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ANTIOXIDANT, ANTIMICROBIAL AND SPF PROTECTIVE ACTIVITY OF *CUCURBITA MOSCHATA, CUCURBITA RETICULATA AND CLITORIA TERNATEA*

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ABSTRACT

Objective: To provide evidence of antioxidant, antimicrobial and sun protective activity of *Cucurbita reticulata*, *Cucurbita moschata* and *Clitoria ternatea*.

Method: *C. reticulata*, *C. moshata* and *C. ternatea* activity were studied by using DPPH scavenging test for antioxidant activity determination. The antimicrobial activities of all the extracts against seven different bacterial strains were determined by using well diffusion test. Sun protective factor were evaluated at different wavelength using UV spectrophotometer.

Result: The *in-vitro* antioxidant studies showed that all the extracts exerted antioxidant activity against DPPH free radical. The antimicrobial studies revealed that *all the extracts possessed antimicrobial activity against* some of the tested bacterial strain. All the extracts showed sun protective activity and C. ternatea showed higher sun protective activity than sunscreen product.

Conclusion: *C. reticulata*, *C. moschata and C. ternatea* are potential natural antimicrobial and antioxidant source. *C. ternatea* may be used as alternative natural sunscreen.

Keywords: Disc diffusion test; Free Radical, Sunscreen

INTRODUCTION

Antioxidants delay or inhibit the cellular oxidation by free radical which is associated with asthma, inflammatory, diabetes, atherosclerosis and cancers [1]. The natural antioxidants provide benefits over synthetic antioxidants as it is free of side effect and scavenge free radical immediately after intake through metabolic activities [2]. Antioxidant compound will intervene the free radical mediated process and prevent body cells from damaging [3]. Antioxidant rich compound also may prevent UV radiation. Sun exposure to human skin or solar ultraviolet (UV) radiation may cause several skin damages which includes sunburn, skin cancer and oxidative stress. Furthermore, the exposure to ultraviolet radiation from the sun can also cause premature aging of the skin [2]. The current study is designed to evaluate the antioxidant activity and sun protective factor of *Cucurbita moschata*, *Cucurbita reticulata* and *Clitoria ternatea* by using DPPH scavenging assay and wavelength measurement. *C. moschata* is traditionally used to kill parasite, for acne treatment and as diuretic agent [4]. In addition, it is widely known for its anti-diabetic properties, where its fruits and seeds have hypoglycemic activity by increasing plasma insulin level. Previous study had showed that *C. moschata* prevent diabetic nephropathy in alloxan-induced diabetics rats and normal animals [5]. *C. moshata* also had demonstrated antihyperlipidemic activity against rat treated with high fat diet [6]. *C. reticulata* is used to treat indigestion and inflammatory symptom of respiratory tract such as asthma and bronchitis [6]. It may reduce apoptosis in human colon and gastric cancer and also decrease proliferation of cancer cells [7]. *C. ternatea* can be used for treating sore throats and abdominal swelling. In ayurvedic medicine, *C. ternatea* is used for treating neurological health problem such as depression and fever [8]. The current study is designed to evaluate the antioxidant, antimicrobial and sun protective factor of *C. moschata*, *C. reticulata* and *C. ternatea* which might be useful for further isolation of bioactive compound from this plant.

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MATERIALS AND METHODOLOGY

Plant materials:

C. reticulata and *C. moshata* were obtained from Kedah region of Malaysia. As for *C. reticulata*, the skin of the fruits were washed and peeled off. The pulps were squeezed and filtered using muslin cloth. A clear aqueous solution obtained was used for further studies. For *C. moshata* and *C. ternatea*, the

outer layer of the fruits were washed and removed. The seeds in the fruit were removed and washed thoroughly. Next, the seed were dried and then crushed into powder and macerated in distilled water for 3 days. The solution was filtered by using filter paper and a colourless filtrate was obtained.

DPPH-free radical scavenging activity:

A stock solution of DPPH (2, 2-diphenyl-1-picryl-hydrazyl) was prepared by dissolving 1.65 mg DPPH in 50 ml methanol. Then, 5 ml of this solution was added to 1 ml of plant extract at different concentration (0.13 mg/ml, 0.25 mg/ml, 0.50 mg/ml and 1 mg/ml). The mixture was shaken vigorously, and the absorbance was measured at 517 nm after 30 minutes [9, 10].

% Antioxidant activity = (Control absorbance-sample absorbance)/(Control absorbance) X 100

Antimicrobial activity:

The antibacterial activity of extracts was determined by well diffusion method. The bacteria were cultivated at 37 ℃ for 24 hour in agar plate to yield 1 x 10^8 colony forming unit/ml. After 24 hour, bacterial culture was spread uniformly on the agar plate (Muller Hinton Agar) using sterile cotton swab. After that, the plant extracts (40 µL) were inserted in the agar well. Next, the plates were incubated at 37 ℃ for 18-24 hours. Ciprofloxacin (40 µg/ml) was used as positive control while sterile distilled water (40 µL) was used as negative control. The experiment was carried out in triplicate for all the samples [11,12].

SPF value determination:

The absorbance of the sample was measured in Ultraviole spectrometer by setting the wavelength from range of 290 nm to 350 nm with 5 nm interval. Test carried out by utilizing 1 cm quartz cell, and ethanol as the blank. The absorbance was obtained and substituted into Mansur equation and SPF value was calculated [13].

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

EE (λ): Erythemal effect spectrum; I (λ): Solar intensity spectrum; Abs (λ): Absorbance of sample; CF: Correction factor =10.

RESULTS AND DISCUSSION

DPPH scavenging activity:

The DPPH scavenging activity was calculated according to the plant extract's absorbance value at 517 nm wavelength. Many radicals of different species were formed during lipid oxidation such as ·OH, O2·, and H2O2. DPPH was a free radical that often used in radical scavenging activity. It reacted by receiving a hydrogen atom from antioxidants to reduce the odd electron in DPPH. DPPH became stable once it accepted an electron. The reduction of DPPH at 517 nm caused formation of non-radical or stable DPPH-H form. The remaining DPPH measured over time is inversely proportional to the radical scavenging activity of the antioxidant [10]. The table below (Table 1) showed the DPPH activity of different plant extracts at different concentrations. All the extracts showed increase in antioxidant activity in a dose dependent manner. *C. reticulata* showed the highest antioxidant activity among all the tested extracts. This might be due to high carotenoid and magnesium content in *C. reticulata*. In addition, citrus essential oil contained β-pinene, α-pinene, α-terpine, α-terpinolene, and limonene that potentiate it radical scavenging activity [14]. Butylated hydroxytoluene (BHT) was used as a positive control in this study and it showed the highest antioxidant activity. *C. moschata* and *C. ternatea* showed a comparable antioxidant activity to *C. reticulata*. This study showed that *C. moshata, C. cucurbita* and *C. ternatea* could reduce the risk of disease associated with lipid oxidation such as cardiovascular disease.

Antimicrobial activity:

Antimicrobial test was done against seven bacterial strains namely *E. coli, S. aureus, S. pyogenes, P. aeruginosa, K. pneumonia, B. cereus* and *E. faecalis* for all the plant extracts. The findings indicated that *C. moschata* exhibited antimicrobial properties against all tested microbial strain (Table 2). This might be due to presence of linalool, decanol, and β-bisobolene and γ-terpinene in *C. moschata* [15, 16]. *C. reticulata* extract showed antimicrobial effect against five tested microbial strain. This was in agreement with previous antimicrobial study by Jayaprakasha et al which indicate the antimicrobial activity of *C. reticulata* ethanolic extract against different gram positive and gram negative bacterial species [17]. The antimicrobial effect of *C. ternatea* against six bacterial species might be due to the presence of saponin, phenolic and flavonoid compound in the flower extract [18].

Table-1: DPPH scavenging activity of plant extracts and standard BHT at different concentrations

Samples	DPPH scavenging activity (%)			
	0.13 mg/ml	0.25 mg/ml	0.5 mg/ml	1 mg/ml
C. reticulata	10.50 ±0.05	71.24±0.05	85.60 ±0.10	88.50±0.05
C. moschata	11.90 ±0.05	59.15±0.10	61.40 ±0.05	78.60 ±0.10
C. ternatea	20.42±0.03	37.42±0.05	77.42±0.05	79.42±0.05
BHT	66.21±0.05	74.44±0.05	80.30±0.10	93.10±0.05

All the values were obtained from means of three replicates ± standard deviation

Table-2: Antimicrobial activity of plant extracts and ciprofloxacin by well diffusion method

Plant extract	*C. reticulata*	*C. moschata*	*C. ternatea*	Ciprofloxacin	Distilled water
E.coli	+	+	-	+	-
S. aureus	+	+	+	+	-
S. pyogenes	-	+	+	+	-
P. aeruginosa	+	+	+	+	-
K. pneumonia	+	+	+	+	-
B. cereus	-	+	+	+	-
E. faecalis	+	+	+	+	-

Table-3: SPF value of plant extracts, *Aloe vera* and sunscreen product

Sample (1 mg/ml)	SPF value
C. reticulata	10.82
C. moschata	11.54
C. ternatea	23.13
A. vera	20.02
Sunscreen product	14.46

Sun protection factor (SPF) is the measure of a sunscreen's ability to prevent skin damaging that is caused by ultraviolet radiation. Application of sunscreen can prevent penetration of ultraviolet radiation [13]. The exposure to ultraviolet radiation from the sun can also cause premature aging of the skin [19]. All the plant extracts were subjected for the determination of SPF value at concentration of 1 mg/ml. *Aloe vera* and sunscreen product were used as positive control. The SPF value obtained for *C. reticulata, C. moschata and C. ternatea* were 10.82, 11.54 and 23.13 respectively (Table 3). The SPF value calculated for *A. vera* and sunscreen product were 20.02 and 14.46 (Table 3). The result obtained showed that *C. ternatea* extract having SPF value higher than the sunscreen product that is available in market. The high level of sun protective factor of *C. ternatea* might be due to its potent antioxidant activity in scavenging DPPH free radical (Table 1). This indicates that *C. ternatea* might be used as natural sunscreen source in the future.

CONCLUSION

C. reticulata, C. moschata and C. ternatea are potential natural antimicrobial and antioxidant source. *C. ternatea* which showed greater sun protective factor than sunscreen product may be used as substitute of sunscreen product.

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