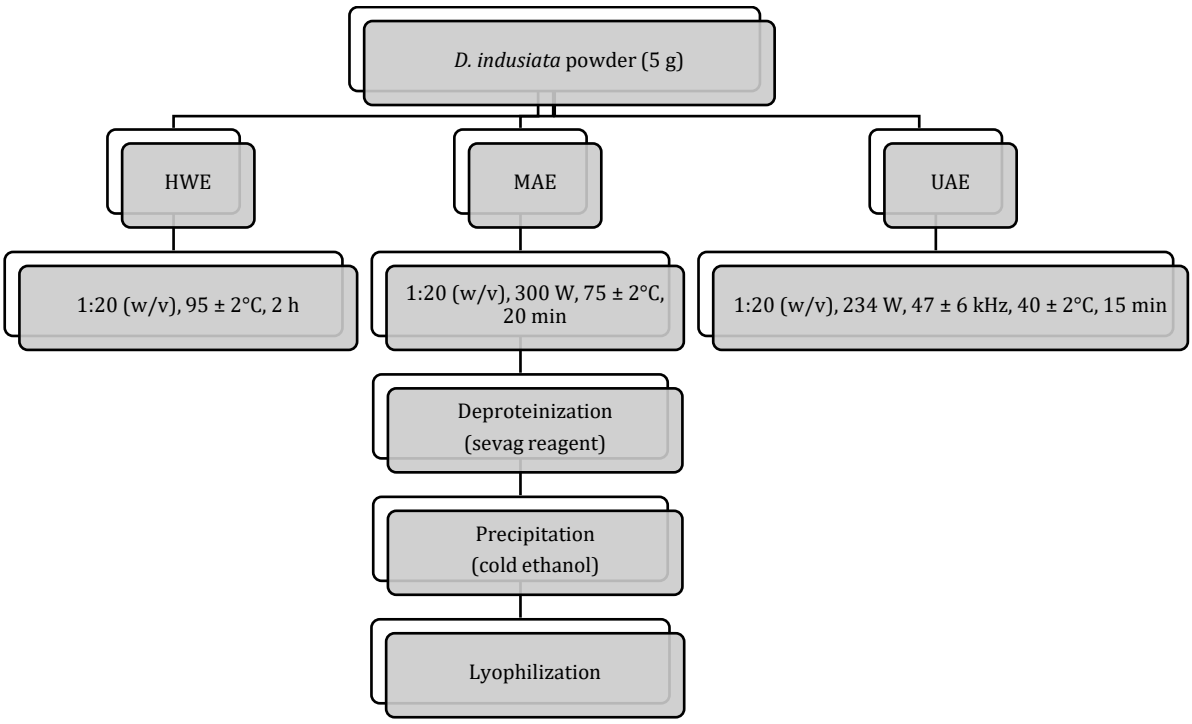

Figure 2. Diagram illustrates the condition of 3 methods used to extract the egg stage of *D. indusiata*

2.3 Quantification of total polysaccharide, total phenolic, and total flavonoid contents

2.3.1 Total polysaccharide content

The total polysaccharide content was determined via the phenol-H_2SO_4 method (Ibrahim, 2014). Briefly, 500 µL of the sample (0.5 mg/mL) was treated with a 5% phenol solution (500 µL) and H_2SO_4 (2.5 mL), and the mixture was allowed to equilibrate to room temperature. The absorbance was subsequently measured at 490 nm using a microplate reader (EPOCH2, Biotek, USA). The total polysaccharide content was quantified in milligrams of D-glucose per gram of extract obtained from a calibration curve of D-glucose.

2.3.2 Total phenolic content

The total phenolic content was assessed utilizing the Folin-Ciocalteu method (Singleton & Rossi, 1965). Briefly, 20 µL of the sample (4 mg/mL) was pipetted into a 96-well plate, and then 100 µL of 10% (v/v) Folin-Ciocalteu reagent and 80 µL of 7.5% sodium carbonate were added. After mixing, the mixture was incubated in the dark for 30 min, and the absorbance was measured at 765 nm using a microplate reader (EPOCH2, Biotek, USA). The total phenolic content was quantified in milligrams of gallic acid per gram of extract from a calibration curve of gallic acid.

2.3.3 Total flavonoid content

The total flavonoid content was determined utilizing the aluminium chloride method (Laczkó-Zöld et al., 2018). Briefly, 20 µL of the sample (4 mg/mL) was dispensed into a 96-well plate, followed by the addition of 15 µL of 2.5% (w/v) $AlCl_3$, 20 µL of 10% (w/v) sodium acetate, and 145 µL of distilled water. The extracts were thoroughly mixed and left at room temperature for 15 min. The absorbance was then recorded at 430 nm using a microplate reader (EPOCH2, Biotek, USA). The total flavonoid content was quantified in mg of quercetin per gram of extract from a calibration curve of quercetin.

2.4 Evaluation of *in vitro* antioxidant activity

2.4.1 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay

The DPPH assay was carried out following the modified methods of Mamah et al. (2017) and Tailor and Goyal (2014). In brief, the DPPH solution was added to the sample (1–10 mg/mL) within a 96-well plate, and the mixture was incubated in the dark at room temperature for 30 min. The absorbance was then measured at 517 nm using a microplate reader (EPOCH2, Biotek, USA). Trolox served as the positive control. The results are depicted as IC_{50} values obtained from an inhibition concentration-

response curve. The percentage of inhibition was calculated via the following Equation (1):

$$\% \text{ Inhibition} = (A_0\text{-}A_1/A_0) \times 100 \quad (1)$$

where A_0 is the absorbance of control, A_1 is the absorbance of standard or sample.

2.4.2 2,2'-Azinobis (3-ethylbenzthiazoline-6-acid) (ABTS) assay

The ABTS assay followed the modified method outlined by Mamah et al. (2017). A solution containing $ABTS^{\bullet+}$ (a mixture of 7 mM ABTS solution and a 2.45 mM potassium persulfate solution) was introduced to the sample (1–10 mg/mL) in a 96-well plate, and the mixture was incubated for 6 min. Following incubation, the absorbance was measured at 734 nm using a microplate reader (EPOCH2, Biotek, USA). Trolox was utilized as the positive standard. The results were then quantified as IC_{50} values with an inhibition concentration-response curve. The percentage of inhibition was calculated according to Equation (1).

2.4.3 Ferric reducing antioxidant power (FRAP) assay

The FRAP assay followed the modified method described by Mamah et al. (2017) and Lee et al. (2013). This assay was carried out in a 96-well plate. In brief, the FRAP reagent (a mixture of 300 mM acetate buffer, 10 mM TPTZ solution, and 20 mM $FeCl_3 \cdot 6H_2O$) was added to the sample (8 mg/mL), and the mixture was incubated at 37°C for 4 min. The absorbance of the mixture was then measured at 593 nm using a microplate reader (EPOCH2, Biotek, USA). The results are expressed in mg Trolox equivalents per gram of extract obtained from a calibration curve of Trolox.

2.5 Statistical analysis

The experiment was conducted in triplicate, and the results are reported as the mean value with its standard deviation (SD). Significant differences between the experimental groups were analyzed using a one-way ANOVA with IBM SPSS Statistics 26, with $p<0.05$ considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Extraction

D. indusiata powder was subjected to extraction using the HWE, MAE, and UAE methods, and the extraction yields are shown in Table 1. The results indicated that all extracts produced a fine powder with a white-grayish color. Each extraction method resulted in differences in the quantity of extraction yield, with MAE resulting in the highest yield at 15.60 ± 1.44%, followed by HWE and UAE. Notably, the extraction process significantly affected the percentage extraction yield. The high yields observed may be attributed to the elevated temperature (Braga et al., 2006). The use of high temperatures (75–95 °C) in MAE and HWE influences extraction efficiency, as higher temperatures promote increased solubility and diffusion into the solvent, resulting in a higher mass transfer rate (Vilas-Boas et al., 2023). Moreover, the extraction results mentioned were consistent with those of studies conducted by He et al. (2020), who extracted polysaccharides from Qingke (*Tibetan hulless* barley) using various extraction methods. Their research revealed that the extraction yields of Qingke obtained via MAE were significantly greater than those achieved through HWE and UAE (He et al., 2020). However, Wu et al. (2021) reported that UAE resulted in the highest extraction yield of the fruiting bodies of *D. indusiata*. The variation in findings may be due to the different conditions in the growth stage of the mushroom, the particle size of the mushroom powder, and the ultrasonic/microwave power used for each extraction method.

Table 1. Extraction yield of the extract from *D. indusiata* during the egg stage prepared by different extraction methods (n = 3)

Extraction method	Extraction yield (%)
HWE	14.48 ± 0.50
MAE	15.60 ± 1.44
UAE	12.33 ± 0.42

3.2 Quantification of total polysaccharide, total phenolic, and total flavonoid contents

The extract from *D. indusiata* during the egg stage was screened for total polysaccharide, total phenolic, and total flavonoid contents, which may have contributed to its antioxidant activities. The results revealed that each extraction method resulted in significant differences in bioactive components ($p<0.05$), as shown in Table 2. The total polysaccharide content was determined using the equation derived from the D-glucose calibration plot ($y = 0.0166x + 0.0619$; $R^2 = 0.9985$). Notably, each extraction method resulted in significant differences in the total polysaccharide content ($p<0.05$). Among these methods, MAE resulted in the highest total polysaccharide content (746.75 ± 3.04 mg glucose/g extract), followed by UAE and HWE. The increased total polysaccharide yields could be attributed to the action of microwave irradiation. When the plant is placed in a solvent transparent to microwaves, the elevated vapour pressure causes destruction of the tissue and cell walls, enabling extractable substances to be displaced into the solvent. This phenomenon enhances the surface area of contact between the solid and liquid phases (Cheng et al., 2015). Additionally, the lower total polysaccharide yields observed with HWE may be attributed to prolonged exposure to high temperatures. Some studies have shown that prolonged high temperatures during extraction may induce alterations in the molecular structure of polysaccharides, leading to a reduced yield (Cai et al., 2008). Additionally, Liu and colleagues investigated the extraction of polysaccharides from the fruiting body of *D. indusiata* using hot water at 90°C for 1–3 h. These findings revealed that the yield of *D. indusiata* polysaccharide (DIP) increased with increasing extraction time and stabilized at 2 h. They also reported that DIP increased when the temperature increased from 70°C to 90°C but slightly decreased at 100°C. This decrease may be due to the relationship between temperature and polysaccharide dissolution. At higher temperatures, the presence of pectin and other components makes polysaccharide dissolution from particles more difficult (Liu et al., 2017). On the basis of these findings, it is possible that using temperatures above 90°C (such as the

95°C used in our study), even with an extraction time of 2 h, may result in a small reduction in the DIP amount. The total phenolic content was subsequently determined via an equation derived from a gallic acid calibration plot (y = 0.0622x + 0.0582; R^2 = 0.9995). The highest total phenolic content was found in UAE (9.73 ± 0.28 mg gallic acid/g extract), followed by MAE and HWE. This may be attributed to the disruption of the plant cell wall caused by the collapse of cavitation bubbles, which leads to the release of extractable compounds and allows greater penetration of the solvent into the sample matrix (Pisoschi et al., 2016; Tomšik et al., 2016). Additionally, our results agree with those reported by Dobros and colleagues, who reported that the total phenolic content extracted by UAE was the highest, followed by maceration and decoction (Dobros et al., 2022). Additionally, UAE is the most effective method for increasing the total phenolic yield, possibly because it alleviates the degradation of phenolic substances induced by high temperatures (Irawan et al., 2021). Furthermore, the total flavonoid content was determined using the equation derived from the quercetin calibration plot (y = 0.0431x + 0.047; R^2 = 0.9998). The total flavonoid content was not significantly different between the HWE and UAE methods. As shown in Table 2, the total flavonoid content was high in HWE (3.96 ± 0.03 mg quercetin/g extract) and UAE (3.96 ± 0.12 mg quercetin/g extract). Interestingly, the HWE method yields a high total flavonoid content but a lower total phenolic content. This may be attributed to gallic acid, which is a phenolic acid that is a subtype of phenolic compounds. It is regularly regarded as a heat-labile compound (Antony & Farid, 2022). During the boiling process at a high temperature of 95°C, phenolic acids may degrade, leading to a reduced amount of phenolic acids. On the other hand, quercetin is a flavanol that is a subtype of flavonoid and is also classified as a phenolic compound (Dias et al., 2021). Sharma et al. (2015) reported that quercetin is more heat resistant. These authors reported that the contents of quercetin and its glucoside increased as the temperature increased to 120°C and then decreased when the temperature reached 150°C. These findings suggest that the HWE method effectively preserves the flavonoid content while reducing the phenolic content. Furthermore, the total phenolic and total flavonoid contents obtained using the HWE method differ from those reported by Nasir and colleagues, who studied the aqueous extract of *D. indusiata*. These differences could result from differences in extraction conditions, such as the growth stage of the mushroom, extraction ratio, temperature, and duration (Nazir et al., 2021).

Table 2. Total polysaccharide, total phenolic, and total flavonoid content of the extract from *D. indusiata* during the egg stage prepared by different extraction methods (n = 3, mean ± SD)

Extraction method	Total polysaccharide (mg glucose/g extract)	Total phenolic (mg gallic acid/g extract)	Total flavonoid (mg quercetin/g extract)
HWE	540.97 ± 2.43	5.11 ± 0.20	3.96 ± 0.03
MAE	746.75 ± 3.04*	6.36 ± 0.12*	3.21 ± 0.15*
UAE	616.87 ± 2.71*	9.73 ± 0.28*	3.96 ± 0.12

Note: * indicates significant difference from HWE ($p<0.05$)

3.3 Evaluation of *in vitro* antioxidant activity

DPPH, ABTS, and FRAP assays were used as tools to measure antioxidant activity. The results revealed that the *D. indusiata* extract during the egg stage scavenged free radicals, as shown in Figures 3 and 4. The results of the DPPH assay revealed that HWE had a significantly greater ability to scavenge free radicals (IC_{50} value of 6.57 ± 0.20 mg/mL) than UAE (IC_{50} value of 9.32 ± 0.12 mg/mL) and MAE (IC_{50} value of 9.90 ± 0.09 mg/mL). In the FRAP assay results, HWE also exhibited the highest efficiency in scavenging free radicals (a reducing power of 5.59 ± 0.10 mg TE/g extract), followed by MAE (a reducing power of 4.72 ± 0.13 mg TE/g extract) and UAE (a reducing power of 4.43 ± 0.09 mg TE/g extract). Our findings revealed that HWE exhibited strong antioxidant activity in both the DPPH and FRAP assays. Our results were also similar to those of Zhang et al. (2023), who reported that *D. indusiata* extracted by HWE at a concentration of 8 mg/mL presented greater DPPH radical scavenging activity than UAE did. The results of the ABTS assay revealed that UAE was better able to scavenge free radicals (IC_{50} value of 5.68 ± 0.09 mg/mL) than HWE (IC_{50} value of 5.89 ± 0.16 mg/mL) and MAE (IC_{50} value of 7.36 ± 0.11 mg/mL). However, the difference between UAE and HWE was not statistically significant in this assay. Notably, the HWE method yields bioactive compounds with potent antioxidant properties, even though it results in a lower total phenolic content. This might be due to heat-resistant substances such as flavonols within the HWE. Flavonols are a subclass of flavonoids classified as phenolic compounds. Flavonoids possess antioxidant properties, primarily due to their ability to scavenge reactive oxygen species (ROS), facilitated by the phenolic hydroxyl groups attached to the flavonoid structure. In a redox reaction, the phenolic groups of flavonoids, which are redox active, can react with ROS and hence donate an electron or a hydrogen atom, which allows them to function as scavengers (Speisky et al., 2022). The antioxidant mechanism of flavonoids also involves the inhibition of ROS formation via metal chelation and the activation of antioxidant enzymes (Dias et al., 2021). Moreover, UAE is an alternative method that provides antioxidant activity comparable to that of HWE. UAE is suitable for phenolic compound extraction because it operates at relatively low temperatures that prevent the degradation of these compounds. Phenolic compounds can be divided into two major classes, namely, flavonoids and phenolic acids. These compounds are known for their antioxidant activity, as they scavenge free radicals, donate electrons and hydrogen atoms, and chelate metal cations (Hassanpour & Doroudi, 2023; Lee & Yoon, 2021). The redox properties of phenolic acids are believed to constitute the main mechanism for their antioxidant activity. Moreover, the findings of this study on antioxidant activity were consistent with those of Ruksiriwanich et al.

(2022), who studied the antioxidant activity of ethanol extracts from the fruiting body of *D. indusiata*. They confirmed that whole mushrooms contain flavonoids (epicatechin) and nonflavonoid compounds (gallic acid and caffeic acid), including polysaccharides with high antioxidant properties. These compounds can act through multiple antioxidative mechanisms, including hydrogen atom transfer, single electron transfer, and the ability to chelate transition metals. Additionally, further studies have provided evidence suggesting that polysaccharides can act as free radical scavengers and may act as natural antioxidants. Wang and colleagues reported that monosaccharides contain either an aldehyde group (aldose) or a ketone group (ketose) and several -OH groups. Consequently, electrophilic groups such as aldehydes or keto functionalities can promote the release of hydrogen from O–H bonds, thereby potentially enhancing the antioxidant capabilities of polysaccharides (Wang et al., 2016). However, polysaccharides can also exhibit antioxidant activity, but their effectiveness is lower than that of other active compounds in the phenolic group (Ru et al., 2012). Therefore, the MAE method may lead to lower antioxidant activity, despite the high polysaccharide content. This resulted from the low to moderate total phenolic and total flavonoid contents present. Overall, the compounds extracted using the HWE method exhibited strong antioxidant activity, which was acted via electron and hydrogen atom donation as well as the reduction of complex compounds. However, UAE is another extraction method that has equivalent properties in scavenging free radicals. The UAE method also operates at lower extraction temperatures and requires shorter extraction times. For this reason, UAE may be an effective method for the extraction of phenolic compounds, which are widely used in cosmetics because of their antioxidant activities. The antimicrobial, anti-inflammatory, and antiaging properties of phenolic compounds are also recommended for use in cosmetology and dermatology (de Lima Cherubim et al., 2020).

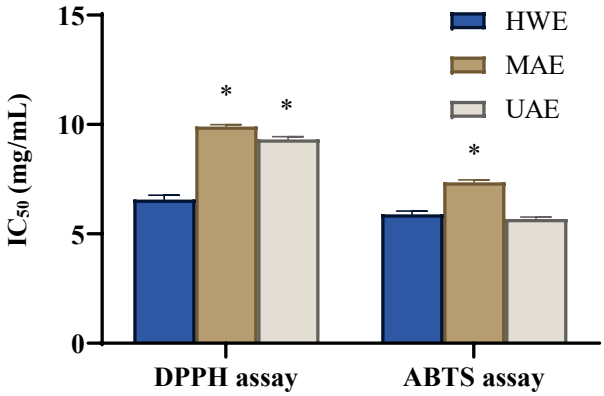

Figure 3. Radical scavenging activity of the extract from *D. indusiata* during the egg stage prepared by different extraction methods in DPPH assay and ABTS assay (n = 3, mean ± SD)
Note: * indicates significant difference from HWE ($p<0.05$)

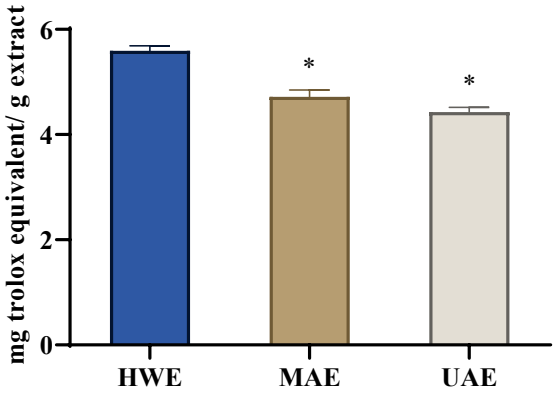

Figure 4. Reducing ability of the extract from *D. indusiata* during the egg stage prepared by different extraction methods in FRAP assay (n = 3, mean ± SD)
Note: * indicates significant difference from HWE ($p<0.05$)

4. CONCLUSION

Different extraction methods significantly affect the extraction yield, bioactive component yield, and antioxidant activity. Among all the extraction methods, *D. indusiata* extracted by MAE presented the highest extraction yields and total polysaccharide content. Furthermore, HWE has notable antioxidant properties and a high total flavonoid content, as evidenced by the results of the DPPH and FRAP assays, but HWE requires a long extraction time and high temperatures. This is a limitation for extracting heat-labile compounds. However, strong antioxidant activities were also observed in UAE, which had the greatest total phenolic content, as evidenced by the ABTS assay. Additionally, UAE offers significant time savings and lower extraction temperatures. It is utilized for phenolic compounds, which are widely used in cosmetic products. These findings conclude that UAE shows promise as an environmentally friendly technology and is an excellent method for extracting bioactive components from *D. indusiata* with potent antioxidant activities. This enables the use of the extracted substances for future applications in cosmeceuticals.

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