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Phytochemical, Antioxidant and Photo-Protective Activity Study of Bunga Kantan (*Etlingera elatior*) Essential Oil

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ABSTRACT

The three main objectives of this study were to evaluate the extracted essential oil in terms of total phenolic and flavonoids contents, radical scavenging activity and sun protection factor. The antioxidant activity of the essential oil was determined by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity while the content of total phenol was determined by using Folin-Ciocalteu reagent. Aluminium chloride colorimetric method was used for flavonoid content determination while photo-protective potential was determined by using Mansur's sun protection factor (SPF) value. The extracted essential oil was found to contain low levels of total phenolic contents (2.30±0.003µg/g as garlic acid equivalent) and total flavonoid contents (254.99±0.64 µg/g as quercetin equivalent) respectively. DPPH free radical scavenging activity of the essential oil was40.73%±1.32 at 200µg/ml. The Mansur's equation of sun protective factor showed that the essential oil possesses some sun protective potentials. This study suggests that the essential oil from *Etlingera elatior* could be potentially used as a new source of natural antioxidant and ingredient that can be incorporated into the sunscreen cosmetic products.

INTRODUCTION

The essential oil is an odorous and volatile product that originates from plant species. These plant species usually produce characteristic aroma from different plant parts: leaves, stems, flowers and roots or rhizomes. Chemically, a single drop of the volatile oil is made up of more than 200 different chemical components, and most of these trace constituents are solely responsible for this characteristic flavour (Doughari, 2009).

Etlingera elatior (EE) or locally known as bunga kantan in Malaysia with its characteristic sweet fragrance has been widely cultivated especially as spices for curry. Bunga Kantan, which belongs to the Zingiberaceae family, is herbaceous perennial plant native to Sumatra and other parts of Indonesia. Besides the name bunga kantan, it is also called as 'torch ginger' or 'red ginger lily' in English and *"kecombrang"* or "*honje"* in Indonesian. The chemical constituents of the essential oil have been categorised into alcohol, aldehyde, ester, hydrocarbon, monoterpene derivative, sesquiterpene and its derivatives (Chan *et al*., 2010). Amongst these constituents, monoterpene hydrocarbon is present as major compounds (Doughari, 2009) while 2-undecanone was associated with insect repellent potential (Khalid and Azman, 2016). Previous studies on various crude extracts from the plant parts of EE have been associated with its pharmacological or medicinal uses; methanol and ethyl acetate extracts were found to be antimicrobial and antifungal agent (Lachumy *et al*., 2010) and ethyl acetate of the flower extract was found to have antioxidant activity as well as a wide range of phyto-constituents (Maimulyanti and Prihadi, 2015). Studies involving the comparison of the plant parts of *EE* collected from different locations in Malaysia also revealed that the flowers exhibited promising anticancer activity, which is attributed to the presence of high amount of phenolic and flavonoid compounds (Ghasemzadeh *et al*., 2015).

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However, according to literature, there was only one study that had reported on the antioxidant properties from the *EE* essential oil (Abdelwahab *et al*., 2010) while no exploration was done on the photo-protective activity. Exploring photo-protective activity from herbal plant resources has been gaining increasing attention in recent years. Rojas *et al*. (2016) reviewed essential oils and plant extracts from more than 40 types botanical sources showed that photo-protective activities of these plants were attributed to the secondary metabolites such as flavonoids, alkaloids, etc. Eleven medicinal plants from Sri Lanka were found to display high sun protection factors (SPF >25) compared to well-known medicinal plant, *Aloe vera* (Napagoda *et al*., 2016). Furthermore, studies on the use of natural antioxidants in sunscreens to provide supplementary photo protective action activity gave rise to the new possibilities for the treatment and prevention of UV-mediated diseases (Costa *et al*., 2015; Iman *et al*., 2015; Silva *et al*., 2014). Since the anti-oxidant properties were not fully studied and the photo-protective potential of the *EE* essential oil were not explored, this research were carried out with three objectives; which are to evaluate, (i) the total phenolic and flavonoids contents, (ii)the radical scavenging activity and,(iii)the sun protection factor of the *EE* essential oil.

MATERIALS AND METHODS

Plant Materials

The *EE* flower and stem in this study were collected from a local market in Ipoh, Perak, Malaysia. Only mature *EE* with flower at full bloom were choose for this study (Choon and Ding, 2016) and identification were performed at Biology Department, Universiti Pendidikan Sultan Idris, Malaysia. The plant materials were washed, chopped into small pieces and air-dried in a shady place at room temperature for 5 days to minimize the losses of antioxidant properties due to thermal degradation. The dried plant materials were then ground into fine powder at a total mass of 210 gram.

Extraction of the Essential Oil

The total of 210 gram fine *EE* powder obtained was subjected to 5-6 hours of hydro-distillation using a simple distillation method (Siddique *et al*., 2012;Jaafar *et al*., 2007). The essential oil was isolated by extraction of the distillate with one liter of dichloromethane using a separating funnel for three times. The mixture of the distillate and dichloromethane was shaken for 5 minutes and allowed to stand over night. The organic layer was separated and concentrated by evaporation under pressure until a constant weight was gained. The weight of the extracted oil was calculated and kept in a sealed vial at 4ºC for further analysis.

Phytochemical Screening

The extracted essential oil was subjected to preliminary phytochemical screening to determine the types of phytochemicals present in the essential oil (Prashant Tiwari *et al*., 2011).

Determination of Total Phenolic Content

Total phenolic content in the essential oil was measured using the Folin–Ciocalteu reagent method (Mohamed *et al*., 2013). Briefly, 200 µL essential oil (5 mg oil/ml ethanol) was made up to 3 ml with distilled water which was then mixed thoroughly with 0.5 ml of Folin-Ciocalteu reagent. After mixing for one min, 2 ml of 20% (w/v) sodium carbonate was added and allowed to stand for one hour in the dark. The absorbance of the reaction mixtures was measured at 650 nm (Lamda 35, UV/Vis Spectrophotometer Perkin Elmer, 190-1100 nm), and the results were expressed as µg Gallic acid (GAE)/g of dry weight. Gallic acid concentrations ranging from 1 to 5 µg/mL were prepared and the standard calibration curve was obtained using a linear fit at r2 =0.9855 (Figure 1).Triplicate measurement were made for each test sample. The result was expressed as gallic acid equivalent.

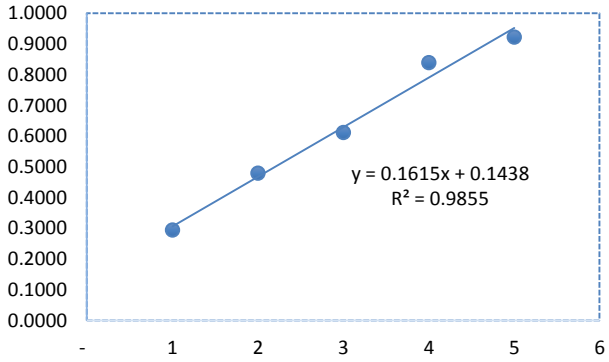

Fig.1: Calibration Curve of Garlic Acid

Determination of Total Flavonoid Content

Total flavonoid content was determined by using aluminium chloride method with quercetin as standard (Seow *et al*., 2016). Briefly, 1 mL of 2% w/v aluminium chloride was added to 200 µL essential oil (5 mg oil/ml ethanol). After vortexing for 10 seconds, the mixture was incubated at room temperature for one hour. The absorbance of the solution was measured at 420 nm. Quercetin concentrations ranging from 5 to 500 µg/mL were prepared and the standard calibration curve was obtained using a linear fit at r2 = 0.9765 (Figure2).Triplicate measurements were made for each test sample. The result was expressed as quercetin equivalent.

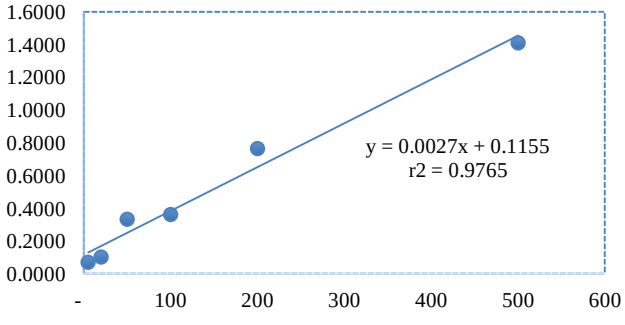

Fig 2: Calibration Curve of Quercetin.

Determination of Radical Scavenging Antioxidant Activity

The radical scavenging anti-oxidant activity of the essential oil was determined by slightly modified DPPH radical scavenging assay (Mohamed *et al*., 2013). Briefly, a 0.1 mM solution of 2, 2- diphenyl-1-picryl-hydrazyl (DPPH) in methanol was prepared and 1 ml of this solution was added to 3 ml of the essential oil at different concentrations (20 – 500 µg/ml). Gallic acid which was used as a positive control was prepared at different concentrations (20-200 µg/ml) and a calibration curve was plotted (Figure 3). Discolouration was measured at 517 nm after incubation for 30 minutes in the dark. Measurements were taken in triplicate. The capacity to scavenge the DPPH radical was calculated following % inhibition = $[(A_0-A_A)/ (A_0)]x100$ where A_0 is absorbance of blank sample and A_A is absorbance of test sample.

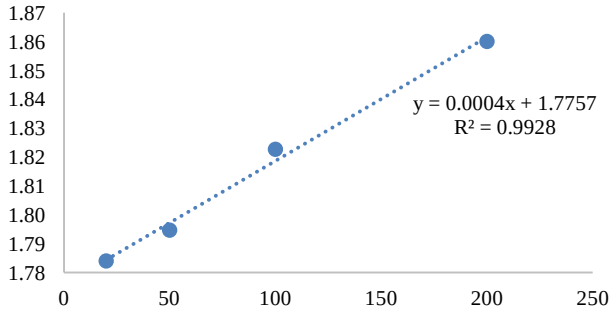

Fig 3: Calibration Curve of Garlic Acid in DPPH

Determination of in-vitro Photo- Protective

The photo-protective potential of the essential oil was indicated by the sun protection factor (SPF) following the Mansur's method (Kaur and Saraf, 2011). Briefly, 200µg/ml essential oil was prepared. The absorbance of the essential oil was measured from 290 – 320 nm, at 5 nm interval. Methanol was used as blank. The observed absorbance values at 5 nm intervals (290-320 nm) were calculated by using the formula SPF spectro-photometric:

$$SPF = CF \times \sum_{290}^{320} EE\ (\lambda) \times I(\lambda) \times Abs(\lambda)$$

Where, CF = correction factor (10), $EE\ (\lambda)$ - erythemal effect spectrum; $I(\lambda)$ - solar intensity spectrum; Abs (λ) – absorbance of sunscreen product.

Statistical Analysis

All results were presented as the mean ± standard deviation. Using Statistical Package for the Social Sciences (SPSS) version. 17, the analysis of variance, correlation and student's t-test were performed. The *p* value ($P<0.05$) was considered as statistically significant level.

RESULT AND DISCUSSION

The hydro-distillation of *EE yielded 0.063% (w/w) of* essential oil. The oil obtained was light yellowish with a strong, pleasant-smell. This result is in consistent with the previous study which reported that a typical sample of hydro-distillation yield from different plant parts ranged from 0.0021% - 0.07% (Jaafar *et al*., 2007).

Compared with other organic solvent distillation, the yield reported in this study was comparatively low; this can be attributed to the availability of extractable components based on different solvent polarity (Maimulyanti and Prihadi, 2015). Although hydro-distillation in this study produced low yield, water as extraction solvent is considered the best choice because it follow the same manner as the traditional medicine is prepared.

The phytochemical results are shown in Table 1. The screening results showed that the *EE* possesses steroids, phenol, flavonoids and trace amount of amino acids. Till now, no data has ever been published on the phytochemical screening of the essential oil. In comparison with crude extracts (Maimulyanti and Prihadi, 2015), this study confirms the presence of alkaloids which had never been reported before. Alkaloids are nitrogenous compounds which function in the defence of plants against herbivores and pathogens. They are widely exploited for their antimicrobial and antibiotic activities (Prashant Tiwari *et al*., 2011). Thus the antimicrobial activity reported in the essential oil of *EE* in previous studies could be partially contributed by the alkaloids.

Assessment of total phenolic and flavonoid contents of the essential oil are presented in Table 2. Phenolic and flavonoids contents were 2.30±0.003 µg/g and 254.99 ±0.64 µg/g respectively; both chemical components occur ubiquitously as natural colour pigments, which contribute to the attractive colour of the *EE* flower.

It is a note worthy that the essential oil contains significantly higher flavonoid content than phenolic content ($P < 0.005$)(Table 2).This result is similar in other plant species (Nurain *et al*., 2013); but contradicted with the *EE* essential oil study reported earlier (Abdelwahab *et al*., 2010). Different results obtained could be due to the plant maturity, seasons and geographical locations of the different of plant materials collected (Çetinkaya *et al*., 2016).

The higher concentration of flavonoids as compared to phenolic content could be specifically attributed to the quercetin or quercetin derivative which is structurally matching to the quercetin standard used in this study. This explanation is also reasonable as quercetin is present in nearly 70% of all the plants (Doughari, 2009) and so this is the reason for the higher probability of its presence in the essential oil extracted from *EE* in this study.

The radical scavenging effect of *EE* is reported in Table 2. At the concentration of 200 µg/g, the essential oil and gallic acid was determined as 40.73% and 94.86% respectively. This result indicated that gallic acid scavenging effects was significantly higher than the essential oil (P=0.00), and it was determined as concentration-dependent (r= 0.9806) (Figure 3). The higher the concentration of the essential oil, the higher the level of inhibitory activity against the free radicals (Figure 4).

Table 1: Phytochemical Screening Result.

Test	
Detection of alkaloids	
Wagner's test	+
Meyer's test	+
Hager's test	+
Detection of carbohydrates	
Molisch's Test	-
Benedict's Test	-
Detection of glycosides	
Legal's Test	+
Detection of phytosterols	
Salkowski's Test	+
Detection of phenols	
Ferric Chloride Test	-
Detection of flavonoids	
Alkaline Reagent Test	+
Detection of proteins and amino acids	
Xanthoproteic test	+

Table 2: Total Phenolic and Flavonoid Contents and Antioxidant Activity of the EE Essential Oil.

Description	**Concentration**	**Regression**
Total phenolic	2.30±0.003 µg/g	$y = 0.1615x + 0.1438$, $R^2 = 0.9855$
Total flavonoid	254.99 ±0.64 µg/g	$y = 0.0027x + 0.1155$, $R^2 = 0.9765$
Essential oil Scavenging effect*	40.73%±1.32	$y = 0.0002x + 0.6968$, $R^2 = 0.9978$
Garlic acid Scavenging effect*	94.86%±0.61	$y = 0.0004x + 1.7757$, $R^2 = 0.9928$

*****at 200 µg/g

Table 3: SPF value of *Etlingera elatior* essential oil.

Wavelength (nm)	**EE (λ) x I(λ)**	**Abs. (A_1)**	**EExIxA**	**Abs. (A_2)**	**EExIxA**	**Abs. (A_3)**	**EExIxA**
290	0.0150	0.441	0.007	0.44	0.007	0.442	0.007
295	0.0817	0.396	0.032	0.396	0.032	0.398	0.033
300	0.2874	0.381	0.109	0.381	0.109	0.381	0.109
305	0.328	0.355	0.116	0.357	0.117	0.354	0.116
310	0.186	0.331	0.062	0.330	0.062	0.331	0.062
315	0.084	0.291	0.024	0.290	0.024	0.291	0.024
320	0.018	0.254	0.005	0.252	0.005	0.252	0.005

SPF = ∑ EE (λ)x I(λ)xAx10 (correction factor)

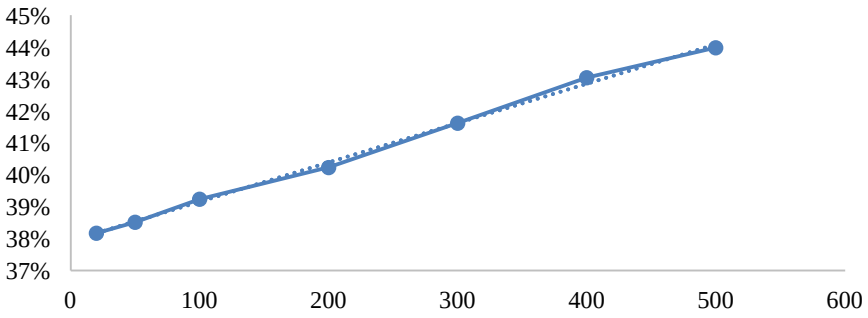

Fig.4: % of Scavenging activity versus concentration of essential oil.

The remarkable scavenging activities of the essential oil can be attributed to the high flavonoid content in comparison to the phenolic content. Flavonoids are polyphenolic compounds which can be categorized into various groups such as flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidins and chalcones (Subedi *et al*., 2014). Hence, it can act asscavengers of various oxidizing species and also act as quenchers of singlet oxygen of different chemical structurally.

This is the first ever study which reports the *in vitro* photo-protective ability of *EE* (Table 3). The *in vitro* photo-protective ability determined by the sun protection factor (SPF) was 3.555±0.025. This result revealed that the essential oil contains natural compounds which is able to provide UV blockage at least at 50% (Iman *et al*., 2015). This natural compounds can be attributed to the present of flavonols such as quercetin, it is believe able to prevent UV radiation-induced damage to plants by increasing quercetin biosynthesis after exposure to the UV rays (Saewan and Jimtaisong, 2013). Besides, the UV energy absorbed could be dissipated as heat, light or through decomposition of flavonols (Saewan and Jimtaisong, 2013). As such, incorporating the *EE* essential oil into any sunscreen product could suggest an alternative way to replace the synthetic compound.

CONCLUSION

This study has successfully reported the phytochemical screening and photo-protective potential of the essential oil from *EE*. Results from this research revealed that the *EE* essential oil is enriched with secondary metabolites, good anti-oxidant activity and sun protection potential. This study further suggests that the essential oil from *EE* could be potentially used as a new source of natural anti-oxidant and ingredient that can be incorporated into the sunscreen cosmetic products.

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