

# POTENTIAL USES OF OIL PALM (*Elaeis guineensis*) LEAF EXTRACT IN TOPICAL APPLICATION

NOR ZULIANA YUSOF\*; SITI SALWA ABD GANI\*\*; YASMEEN SIDDIQUI†; NOR FADZILLAH  
MOHD MOKHTAR‡ and ZAFARIZAL ALDRIN AZIZUL HASAN\*

## ABSTRACT

The antioxidant, anti-microbial activity and potential application of oil palm (*Elaeis guineensis*) leaf extract (OPLE) as a skin lightening and sunscreen agent in topical application were evaluated. Currently, there are no evidence of skin lightening effect and protection against ultraviolet (UV) irradiation of OPLE. OPLE contains  $9.84 \pm 0.06$  mg gallic acid equivalent per gram of dry extract and shows antioxidant activity through 2,2-diphenyl-1-picrylhydrazyl assay with  $IC_{50}$  of  $247 \pm 0.58 \mu\text{g ml}^{-1}$ . OPLE was effective only against gram-positive bacteria and the minimum inhibitory concentration was  $12.5 \text{ mg ml}^{-1}$ . OPLE also inhibited mushroom tyrosinase enzyme with  $IC_{50}$   $254.88 \mu\text{g ml}^{-1}$ . Determination of UV spectrophotometric and in vitro sun protection factor (SPF) indicated that OPLE provides a protection against ultraviolet B (UVB) and ultraviolet A (UVA) irradiation. OPLE were mixed with glycerine at 1%, 5% and 10% concentrations and showed slightly increase in SPF values from 0.96, 1.48 and 1.87, respectively. The UVA/UVB ratios were significantly increase from 0.076 for glycerine to 0.645, 0.906 and 0.910 for OPLE at 1%, 5% and 10% in glycerine, respectively. OPLE showed potentially good UV absorbance at UVB and UVA range wavelength and can be used as natural sunscreen and skin lightening agent for topical application.

**Keywords:** oil palm leaf extract, antioxidant, anti-microbial, anti-tyrosinase, ultraviolet radiation.

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## INTRODUCTION

Malaysia is one of the largest producers and exporters of palm oil and its products in the world. In 2015, the total planted area has reached to 5.64

million hectares, an increase of 4.6% against 5.39 million hectares recorded in the previous year, 2014 (MPOB, 2015). Apart from palm oil production, oil palm industries yield several by-products such as oil palm trunk, oil palm fronds, empty fruit bunches, palm kernel cake, palm oil mill effluent and palm pressed fibre (Abdullah and Sulaiman, 2013). Several of these by-products are utilised in generating compost and fertilisers. However, the process is time-consuming. The major contribution of the oil palm waste in Malaysia is derived from the oil palm fronds (Basiron and Chan, 2004). Normally, oil palm fronds are left rotting between the rows of palm trees, mainly for soil conservation and long-term benefit of nutrient recycling. Oil palm fronds also can be used as ingredients for livestock

\* Malaysian Palm Oil Board, 6 Persiaran Institusi, Bandar Baru Bangi, 43000 Kajang, Selangor, Malaysia.  
E-mail: zuliana@mpob.gov.my

\*\* Halal Products Research Institute, Universiti Putra Malaysia, Putra Infoport, 43400 UPM Serdang, Selangor, Malaysia.

† Laboratory for Food Crops, Institute of Tropical Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.

‡ Department of Mathematics, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.

feeding (Wan Zahari *et al.*, 2002; Wan Zahari and Mohd Farid, 2011). In addition, palm leaves are abundant and are under-utilised. Several studies have reported that oil palm leaf extract (OPLE) are rich in antioxidant activity (Ng and Choo, 2010; Jaffri *et al.*, 2011; Vijayarathna and Sasidharan, 2012). In addition, the antioxidative activities are higher in the dried leaves extract compared to wet leaves extract (Ng and Choo, 2010).

Numerous studies were carried out to characterise the chemical compositions of OPLE. It largely consists of phenolic compounds such as glycosylated flavonoids, catechins, (-)-catechin gallate, ferulic acid, gallic acid, protocatechuic acid and carotenoids (Runnie *et al.*, 2003; Phang *et al.*, 2009).

Phenolic compounds are the most common water-soluble antioxidant (Macheix *et al.*, 1990) and have anti-microbial properties (Jurd *et al.*, 1971). Sato and Toriyama (2009) reported that catechin group has anti-melanogenic agent properties and that it might be effective in hyperpigmentation disorders. Therefore, the total phenolic content, antioxidant, anti-microbial properties and the effect of OPLE on inhibition of tyrosinase enzyme through *in vitro* tyrosinase assay were determined in this study.

The ultraviolet (UV) radiation is the waveband that affects skin pigmentation and can be divided into three main wavelengths *i.e.*, UVC (200-290 nm), UVB (290-320 nm) and UVA (320-400 nm). UVA is further subdivided into UVA II (320-340 nm) and UVA I (340-400 nm) (ISO 24444:2010). UVB contributes for about 5% while UVA for about 95% of the ultraviolet spectrum and both UVB and UVA can penetrate the ozone layer (Maria *et al.*, 2012). UVB will penetrate the epidermis, but it is mostly absorbed just above the dermis. On the other hand, UVA radiation penetrates deeper in the skin, reaching the dermis (Dupont *et al.*, 2013). Exposure to UVA radiation results in damage to the elastic and collagen fibres of the connective tissue of the skin, which causes premature ageing, while UVB radiation induces acute inflammation or sunburn and intensification of photo-ageing (United States Environmental Protection Agency, 2010).

There are two main types of active ingredients used in sun care formulations to control the amount of UV light penetrating the skin: chemical (organic) sunscreens and physical (inorganic) sunscreens. Chemical sunscreens are incorporated in skin care products to absorb the UV light. Although they are effective UV filters, several UV filters were shown to trigger skin allergic reactions when applied onto the skin (Collaris and Frank, 2008). On the other hand, physical sunscreens such as titanium dioxide (TiO<sub>2</sub>) and zinc oxide (ZnO) work by reflecting and scattering the UV light. Disadvantages of these inorganic sunscreens are their unappealing visual appearance because of the solid consistency that

leave unpleasant white marks onto the skin when high quantities are incorporated into sunscreen products (Anderson *et al.*, 1997).

The application of sunscreens is an efficient method of protecting the skin against UV radiations. The efficacy of sunscreens is characterised by the sun protection factor (SPF), a ratio calculated from the energies required to induce a minimum erythema response with and without sun product applied to the skin of human volunteers, using UV radiation usually from an artificial source. The SPF is a numerical rating system to indicate the degree of protection provided by sun care products like sunscreen (COLIPA, 2006).

Determination of SPF can be carried out through *in vivo* and *in vitro* methods. Due to the high costs and time-consuming of *in vivo* SPF determination, the *in vitro* SPF test method is the preferred test (Maria *et al.*, 2012). At present, there are three *in vitro* methods for evaluation of protection from UVA, namely from the United States (Food and Drug Administration-FDA), European Union (Cosmetics Europe - The Personal Care Association - COLIPA) and United Kingdom (Beauty Health Pharmacy and Prescriptions -Boots) (Maria *et al.*, 2012). The Boots star rating system (Boots the Chemist Ltd, 1992) and critical wavelength (Diffey, 1994) were used to measure the protection against UVA.

Recently, the use of natural sunscreen has been gaining significant attention due to its safety, multiple biological actions on skin and cost effectiveness. The plant actives are preferred over chemical sunscreen due to the broad spectrum of UV absorption, protection against oxidative stress, inflammation and cancer (Anitha, 2012; Goswami *et al.*, 2013; Korac and Khambholja, 2011).

Based on the literature search carried out, no specific reports have been published on the protection against UV irradiation of phenolic compounds derived from OPLE. In this study, *in vitro* SPF and UVA/UVB ratio were used to investigate the effects of OPLE on protection against UVB and UVA irradiation. This will eventually lead to development of oil palm (*Elaeis guineensis*) leaf extract as an active ingredient for topical application.

## MATERIALS AND METHODS

### Chemical Material

The reagents Folin-Ciocalteu's phenol reagent, gallic acid, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 6-Hydroxy-2,5,7,8-Tetramethylchroman-2-Carboxylic acid (TROLOX), 3,4-dihydroxy-L-phenylalanine (L-DOPA), lyophilised mushroom tyrosinase and kojic acid were purchased from Sigma-Aldrich, Steinheim, Germany. The remaining reagents were supplied by different companies, as follow: ethanol

(Friedemann Schmidt, Parkwood WA, Australia), methanol and dimethylsulfoxide (DMSO) (Fisher Chemical, Loughborough, United Kingdom), sodium carbonate anhydrous (R&M Chemicals, Essex, United Kingdom), phosphate buffer solution (PBS) (Gibco by Life Technologies, New York, USA) and glycerine (Croda, Seraya Avenue, Singapore). All reagents and solvents were of analytical grade. ABS Green Tea Extract (GTE) was purchased from Active Concepts LLC, North Carolina, USA and used as a benchmark in determination of anti-tyrosinase activity and *in vitro* SPF.

### Biological Material

The Mueller-Hinton agar was obtained from Oxoid (UK). Sabaroud dextrose agar, Mueller-Hinton broth and potato dextrose broth were purchased from Difco (USA). American Type Culture Collection (ATCC) bacterial strains were used; *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 15442 and *Escherichia coli* ATCC 8739.

### Preparation of Oil Palm Leaf Extract

Fresh oil palm (*Elaeis guineensis*) leaves from mature palm, aged 6 years were obtained from the Malaysian Palm Oil Board (MPOB), Bangi, Selangor, Malaysia. Three batches of oil palm leaves were collected from different oil palm fronds (OPF) of different trees. The sampling was performed from the middle part of OPF, followed by the middle part of oil palm leaves. The leaves collected were washed with tap water and chopped coarsely and left to dry in the oven at 40°C for 24 hr. The dried leaves were ground to powder using a mechanical blender and 20 g of the leaf powder were extracted with absolute ethanol at a 1:10 (w/v) under soxhlet extractor for 2 hr at 78.0°C. The extracts were then filtered through filter paper (Whatmann No. 1) and vacuum-dried in a rotary evaporator (Premium 7, Heidolph) at 40°C, until the solvent was completely removed or one-tenth its volume to yield dark green waxy material.

### Determination of Total Phenolics

Total phenolic compounds in the crude ethanolic extract OPLE were determined using the Folin-Ciocalteu method (Singleton *et al.*, 1999). In brief, 0.1 ml of ethanolic OPLE solution (1:10, w/v) was added to 0.5 ml Folin-Ciocalteu reagent, followed by 7 ml distilled water. The mixture was left standing at room temperature, in the dark for 5 min. Then, 1.5 ml sodium carbonate (15% Na<sub>2</sub>CO<sub>3</sub>/H<sub>2</sub>O) solution was added and the mixture was left at room temperature for another 2 hr. UV absorbance at 765 nm was measured using microplate reader spectrophotometer (Synergy H1, BioTek, USA).

Samples were measured in three replicates. Gallic acid was used as reference. Standard curve of gallic acid solution (0, 10, 20, 40, 60, 80 and 100 ppm) was prepared using the similar procedure. The results were expressed as mg gallic acid equivalent (GAE) g<sup>-1</sup> of extract.

$$T = C \times V/M \quad \text{Equation (1)}$$

where *T* is the total phenolic content in mg g<sup>-1</sup> of the extract as GAE;

*C* is the concentration of gallic acid established from the calibration curve in mg ml<sup>-1</sup>;

*V* is the volume of the extract solution in ml; and

*M* is the weight of the extracts in grams.

### DPPH Radical Scavenging Activity

The DPPH free radical scavenging activity of OPLE was determined according to the method described by Blois (1958) with slight modifications. Briefly, 4.2 mg of DPPH powder was dissolved in 50 ml of methanol (0.2 mM). Then, 50 μl OPLE at different concentrations of 62.5, 125, 250 and 500 μg ml<sup>-1</sup> in methanol were prepared and reacted with 195 μl of DPPH methanolic solution in 96-well microtitre plate. After 60 min of incubation, the absorbance values of the samples were read at 540 nm using a microplate reader (Synergy H1, BioTek, USA). Trolox was used as the standard. The absorbance of the DPPH and methanol was used as a negative control. The analysis was done in triplicate to confirm the reproducibility of the data. The antioxidant activity, expressed as the percentage of DPPH radical scavenging, was calculated by using Equation (2).

$$\% \text{ DPPH scavenging activity} = \frac{[\text{Abs}_{\text{negative control}} - \text{Abs}_{\text{sample/standard}}]}{\text{Abs}_{\text{negative control}}} \times 100 \quad \text{Equation (2)}$$

The IC<sub>50</sub> of DPPH assay represents the concentration of the tested sample needed to reduce the DPPH by 50% where the value obtained from linear regression graph.

### Determination of Anti-tyrosinase Activity

The anti-tyrosinase activity of OPLE was evaluated based on inhibition of mushroom tyrosinase by the test sample with L-DOPA as substrate using a method described by Chiari *et al.* (2010). The test solution was serially diluted two-fold from 500 μg ml<sup>-1</sup> using DMSO and 20 μl was pipetted into a 96-well plate, followed by addition of 138 μl phosphate buffer solution and 2 μl mushroom tyrosinase solution (2500 U ml<sup>-1</sup>, in PBS). After incubation at 37 °C for 90 min, 40 μl of L-DOPA (2.5

mM in PBS) was added, and measurement at 450 nm monitored for 20 min. Commercial GTE was used as a benchmark active for comparison purposes and kojic acid was used as a standard positive control. The tyrosinase inhibitory activities of the tested samples were expressed as IC<sub>50</sub> values which are the concentration required inhibiting 50% of tyrosinase activity.

### Anti-microbial Activity Assays

The minimum inhibitory concentrations (MIC) of plant extracts were determined using the broth microdilution method in 96-well plates as described by Eloff (1998). The MIC was tested on *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. The OPLE were dissolved in 10% DMSO at 100, 50, 25, 125, 6.25, 3.13, 1.56, 0.78 and 0.39 mg ml<sup>-1</sup>. The bacteria were cultured in Mueller-Hinton (MH) broth for 24 hr. Streptomycin disc (10 µg) was used as the positive control whereas broth, 10% DMSO and water were used as negative controls. In brief, 100 µl of water and 100 µl of test samples were added in each well. The optical density of *S. aureus*, *P. aeruginosa* and *E. coli* were adjusted with MH broth to match that McFarland standard, equivalent to 10<sup>8</sup> colony forming units at 600 nm. After that, 100 µl of the adjusted culture were added to all the wells. The microtitre plates were then incubated for 24 hr at 37°C and the results were observed. The clear wells were considered as the MIC values.

Minimum bactericidal concentration (MBC) was determined by pipetting 100 µl of bacterial cells from the MIC test plate onto MH agar and spread on the surface of the agar. The plates were then incubated at 37°C for 24 hr. Colony of each bacteria at 100, 50, 25, 125, 6.25, 3.13, 1.56, 0.78 and 0.39 mg ml<sup>-1</sup> in 10% DMSO were observed and the lowest concentration of sample required to kill 99.9% the tested microorganism was recorded as MBC.

### Determination of *in vitro* Sun Protection Factor

The determinations of *in vitro* sun protection factor (SPF) for UVB and UVA of all samples were performed by Labsphere UV-2000S Ultraviolet Transmittance Analyser using polymethyl methacrylate plate with 6 micron roughness and 1.30 mg cm<sup>-2</sup> of product application (COLIPA, 2011). The *in vitro* technique involves measuring the spectral transmittance at UV wavelengths from 280 nm to 400 nm and the data are used to determine the SPF. Physical measurements have shown that the solar erythral response is inversely proportional to the radiation wavelength. The shorter wavelength or UVB region at 280 – 315 nm can induce erythral reaction. No erythral reaction is observed for the UVA region at 315 – 400 nm (Diffey and Robson, 1989).

SPF is defined as the ratio of the minimal erythema dose (MED) with applied sunscreen and the MED without sunscreen (COLIPA, 2006). The SPF is an indicator of protection against UVB radiation and is calculated based on Equation (3):

$$SPF_{in\ vitro} = \frac{\int_{290\ nm}^{400\ nm} E(\lambda) \cdot S(\lambda) \cdot d\lambda}{\int_{290\ nm}^{400\ nm} E(\lambda) \cdot S(\lambda) \cdot T(\lambda) \cdot d\lambda} \quad \text{Equation (3)}$$

where, E(λ) = erythema action spectrum, S(λ) = solar spectral irradiance, T(λ) = spectral transmittance of the sample.

The equation shows that the higher the amount of transmittance, the lower the SPF value. The transmittance spectrum of a sunscreen in either region is averaged in order to produce one value, which describes the amount of UVA or UVB blocking. The average transmittance in each region is given by Equations (4) and (5), respectively:

$$T(UVA)_{av} = \frac{\sum_{315\ nm}^{400\ nm} T\lambda \cdot \Delta\lambda}{\sum_{315\ nm}^{400\ nm} \Delta\lambda} \quad \text{Equation (4)}$$

and,

$$T(UVB)_{av} = \frac{\sum_{280\ nm}^{315\ nm} T\lambda \cdot \Delta\lambda}{\sum_{280\ nm}^{315\ nm} \Delta\lambda} \quad \text{Equation (5)}$$

where, Δλ = measured wavelength interval. Consequently, the percent blocking for UVA and UVB, respectively, is calculated as in Equation (6);

$$100\% - T(UVA)_{av} \text{ or } 100\% - T(UVB)_{av} \quad \text{Equation (6)}$$

where, T(UVA)<sub>av</sub> or T(UVB)<sub>av</sub> is expressed as a percentage.

### Determination of UVA/UVB Ratio

In addition to its ability to determine the SPF of a sunscreen, the *in vitro* technique can also measure the UVA protection of the sunscreen.

The spectral transmittance values, Tλ, are converted to spectral absorbance values Aλ = -log(Tλ). A term called the UVA/UVB ratio is calculated, which is the ratio of the total absorption in the UVA to that in the UVB as in Equation (7):

$$\frac{aUVA}{aUVB} = \frac{\int_{320\ nm}^{400\ nm} E(\lambda) \cdot S(\lambda) \cdot d\lambda}{\int_{290\ nm}^{320\ nm} A(\lambda) \cdot d\lambda} \quad \text{Equation (7)}$$

The star rating for UVA protection is determined from the measured UVA ratio as shown in the *Table 1* (Boots the Chemists Ltd, 1992).

**Statistical Analysis**

All experiments were carried out in triplicate. The results are given as mean and standard deviation. Statistical analysis, analysis of variance (ANOVA) and Tukey tests, were carried out when necessary. The level of significance is 95% (p<0.05).

**RESULTS AND DISCUSSION**

**Total Phenolic Content and DPPH Radical Cavenging Activity**

In brief, 20 g of the dried leaves were extracted using ethanol and the final dried extract weight was 2 g. Therefore, the extraction yield of OPLE was 10%. *Figure 1* depicts the standard curve for the total phenolic content assay, whereby it demonstrated that as the concentration of gallic acid increased, the absorption also increased. It was observed that the reduction of Folin Ciocalteu reagent by phenolic ion will change its solution colour from yellow to blue (Prior *et al.*, 2005). The graph produce a good correlation (R<sup>2</sup>=0.9934) between concentration and its absorption spectrophotometrically at 765 nm and the equation for this graph: y=0.0017x + 0.0068 was used to calculate GAE of OPLE. From this study, OPLE contains 9.84±0.06 mg GAE g<sup>-1</sup> of extract. Previous study reported that total phenolic content of dried OPLE was 10.2 mg GAE g<sup>-1</sup> (Ng and Choo, 2010) and 70.07±1.501 mg GAE g<sup>-1</sup> (Ng *et al.*, 2013). The extraction using more polar solvents such as methanol resulted in a higher amount of total

phenolic content with 24.3 mg GAE g<sup>-1</sup> as compared to green tea (22.5 mg GAE g<sup>-1</sup>) (Runnie *et al.*, 2003).

Results shown in *Table 2* and *Figure 2* demonstrate the antioxidant activity of OPLE through DPPH scavenging assay from 62.5, 125, 250 and 500 µg ml<sup>-1</sup> of concentration. The standard curve demonstrated a good correlation, as the concentration of OPLE increased, the antioxidant activity also increased. OPLE at 500 µg ml<sup>-1</sup> shows higher antioxidant activity, 94.36±0.29% with an IC<sub>50</sub> value of 247.00±0.58 µg ml<sup>-1</sup>. In 2010, Ng and Choo reported that dried leaf extract showed antioxidant activities ranged from 56%-93% when determined by the DPPH assay. The IC<sub>50</sub> value in this study is much lower than values reported by Vijayarathna and Sasidharan (2012) with IC<sub>50</sub> 814 µg ml<sup>-1</sup> and Ng *et al.* (2013) with IC<sub>50</sub> 646 µg ml<sup>-1</sup>. Lower IC<sub>50</sub> value indicates higher antioxidant activity.

**Anti-bacterial Assays**

The anti-bacterial assay was carried out on gram positive bacteria *S. aureus* ATCC 6538, and two gram negative bacteria *P. aeruginosa* ATCC 15442 and *E. coli* ATCC 8739. A two-fold serially diluted OPLE concentrations were prepared at 100, 50, 25, 12.5, 6.25, 3.13, 1.56, 0.78 and 0.39 mg ml<sup>-1</sup>. The MIC result of OPLE against *S. aureus* ATCC 6538, *P. aeruginosa* ATCC 15442 and *E. coli* ATCC 8739 are presented in *Table 3*. The OPLE exhibits good anti-bacterial activity against *S. aureus* with lowest concentration that inhibited bacterial growth was 12.5 mg ml<sup>-1</sup>. The results were in accordance with the studies by Chong *et al.* (2008) and Vijayarathna *et al.* (2012) where the MIC values for the methanol extract of OPLE on *S. aureus* were 6.25 mg ml<sup>-1</sup> and 12.5 mg ml<sup>-1</sup>, respectively. Gram negative bacteria, *P. aeruginosa* and *E. coli* were quite resistant to OPLE

**TABLE 1. STAR RATING FOR ULTRAVIOLET (UVA) PROTECTION**

Mean UVA/UVB ratio	Star rating category	Category description
0.0 to 0.2	-	Too low /no claim
0.21 to 0.4	*	Minimum
0.41 to 0.6	**	Moderate
0.61 to 0.8	***	Good
0.81 to 0.9	****	Superior
0.91 and above	*****	Ultra

**TABLE 2. ANTIOXIDANT ACTIVITY OF OIL PALM LEAF EXTRACT (OPLE) ON SCAVENGING EFFECT USING DPPH ASSAY**

Assay	Concentration, µg ml <sup>-1</sup>				IC <sub>50</sub> , µg ml <sup>-1</sup>
	62.5	125	250	500	
DPPH assay (%)	20.40±3.64	30.65±0.41	60.46±0.64	94.36±0.29	247.00±0.58

Note: DPPH – 2,2-Diphenyl-1-picrylhydrazyl.

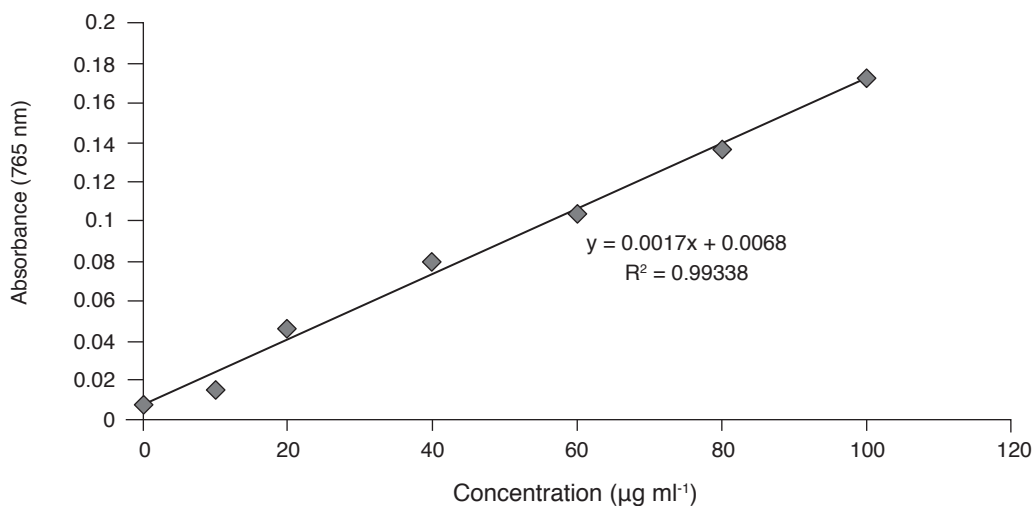


Figure 1. Folin Ciocalteu' gallic acid standard curve.

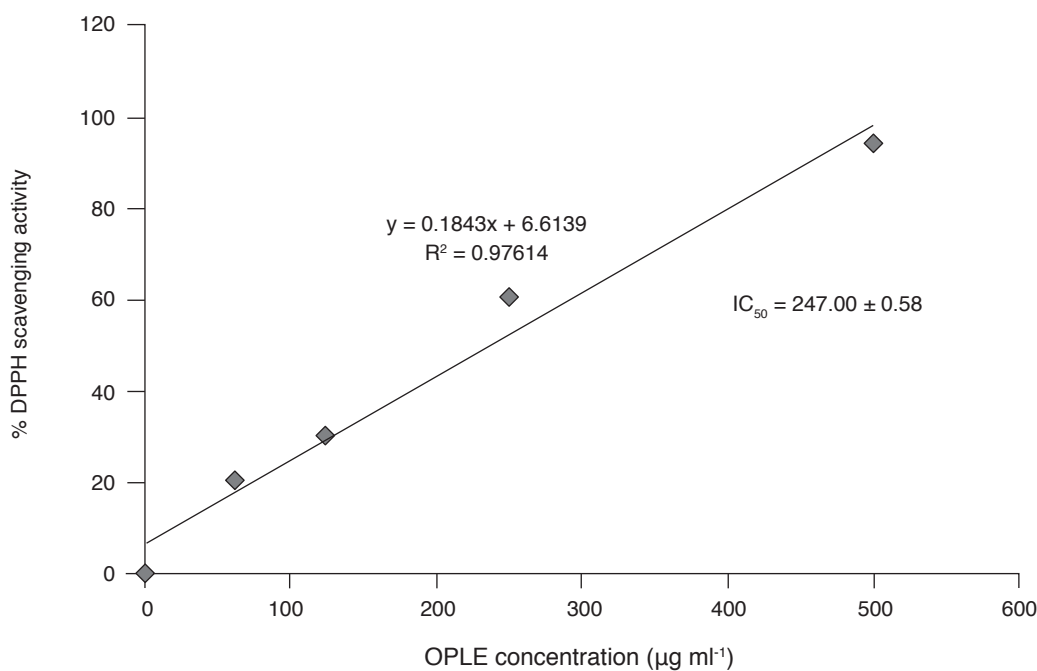


Figure 2. The 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity (%) of oil palm leaf extract (OPLE) (µg ml<sup>-1</sup>).

TABLE 3. MINIMUM INHIBITORY CONCENTRATION (MIC) OF OPLE AGAINST DIFFERENT BACTERIA

Bacteria	OPLE concentration (mg ml <sup>-1</sup> )								
	100	50	25	12.5	6.25	3.13	1.56	0.78	0.39
Gram positive									
<i>Staphylococcus aureus</i> ATCC 6538	C	C	C	C	NC	NC	NC	NC	NC
Gram negative									
<i>Pseudomonas aeruginosa</i> ATCC 15442	NC	NC	NC	NC	NC	NC	NC	NC	NC
<i>Escherichia coli</i> ATCC 8739	NC	NC	NC	NC	NC	NC	NC	NC	NC

Note: \*C - clear (no bacterial growth), NC - not clear (bacterial growth).

OPLE – oil palm leaf extract.

and no inhibitory were recorded in this study. The results were supported by the finding from Ng *et al.* (2013) where methanolic extract from oil palm leaves showed anti-microbial activity with the exception to gram negative bacteria *E. coli*.

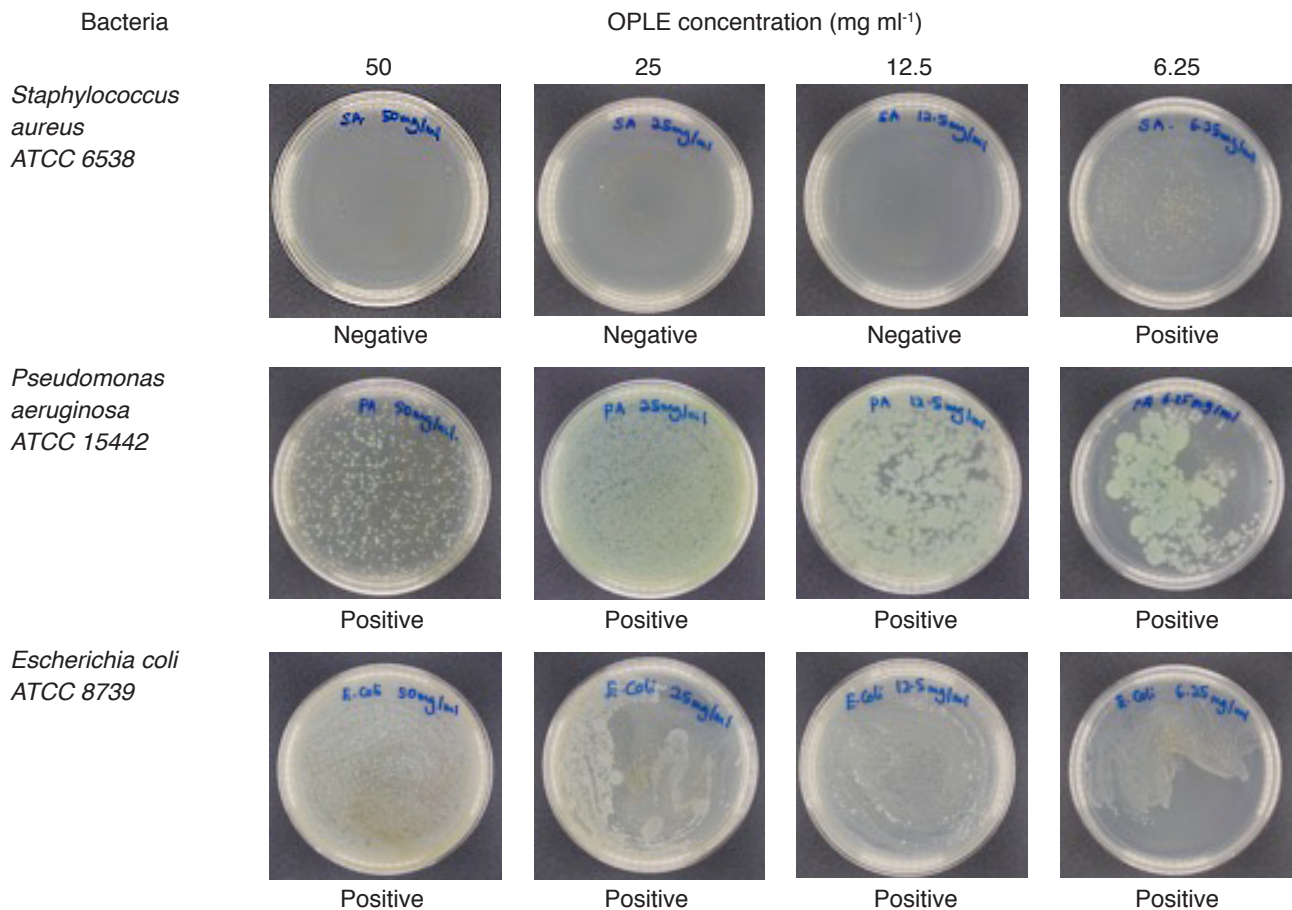
Results from the MBC assay supported the data obtained from the MIC determination assay where OPLE shows bactericidal activity towards *S. aureus* at 12.5 mg ml<sup>-1</sup> and no inhibition were recorded for *P. aeruginosa* and *E. coli* (Figure 3). These results indicated that OPLE has anti-microbial activity against gram positive bacteria but no inhibition towards gram negative bacteria.

**Determination of Anti-tyrosinase Activity**

Skin pigmentation in human is caused by melanin synthesis in UV irradiated melanocytes. Upon exposure of the skin to UV radiation, melanogenesis is enhanced by the activation of the key enzyme of melanogenesis, tyrosinase (Gillbro and Olsson, 2011). Tyrosinase is a key enzyme in melanin synthesis that can catalyse three different reactions: the hydroxylation of tyrosine to DOPA, the oxidation of DOPA to DOPA quinone and the oxidation of 5,6-dihydroxyindole (DHI) to indole-

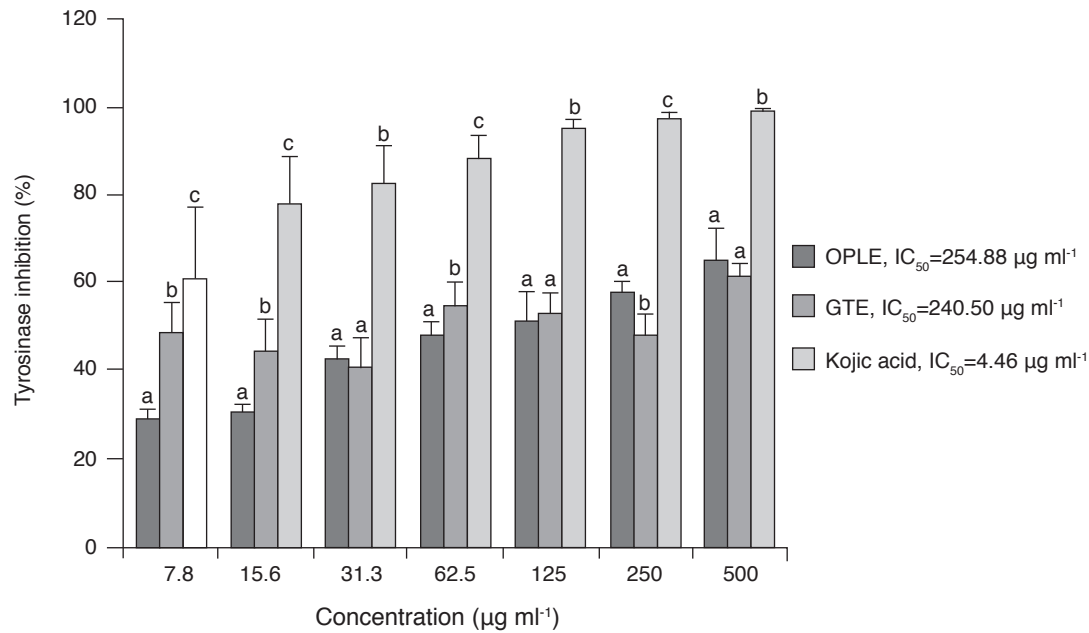
quinone (Hearing and Tsukamoto, 1991). The capability of OPLE to inhibit tyrosinase activity can be translated to its potential as skin lightening agent. When tyrosinase enzyme activity is inhibited, melanin production is reduced, resulting in a fairer skin. Kojic acid, from mushrooms and other plant materials, is a common ingredient in skin lightening products. Green tea (*Camellia sinensis* L.) with major active constituents; epicatechin gallate, gallic acid, epigallocatechin gallate were reported to have stronger tyrosinase inhibitory activity (No *et al.*, 1999; Jo *et al.*, 2012) and therefore was used as a benchmark.

Figure 4 shows the anti-tyrosinase activity of OPLE, commercial GTE and kojic acid as a positive control. Anti-tyrosinase activity of kojic acid was significantly higher and gradually increased from 60.53% to 99.10% with an increment of concentration of test sample (Figure 4). The anti-tyrosinase activity of OPLE and commercial GTE ranged between 29.69%-64.62% and 48.36%-60.93%, respectively. At highest concentration of 500 µg ml<sup>-1</sup>, the anti-tyrosinase activity of OPLE and GTE were not significantly different with p>0.05. Therefore, OPLE exhibited an anti-tyrosinase activity as compared to GTE. Oil palm leaves were reported to have 8%



Note: \*Negative - no bacterial growth; Positive - bacterial growth.

Figure 3. Minimum bactericidal concentration (MBC) of oil palm oil palm leaf extract (OPLE) against different organisms.



Note: GTE – green tea extract.

Figure 4. Anti-tyrosinase activity (%) of oil palm leaf extract (OPLE) using mushroom tyrosinase against kojic acid, values with different superscript letters within the same concentration are statistically different ( $p < 0.05$ ).

higher total polyphenols content than GTE and contain epigallocatechin, catechin, epicatechin, epigallocatechin gallate and epicatechin gallate (Mohamed, 2014).

In this study, the tyrosinase inhibition effect of OPLE and GTE might be due to the presence of the catechin group whereby Sato and Toriyama (2009) reported that catechin group inhibited melanin synthesis in B16 melanoma cells. Besides the catechin group, the presence of gallic acid in OPLE might also contribute to anti-tyrosinase activity. Gallic acid significantly inhibited both melanin synthesis and tyrosinase activity (Su *et al.*, 2013). Kojic acid as a stronger inhibitor exhibited the lowest  $IC_{50}$  4.46  $\mu\text{g ml}^{-1}$ , whereby OPLE and GTE exhibited a comparable  $IC_{50}$  value of 254.88 and 240.50  $\mu\text{g ml}^{-1}$ , respectively. The result suggested that OPLE has the potential to be used as natural skin lightening agent in topical applications.

#### *In vitro* SPF

The *in vitro* SPF determination is an indicator of protection against UVB radiation (290-320 nm), while UVA/UVB ratio measures the protection against UVA radiation (320-400 nm). OPLE which is a waxy paste with dark green colour was mixed with glycerine at 1%, 5% and 10% concentrations. Table 4 shows the results of mean SPF, UVA/UVB ratio and Boots Star Rating for glycerine and glycerine containing OPLE at 1%, 5% and 10%. For a comparison, similar commercial GTE used in anti-tyrosinase activity were tested at same

concentrations as OPLE. The test results show that glycerine has mean SPF of 0.99 and very low UVA/UVB ratio. However, the increment of OPLE in glycerine gradually decreased the percentage of transmittance where 1% OPLE recorded a transmittance at 104.09%, 5% OPLE at 66.92% transmittance and 10% OPLE at 52.62% (Figure 5). The reduction in transmission profiles indicated an UVB absorbance by OPLE and thus showed an increment in SPF value (Table 4). There were no significant differences in percentage of transmittance and SPF values for glycerine containing GTE at 1%, 5% and 10%. Besides SPF, increasing concentration of OPLE also increased the UVA/UVB ratio where 5% and 10% OPLE were categorised as having superior protection against UVA radiation and the results were similar to GTE. OPLE shows potentially good UV absorbance at UVB and UVA range wavelength. Therefore, OPLE showed a good potential to be used as natural sunscreen agent for topical application.

#### CONCLUSION

The oil palm leaves are abundant, under-utilised and one of the by-products of the palm oil industry. Based on these findings, OPLE shows antioxidant activity and anti-microbial effect towards gram positive bacteria. OPLE also provides good protection against UVA and UVB irradiation and has the potential to be used as skin lightening agent through inhibition

**TABLE 4. MEAN SPF, UVA/UVB RATIO OF GLYCERINE, 1%, 5% AND 10% OPLE AND COMMERCIAL GREEN TEA EXTRACT IN GLYCERINE**

Samples	UVB protection	UVA protection	
	Mean SPF (mean ± std. dev)	UVA/UVB ratio	Category
Glycerine	0.99±0.01	0.076	Too low / no claim
Glycerine + 1% OPLE	0.96±0.01	0.645	Good
Glycerine + 5% OPLE	1.48±0.01	0.906	Superior
Glycerine + 10% OPLE	1.87±0.01	0.910	Superior
Glycerine + 1% GTE	1.01±0.01	0.634	Good
Glycerine + 5% GTE	0.98±0.01	1.040	Ultra
Glycerine + 10% GTE	1.00±0.01	1.278	Ultra

Note: \*GTE - commercial green tea extract. UV – ultraviolet.  
SPF - sun protection factor.

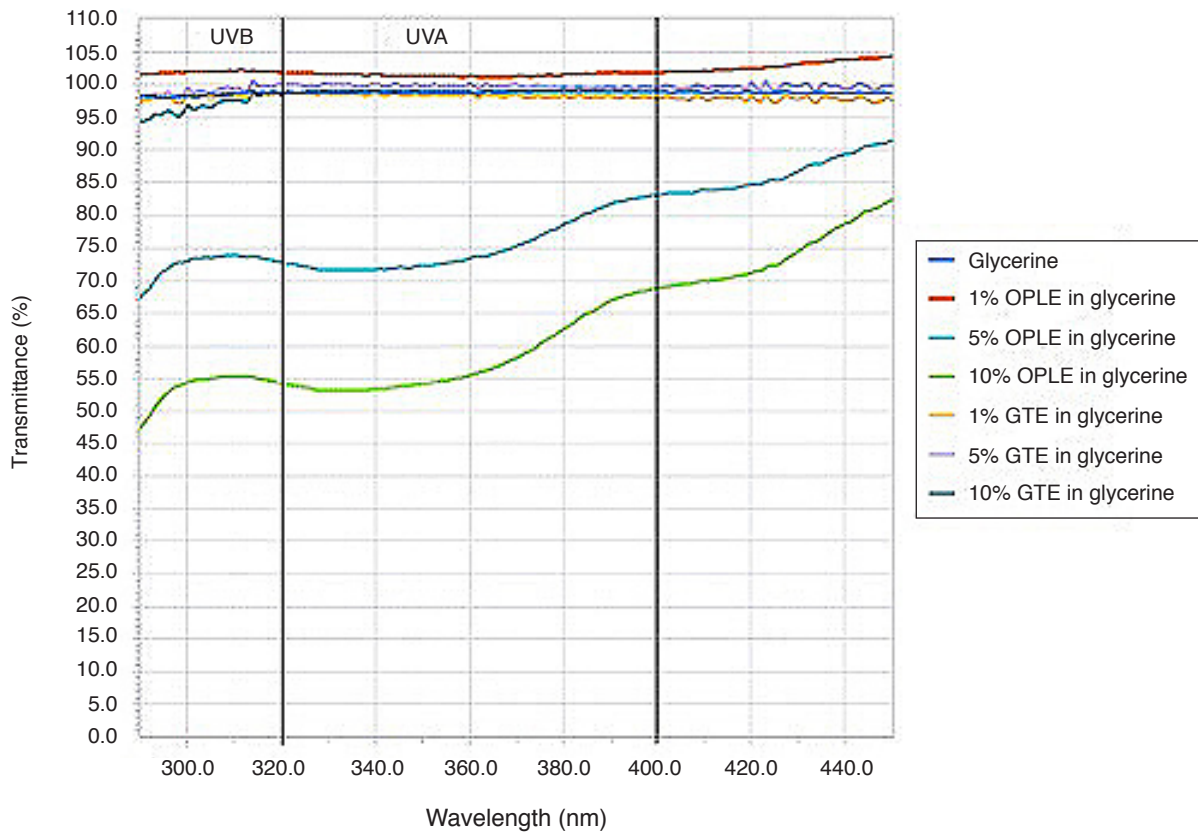


Figure 5. Transmittance profile of different concentrations of oil palm leaf extract (OPLE) and commercial green tea extract (GTE) in glycerine.

of tyrosinase enzyme. Therefore, OPLE can be further developed in topical applications as natural sunscreen and skin lightening agent.

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