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Antioxidant and free radical scavenging properties of *Dacryodes edulis* leaf and bark extracts

Iniobong Enengedi, Okon Ekpa and Ukana Akpabio

Abstract

Photoaging is of the increase in sunny geographical areas due to long-term effects of repeated exposure to ultra violet (UV) radiation. The antioxidant properties of *Dacryodes edulis* leaf and bark extracts were determined using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. All the ethanol and aqueous extracts of *D. edulis*: Uyo leaf ($IC_{50} = 148.43 \pm 0.04 \mu\text{g}/\text{cm}^3$ and $161.72 \pm 1.02 \mu\text{g}/\text{cm}^3$), Uyo bark ($IC_{50} = 152.29 \pm 2.04 \mu\text{g}/\text{cm}^3$ and $171.34 \pm 0.04 \mu\text{g}/\text{cm}^3$), Ikom leaf ($IC_{50} = 153.45 \pm 0.05 \mu\text{g}/\text{cm}^3$ and $142.08 \pm 0.01 \mu\text{g}/\text{cm}^3$) and Ikom bark ($IC_{50} = 126.37 \pm 0.02 \mu\text{g}/\text{cm}^3$ and $129.60 \pm 5.07 \mu\text{g}/\text{cm}^3$) respectively, except the aqueous extract of *D. edulis*-Uyo bark, significantly ($p < 0.05$) exhibited stronger antioxidant activities than the standard antioxidant, Butylated hydroxyanisole. This high antioxidant activity could be attributed to their significantly ($p < 0.05$) higher phenolic, flavonoid and tannin contents. These extracts could be used as active ingredients in photo-protective personal care products to counteract the effect of UV light on the skin.

Keywords: Antioxidant, free radical scavenging, photoaging, active ingredients

1. Introduction

Aging is a complex physiological process that involves both morphological and biochemical changes occurring, with the passage of time, in single cells and the whole organism [1]. Skin aging, both intrinsic and extrinsic, is a dynamic process which depends on many endogenous and exogenous factors, and results in a variety of functional and aesthetic skin changes [2]. It occurs through two different independent biological mechanisms, namely, chronological aging and photoaging. Chronological (intrinsic) aging, which is largely genetically determined, depends on passage of time and is inevitable; it is characterized by slow and irreversible tissue degeneration and affects the skin as well as the whole body [3]. Photoaging (extrinsic aging) depends on the degree of sun exposure and individual skin pigmentation and it is avoidable. Individuals who have a history of intensive sun exposure, live in sunny geographical areas, and have fair skin will experience the greatest amount of ultraviolet (UV) radiation skin load and consequently severe photoaging [4]. The effects of photodamage are often evident many years before intrinsic aging is apparent. Young people who are exposed to a great amount of UV rays appear prematurely aged [5].

Despite the fact that skin epidermis possesses an extremely efficient natural antioxidant defense, including different types of antioxidant enzymes in the skin, such as peroxidase, catalase and glutathione, the protective effect that these offer may be limited by a high production of reactive oxygen species (ROS), generating cellular oxidative stress as a result of the imbalance between antioxidant and oxidant species of living organisms, and this reduction may be the cause of skin aging. Therefore, antioxidants with free radical scavenging activities may have great significance in the protection and therapeutics of age-related diseases involving free radicals [6].

The most promising topical treatments incorporate antioxidants, estrogen, vitamins and minerals, they scavenge free radicals that are known to contribute to aging [7]. Natural skin care products are quickly absorbed by the superficial layers of the skin and are usually hypo-allergenic in nature. Many herbs, particularly fruits, vegetables, and whole grains contain antioxidants and polyphenols that scavenge free radicals and eliminate byproducts of metabolism. They are also useful as diet that is both healthy for the body in general and helpful for avoiding the typical signs of aging; a few are astonishingly high in these beneficial antioxidant food molecules [8]. Topical application of antioxidants have far-reaching benefits for protecting and improving UV-damaged and aging skin [9].

This research aimed at assessing the antioxidant properties of *D. edulis* leaf and bark extracts, for industrial application as photo protective ingredient in personal care products.

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2. Materials and methods

2.1 Sample Collection and Identification

Dacryodes edulis (leaf and bark) were collected from Uyo in Akwa Ibom State and Ikrom in Cross River State, Nigeria. The samples were transferred into polyethylene bags, labelled properly and taken to the laboratory for identification and preparation. The plant materials were identified and authenticated by a Taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Akwa Ibom State. Voucher specimen was deposited at the herbarium with the number, UUH 3541.

2.2 Extraction and Fractionation of Plant Extracts

The method of [10] was used for the extraction of plant materials. *D. edulis* leaf and bark were washed, chopped into pieces and dried at room temperature for about 14 days to a constant weight. The samples were coarsely powdered, each of the coarsely powdered samples was weighed, placed in a big glass jar and extracted by maceration with 80% ethanol for 72 hours (h) at room temperature. Also, wet samples were extracted by maceration with water for 8 h. The glass jar was covered with aluminum foil and the content stirred at interval. Then the extracts were filtered. The ethanol and aqueous extracts were concentrated in a rotary evaporator at a reduced pressure at 45°C, and the solvent removed completely by evaporation in the water bath. Some amounts of the ethanol extracts were suspended in 200 cm³ of distilled water and subjected to sequential liquid-liquid extraction with a solvent series of increasing polarity: dichloromethane (DCM), ethyl acetate (EA) and n-butanol (BuOH). The fractionation was performed until the organic solvent became colourless in 1000 ml glass separatory funnels by mixing 200 cm³ of solvent with the aqueous phase and the content shaken. The separatory funnel was supported on a ring clamp, allowing the layers to separate. The pooled fractions: dichloromethane fraction, ethyl acetate fraction, n-butanol fraction and the remaining aqueous fraction were concentrated in a rotary evaporator and evaporated to dryness. Aqueous extracts, parts of the ethanol extract and fractions were stored in a functional refrigerator until used for analysis.

2.3 Quantitative Determination of Phytochemicals

2.3.1 Determination of Total Phenolics

Total phenolic content (TPC) of the extracts and their different fractions were determined using Folin-Ciocalteu phenol reagent as described [11]. Briefly, each of the extracts and fractions respectively were dissolved in methanol to obtain a solution with concentration of 1 milligram per centimeter cubed (1mg/cm³). The extract or fraction (0.1 cm³) was added to 0.5 cm³ (1/10 dilution with water) of Folin-Ciocalteu phenol reagent and 1 cm³ of distilled water in a vial. The solution was thoroughly mixed and incubated at 25°C for 1 minute. After 1 minute, 1.5 cm³ of 20 % sodium carbonate (Na₂CO₃) solution was added. The mixture was shaken and incubated in the dark for 2 hours. The absorbance of the reaction mixture was measured at 760 nm using a Jenway 7305 spectrophotometer. Distilled water was used as blank. A set of reference standard solutions of gallic acid (10, 100, 200, 300, 400, 500 and 600 µg/cm³) respectively were prepared and a standard curve was plotted. The absorbance of each sample was compared with a standard curve plotted from gallic acid. The total phenolic content was calculated from linear regression equation from gallic acid calibration curve (Figure 1), expressed in terms of microgram of gallic acid equivalent per centimeter cubed (µg of GAE/cm³) of extract

or fraction.

2.3.2 Determination of Total Flavonoids

Total flavonoids content (TFC) of the extracts and their different fractions was determined according to the colorimetric method described [12]. Briefly, each of the extract and fractions was dissolved in methanol to obtain a solution with concentration of 1 milligram per centimeter cubed (1mg/cm³). The extract or fraction (0.5 cm³) was added to a vial, then 2 cm³ of distilled water was added and mixed. Sodium nitrite (0.15 cm³ of 5% w/v) solution was added into the bottle and the reaction mixture was allowed to stand for 6 minutes. Then 0.15 cm³ aluminium trichloride (AlCl₃, 10% w/v) was added and allowed to stand for another 6 minutes, followed by addition of 2 cm³ of sodium hydroxide (1M NaOH, 4% w/v) to the reaction mixture. Then 0.2 cm³ of distilled water was added to the reaction mixture to bring the final volume to 5 cm³. The reaction mixture was mixed thoroughly and allowed to stand for 15 minutes. The absorbance of the reaction mixture was measured at 510nm using a Jenway 7305 spectrophotometer. Distilled water was used as blank. A set of reference standard solutions of quercetin (10, 100, 200, 300, 400, 500, 600, 700 and 800 µg/cm³) respectively were prepared and a standard curve was plotted. The absorbance of each sample was compared with a standard curve plotted from quercetin. The total flavonoids content was calculated from linear regression equation from quercetin calibration curve (Figure 2), expressed in terms of microgram of quercetin equivalent per centimeter cubed (µg of QE/cm³) of extract or fraction.

2.3.3 Determination of Total Tannins

The tannin content (TTC) of the extracts and their different fractions was determined using Folin - Ciocalteu phenol reagent as described [13]. Briefly, each of the extract and fractions was dissolved in methanol to obtain a solution with concentration of 1 milligram per centimeter cubed (1mg/cm³). The extract or fraction (0.1 cm³) was added to a volumetric flask (10 cm³) containing 7.5 cm³ of distilled water and 0.5 cm³ of Folin-Ciocalteu phenol reagent. Na₂CO₃ solution (1 cm³ of 35 %) was added to the flask and the mixture diluted to 10 cm³ with distilled water. The mixture was shaken well and kept at 25 °C for 30 min. A set of reference standard solutions of tannic acid (50, 100, 150, 200 and 250 µg/cm³) respectively were prepared. Absorbance for test and standard solutions were measured against the blank at 725 nm with a Jenway 7305 spectrophotometer. Water was used as blank. The tannin content was calculated from linear regression equation from tannic acid calibration curve (Figure 3), expressed in terms of microgram of tannic acid equivalent per centimeter cubed (µg of TAE /cm³) of extract or fraction.

2.4 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) Free Radical Scavenging Assay

The antioxidant activity of extract or fraction was determined using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay according to the method described [14]. DPPH solution (0.004% w/v) was freshly prepared in methanol. The crude extract or fraction was mixed with methanol to prepare the stock solution (1000 µg/cm³). From the stock solution 2 cm³ and 1.6 cm³ were taken in two vials and by serial dilution with methanol to 8 cm³ to prepare another stock solutions (250 µg/cm³ and 200 µg/cm³) respectively. Each of the stock solutions (1 cm³, 0.8 cm³, 0.6 cm³, 0.4 cm³ and 0.2 cm³) respectively were taken in five

vials and by serial dilution with methanol, was made the final volume of each vial up to 1 cm³ to have concentrations (1000 µg/cm³, 800 µg/cm³, 600 µg/cm³, 400 µg/cm³ and 200 µg/cm³); (250 µg/cm³, 200 µg/cm³, 150 µg/cm³, 100 µg/cm³ and 50 µg/cm³); (200 µg/cm³, 160 µg/cm³, 120 µg/cm³, 80 µg/cm³ and 40 µg/cm³) respectively. The freshly prepared DPPH solution (1 cm³ of 0.004% w/v) was added in each of these vials containing varying concentrations of the extract or their different fractions. The mixture was shaken vigorously and allowed to stand for 30 min at room temperature in the dark. The reduction of the purple colour of DPPH to yellow was determined by measuring the absorbance at 517 nm. The radical scavenging activity of the extract or fraction was calculated using Equation (1):

$$\text{DPPH scavenging effect (\%)} = \frac{\text{A}_{\text{control}} - \text{A}_{\text{sample}}}{\text{A}_{\text{control}}} \times 100 \quad \text{Equation (1)}$$

Where A = absorbance at 517 nm.

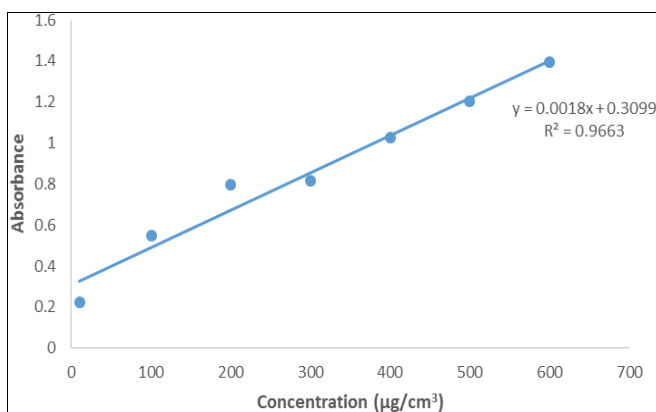


Fig 1: Calibration curve for Gallic acid

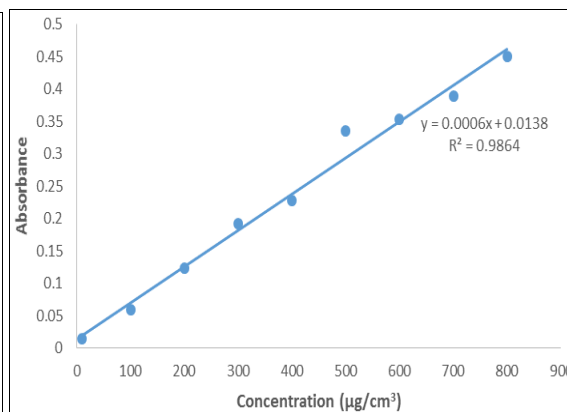


Fig 2: Calibration curve for Quercetin

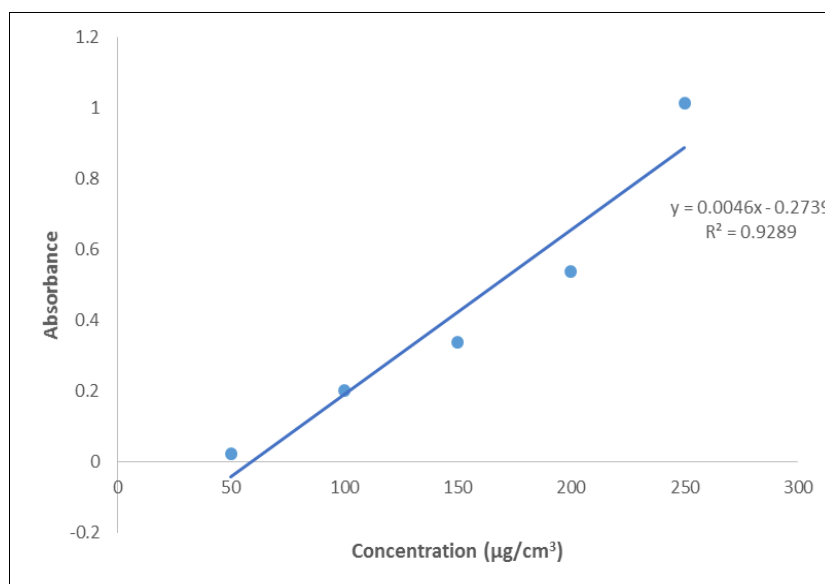


Fig 3: Calibration curve for Tannic

The commercially known antioxidant, butylated hydroxyanisole (BHA) of the same concentrations and serial dilutions were prepared and used for comparison or as a positive control. The DPPH solution in the absence of extract or fraction was used as control and methanol was used as blank. Fifty percent inhibitory concentration (IC₅₀) was calculated from linear regression equation of DPPH scavenging activity of BHA, plants' extracts and fractions (Figures 4, 5, 6, 7, 8, 9, 10 and 11). The IC₅₀ value is the concentration (µg/cm³) of extract/fraction/standard that causes a decrease in the initial amount of DPPH radicals by Fifty percent (50%). Lower IC₅₀ value indicates higher antioxidant capacity [15].

3. Results and Discussion

Total phenolic, total flavonoid and total tannin contents of the extracts were calculated from their respective calibration curves (Figures 1, 2 and 3).

Phenolics have been known to possess a capacity to scavenge free radicals and are always associated with strong antioxidant properties [16]. A higher phenolic contents in *D. edulis*-Uyo leaf and bark extracts than *D. edulis*-Ikom leaf and bark extracts (Tables 1 and 2), is an indication that *D. edulis*-Uyo leaf and bark extracts with higher total phenolic contents could be better ingredient in cosmetic formulations for post-sun skin care. These extracts could be good scavenger of free radicals caused by repeated exposure of the skin to UV

radiation, thereby preventing, delaying and remedying oxidative stress-mediated extrinsic aging (photoaging), skin hyper-pigmentation and skin diseases. [17] Studied the effects of pre- and post-treatment with plant polyphenols on human keratinocyte responses to solar UV and suggested that polyphenols may be important component in cosmetic formulations for post-sun skin care. Their topical application is efficient to inhibit erythema [18].

Table 1: Total phenolic, total flavonoid and total tannin contents of *D. edulis*-Uyo leaf, bark and their respective fractions.

Sample	Leaf			bark		
	TPC ($\mu\text{g GAE}/\text{cm}^3$)	TFC ($\mu\text{g QE}/\text{cm}^3$)	TTC ($\mu\text{g TAE}/\text{cm}^3$)	TPC ($\mu\text{g GAE}/\text{cm}^3$)	TFC ($\mu\text{g QE}/\text{cm}^3$)	TTC ($\mu\text{g TAE}/\text{cm}^3$)
D. e (U)	497.28 \pm 0.02 ^a	445.33 \pm 0.06 ^b	100.85 \pm 0.06 ^b	330.61 \pm 0.02 ^b	117.00 \pm 0.09 ^b	87.80 \pm 0.01 ^a
D. e (U) DCM	47.83 \pm 0.02 ^e	12.00 \pm 0.12 ^d	60.63 \pm 0.04 ^c	35.61 \pm 0.03 ^e	47.00 \pm 0.03 ^c	60.41 \pm 0.06 ^b
D. e (U) EA	462.28 \pm 0.04 ^b	480.33 \pm 0.04 ^a	59.98 \pm 0.01 ^c	435.61 \pm 0.03 ^a	213.67 \pm 0.02 ^a	91.93 \pm 0.04 ^a
D. e (U) BuOH	65.61 \pm 0.10 ^d	15.33 \pm 0.07 ^d	127.59 \pm 0.06 ^a	46.17 \pm 0.02 ^d	42.00 \pm 1.00 ^c	59.76 \pm 0.05 ^c
D. e (U) AqF	13.94 \pm 0.03 ^f	18.67 \pm 0.02 ^d	59.76 \pm 0.02 ^c	430.61 \pm 0.02 ^a	15.33 \pm 0.06 ^e	59.98 \pm 0.10 ^c
D. e (U) AqE	382.28 \pm 0.04 ^c	240.33 \pm 0.05 ^c	97.80 \pm 0.04 ^b	72.83 \pm 0.04 ^c	38.67 \pm 0.03 ^d	86.72 \pm 0.03 ^a

Same letters along the column means not significantly different ($p>0.05$) while different letters along the column means significantly different ($p<0.05$).

D. e (U) = *Dacryodes edulis*-Uyo extract, D. e (U) DCM = dichloromethane fraction, D. e (U) EA = ethyl acetate fraction, D. e (U) BuOH = n-butanol fraction, D. e (U) AqF = aqueous fraction from the D. e (U), D. e (U) AqE = *Dacryodes edulis*-Uyo aqueous extract.

$\mu\text{g GAE}/\text{cm}^3$ = microgram of gallic acid equivalent per centimeter cubed of extract, $\mu\text{g QE}/\text{cm}^3$ = microgram of quercetin equivalent per centimeter cubed of extract, $\mu\text{g TAE}/\text{cm}^3$ = microgram of tannic acid equivalent per centimeter cubed of extract.

Table 2: Total phenolic, total flavonoid and total tannin contents of *D. edulis*-Ikom leaf, bark and their respective fractions.

Samples	Leaf			bark		
	TPC ($\mu\text{g GAE}/\text{cm}^3$)	TFC ($\mu\text{g QE}/\text{cm}^3$)	TTC ($\mu\text{g TAE}/\text{cm}^3$)	TPC ($\mu\text{g GAE}/\text{cm}^3$)	TFC ($\mu\text{g QE}/\text{cm}^3$)	TTC ($\mu\text{g TAE}/\text{cm}^3$)
D. e (I)	392.28 \pm 0.05 ^b	400.33 \pm 0.04 ^a	99.98 \pm 0.02 ^c	250.06 \pm 0.02 ^b	210.33 \pm 0.01 ^b	88.89 \pm 0.02 ^c
D. e (I) DCM	6.72 \pm 0.06 ^f	85.33 \pm 0.03 ^d	60.20 \pm 0.04 ^d	8.39 \pm 0.04 ^e	12.00 \pm 0.02 ^e	110.85 \pm 0.03 ^a
D. e (I) EA	381.72 \pm 0.04 ^c	240.33 \pm 0.07 ^b	61.07 \pm 2.06 ^d	376.72 \pm 0.04 ^a	215.33 \pm 0.03 ^a	59.76 \pm 0.03 ^d
D. e (I) BuOH	52.83 \pm 0.06 ^d	65.33 \pm 0.06 ^e	103.24 \pm 4.03 ^b	15.06 \pm 0.02 ^f	30.33 \pm 0.03 ^d	60.63 \pm 0.03 ^d
D. e (I) AqF	47.28 \pm 0.07 ^e	47.00 \pm 0.03 ^f	59.76 \pm 4.05 ^d	17.83 \pm 0.04 ^d	5.33 \pm 0.04 ^f	59.98 \pm 0.09 ^d
D. e (I) AqE	452.83 \pm 0.04 ^a	260.33 \pm 0.03 ^c	103.24 \pm 0.02 ^a	192.83 \pm 0.09 ^c	98.67 \pm 0.02 ^c	96.07 \pm 0.08 ^b

Same letters along the column means not significantly different ($p>0.05$) while different letters along the column means significantly different ($p<0.05$).

D. e (I) = *Dacryodes edulis*-Ikom ethanol extract, D. e (I) DCM = dichloromethane fraction, D. e (I) EA = ethyl acetate fraction, D. e (I) BuOH = n-butanol fraction, D. e (I) AqF = aqueous fraction from the D. e (I), D. e (I) AqE = *Dacryodes edulis*-Ikom aqueous extract.

$\mu\text{g GAE}/\text{cm}^3$ = microgram of gallic acid equivalent per centimeter cubed of extract, $\mu\text{g QE}/\text{cm}^3$ = microgram of quercetin equivalent per centimeter cubed of extract, $\mu\text{g TAE}/\text{cm}^3$ = microgram of tannic acid equivalent per centimeter cubed of extract.

Significantly higher total flavonoid contents ($p<0.05$) was observed in all the ethanol extracts of the samples than the aqueous extracts (Tables 1 and 2). Flavonoids have been widely incorporated into cosmetic and dermatological formulations, affording benefits such as antioxidant action, improved skin tone and fewer lines and wrinkles [19]. The high flavonoids contents in *D. edulis* leaf and bark extracts is an indication that *D. edulis* leaf and bark extracts can possess a good antioxidant activity, antimicrobial activity and tyrosinase inhibition effect. Quercetin and quercetrin (flavonoids) might have contributed to the high flavonoids

contents in *D. edulis* leaf and bark extracts. Quercetin and quercetrin have been isolated from *D. edulis* leaf and bark [20, 21]. Quercitrin from methanol extract of *Lindera obtusiloba* leaves have been reported to possess potent antioxidant activity and inhibitory effect of melanogenesis [22]. Also quercetin isolated from *Morus alba* leaves was shown to significantly inhibit tyrosinase activity and melanin content in B6F10 melanoma cells [23]. Flavonoids are multi-active components used in specialised cosmetic products, primarily for antioxidant and soothing actions [24].

The high total tannin contents observed in these extracts and fractions reveal that the total tannin contents of these extracts or fractions depended on the extracting or fractionating solvent as well as the chemical compositions of the extracts or fractions. These extracts or fractions, if incorporated into personal care products can exhibit skin tightening effect and prevent infections, leading to healthy skin. Tannins are incorporated into personal care products to create the effect of firmness and toning of the skin due to high astringent effect [25].

Table 3: IC₅₀ values of *D. edulis*-Uyo leaf and bark

Scavenging activity	IC ₅₀ value ($\mu\text{g}/\text{cm}^3$) Uyo leaf	IC ₅₀ value ($\mu\text{g}/\text{cm}^3$) Uyo bark
BHA	163.69 \pm 3.01 ^d	163.69 \pm 3.01 ^d
Ethanol extract	148.43 \pm 0.04 ^e	152.29 \pm 2.04 ^e
Aqueous extract	161.72 \pm 1.02 ^d	171.34 \pm 0.04 ^c
Dichloromethane fraction	365.51 \pm 0.03 ^b	450.04 \pm 0.03 ^a
Ethyl acetate fraction	207.36 \pm 0.04 ^c	127.79 \pm 0.10 ^f
n-butanol fraction	125.31 \pm 0.03 ^f	156.06 \pm 9.04 ^e
Aqueous fractions	415.91 \pm 0.02 ^a	183.31 \pm 0.03 ^b

Same letters along the column means not significantly different ($p>0.05$) while different letters along the column means significantly different ($p<0.05$).

Table 4: IC₅₀ values of *D. edulis*-Ikom leaf and bark

Scavenging activity	IC ₅₀ value (µg/cm ³) Ikom leaf	IC ₅₀ value (µg/cm ³) Ikom bark
BHA	163.69±3.01 ^d	163.69±3.01 ^b
Ethanol extract	153.45±0.05 ^e	126.37±0.02 ^e
Aqueous extract	142.08±0.01 ^f	129.60±5.07 ^e
Dichloromethane fraction	281.26±0.02 ^a	292.47±4.02 ^a
Ethyl acetate fraction	150.29±0.03 ^g	112.01±0.02 ^f
n-butanol fraction	181.81±0.02 ^c	145.44±0.03 ^c
Aqueous fractions	213.44±0.04 ^b	131.53±0.06 ^d

Same letters along the column means not significantly different ($p > 0.05$) while different letters along the column means significantly different ($p < 0.05$).

The DPPH method has been widely applied for estimating antioxidant activity in recent years [26]. DPPH, a stable free radical with a purple colour, changes to a stable yellow compound upon reacting with an antioxidant. The DPPH radical scavenging assay was assessed by IC₅₀ value. Lower IC₅₀ value indicates higher antioxidant activity.

Both the ethanol and aqueous extracts of *D. edulis*-Uyo leaf (IC₅₀ = 148.43±0.04 µg/cm³ and IC₅₀ = 161.72±1.02 µg/cm³); and ethanol and aqueous extracts of *D. edulis*-Ikom leaf (IC₅₀ = 153.45±0.05 µg/cm³ and IC₅₀ = 142.08±0.01 µg/cm³) as shown in Tables 3 and 4 respectively, revealed higher and stronger antioxidant activity than the standard antioxidant BHA (IC₅₀ = 163.69±3.01 µg/cm³). However, there was no significant difference ($p > 0.05$) between the aqueous extract of *D. edulis*-Uyo leaf and the BHA (Table 3). The high antioxidant activity of *D. edulis*-Uyo and Ikom leaf extracts could be due to their high phenolics, flavonoids and tannin contents (Tables 1 and 2). Thus, the extracts can be used as active ingredients in photo-protective personal care products to counteract the effect of ultra violet (UV) light on the skin. The n-butanol and ethyl acetate fractions of *D. edulis* leaf were stronger radical scavengers (Tables 3 and 4) among the fractions. This high antioxidant activity of n-butanol fraction and ethyl acetate fraction could be due to the high tannin, phenolics and flavonoids contents of these fractions (Tables 1 and 2).

The ethanol extract of *D. edulis*-Uyo bark (IC₅₀ = 152.29±2.04 µg/cm³); and both the ethanol (IC₅₀ = 126.37±0.02 µg/cm³) and aqueous (IC₅₀ = 129.60±5.07 µg/cm³) extracts of *D. edulis*-Ikom bark as shown in Tables 3 and 4 respectively, revealed stronger antioxidant activity than the standard BHA (IC₅₀ = 163.69±3.01 µg/cm³). From the results, the ethanol and aqueous extracts of *D. edulis*-Ikom bark had better antioxidant potentials than its counterpart from Uyo.

The extracts and fractions of *D. edulis* leaf and bark have good antioxidant activity. All the ethanol and aqueous

extracts of the leaf and bark except aqueous extract of *D. edulis*-Uyo bark exhibited stronger antioxidant activity than the standard antioxidant, BHA. The strong antioxidant activity of the extracts could be attributed to the flavonoids (quercetin, quercitrin and afzelin), phenolics (methyl gallate) and Sitosterol 3-O-β-D-glucopyranoside sterol present in *D. edulis*. These compounds had been reported as some of the major constituents of *D. edulis* leaf and stem bark extracts [20, 21]. Afzelin was reported to protect human keratinocytes from the deleterious effects of UV irradiation through its biological properties (DNA-protective, antioxidant, and anti-inflammatory) as well as acting as a UV absorber. Thus, afzelin may prevent photoaging and the development of skin cancer [27]. Quercetin has a skin protective effect against damage caused by UV radiation, histamine, or contact with toxic chemical compounds. Antioxidant activity of methyl gallate had been reported [28]. Antioxidants protect cells against the damaging effects of reactive oxygen species, otherwise known as free radicals, such as singlet oxygen, super oxide, peroxy radicals, hydroxyl radicals and peroxynite which results in oxidative stress leading to cellular damage. Oxidative stress is one of the major mechanisms for skin aging and dermatological conditions [29].

4. Conclusions

The results of this research have established that the extracts of *D. edulis* leaf, bark and their respective fractions could serve as free radical inhibitors or scavengers, acting possibly as antioxidants. Topical application of antioxidants can provide an efficient strategy to enrich the endogenous cutaneous system, leading to a decrease in the UV-radiation mediated oxidative damage and prevent oxidative stress-mediated diseases. *D. edulis* leaf and stem bark can therefore be effective as anti-aging and skin lightening ingredients in personal care products. They can also prevent damage from UV radiation, which effect leads to reactive oxygen species generation.

5. Appendix

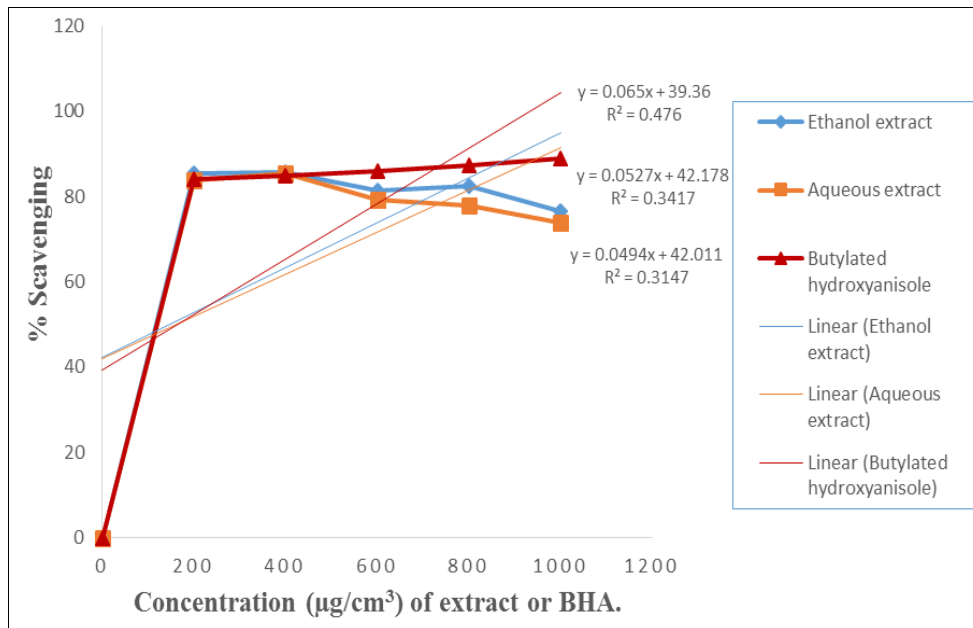


Fig 4: DPPH scavenging activity of *D. edulis*-Uyo leaf extracts and BHA.

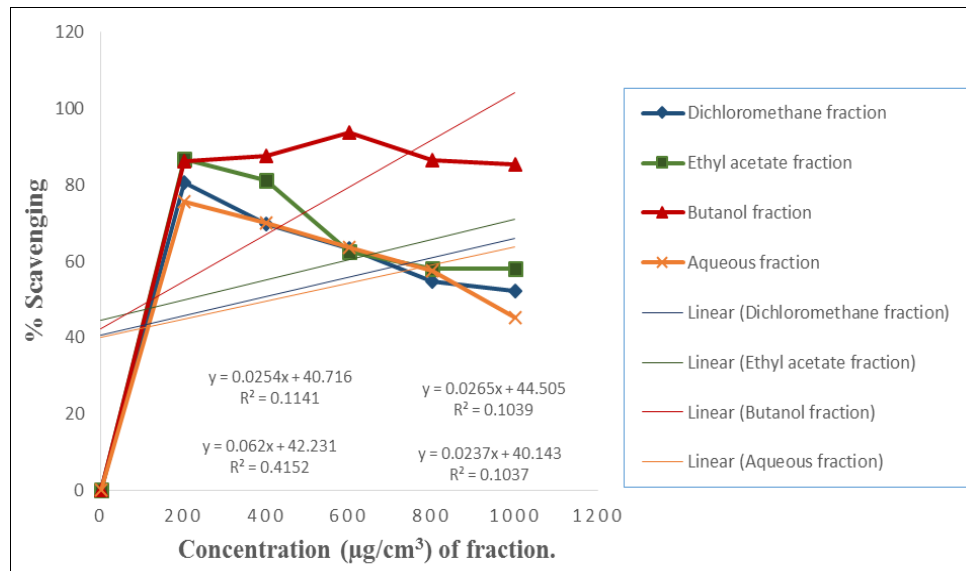


Fig 5: DPPH scavenging activity of *D. edulis*-Uyo leaf fractions.

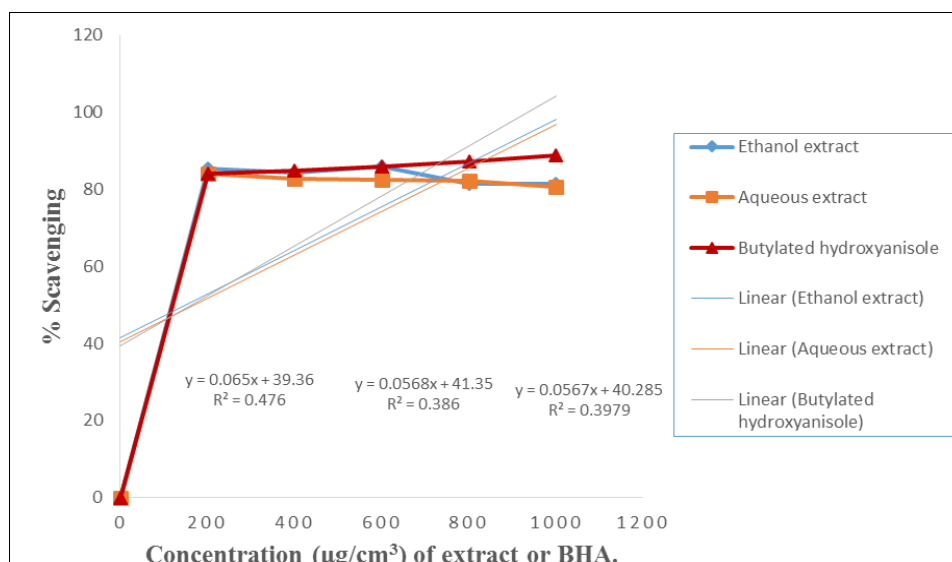


Fig 6: DPPH scavenging activity of *D. edulis*-Uyo bark extracts and BHA.

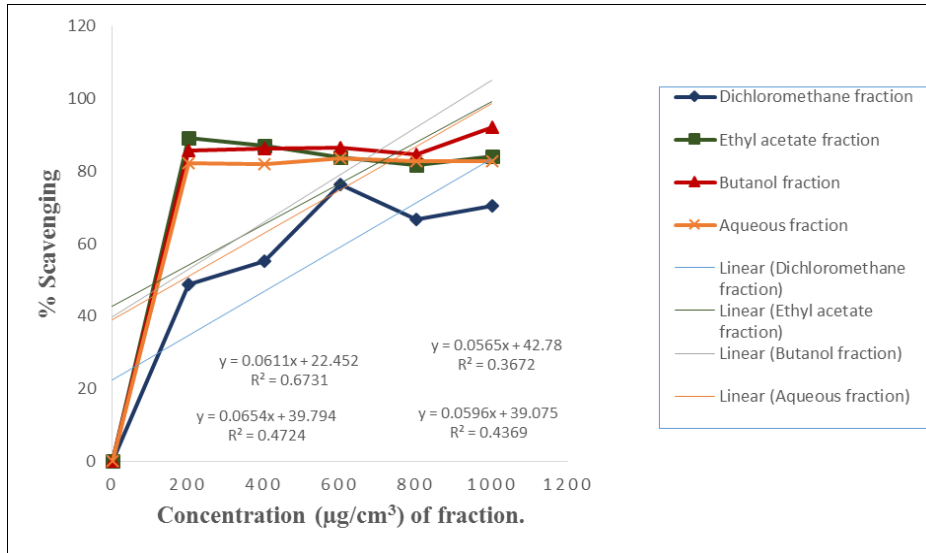


Fig 7: DPPH scavenging activity of *D. edulis*-Uyo bark fractions.

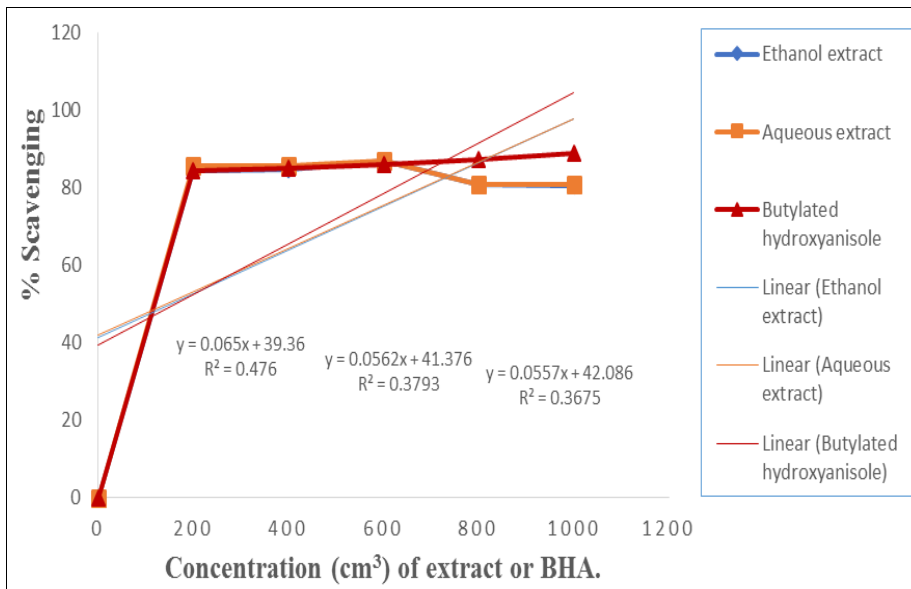


Fig 8: DPPH scavenging activity of *D. edulis*-Ikom leaf extracts and BHA.

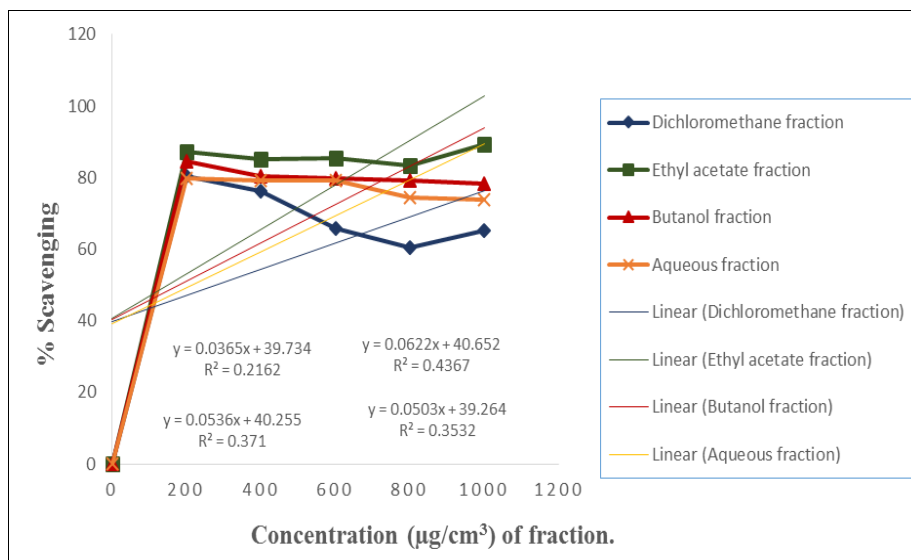


Fig 9: DPPH scavenging activity of *D. edulis*-Ikom leaf fractions.

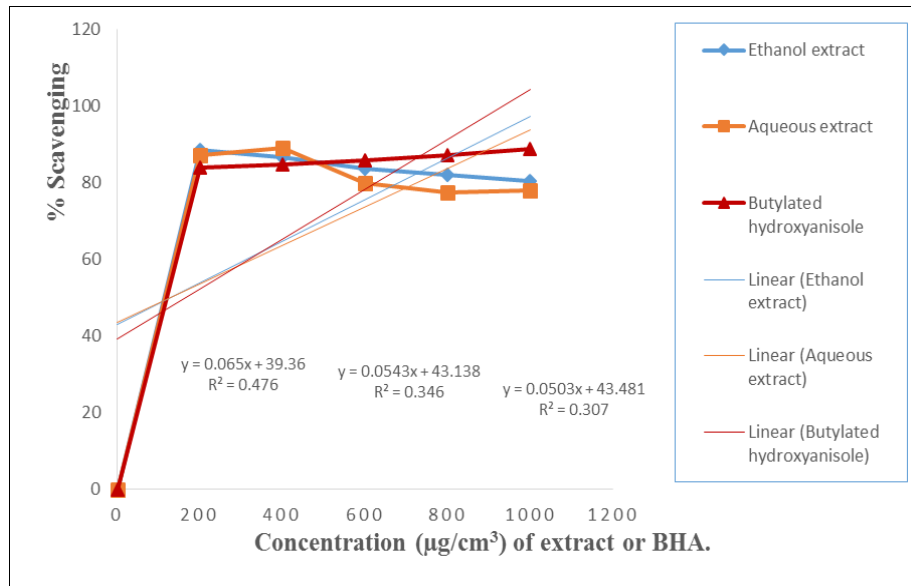


Fig 10: DPPH scavenging activity of *D. edulis*-Ikom bark extracts and BHA.

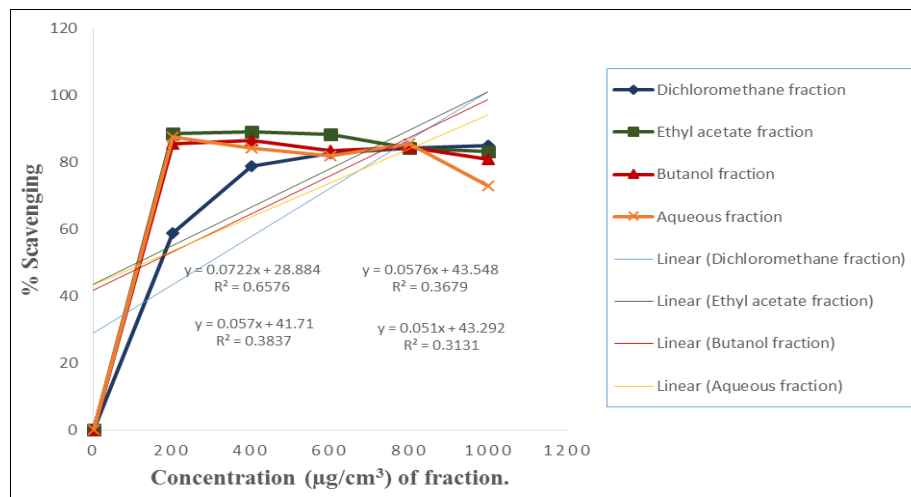


Fig 11: DPPH scavenging activity of *D. edulis*- Ikom bark fractions.

6. References

- Iriti M, Vitalini S, Fico G, Faoro F. Neuroprotective herbs and foods from different traditional medicines and diets. *Molecules*. 2010; 15:3517-3555.
- Rabe JH, Mamelak AJ, McEiquinn PJ, Morison WL, Sauder DN. Photoaging: mechanisms and repair. *Journal of American Academy Of Dermatology*. 2006; 55(1):1-19.
- Sakura M, Chiba Y, Kamiy E, Furukawa A, Kawamura N, Niwa M *et al*. Differences in the histopathology and cytokine expression pattern between chronological aging and photoaging of hairless mice skin. *Modern Research in Inflammation*. 2014; 3:82-89.
- Fisher GJ, Kang S, Varani J, Bata-Csorgo Z, Wan Y, Datta S *et al*. Mechanism of photoaging and chronological skin aging. *Archives of Dermatology*. 2002; 138(11):1462-1470.
- Sjerobabski-Masneć I, Situm M. Skin aging. *Acta Clinica Croatica*. 2010; 49(4):515-519.
- Stojiljkovic D, Pavlovic D, Arsic I. Oxidative stress, skin aging and antioxidant therapy. *Scientific Journal of the Faculty of Medicine in Nis*. 2014; 31(4):207-217.
- Mukherjee PK, Maitya N, Nema NK, Sarkar BK. Bioactive compounds from natural resources against skin aging. *Phytomedicine*. 2011; 19:64-73.
- Schagen SK, Zampeli VA, Makrantonaki E, Zouboulis CC. Discovering the link between nutrition and skin aging. *Dermatoendocrinology*. 2012; 4(3):298-307.
- Uwa LM. The anti-aging efficacy of antioxidants. *Current Trends in Biomedical Engineering and Bioscience*. 2017; 7(4):1-3.
- Apu AS, Al-Baizyd AH, Ara F, Bhuyan SH, Matin M, Hossain MF. Phytochemical analysis and bioactivities of *Argemone mexicana* Linn leaves. *Pharmacology Online*. 2012; 3:16-23.
- Djeridane A, Yousfi M, Nadjemi B, Boutassouna D, Stocker P, Vidal N. Antioxidant activity of some algerian medicinal plants extracts containing phenolic compounds. *Food Chemistry*. 2006; 79(4):654-660.
- Jothy SL, Zuraini Z, Sasidharan S. Phytochemicals screening, DPPH free radical scavenging and xanthine oxidase inhibitory activities of *Cassia fistula* seeds extract. *Journal of Medicinal Plants Research*. 2011; 5(10):1941-1947.
- Tambe VD, Bhambar RS. Estimation of total phenol, tannin, alkaloid and flavonoid in *Hibiscus tiliaceus* Linn. *Wood Extracts. Research and Reviews: Journal of Pharmacognosy and Phytochemistry*. 2014; 2(4):41-47.
- Hamid K, Saha MR, Urmi KF, Habib MR, Rahman MM. Screening of different parts of the plant *Pandanus odoros*

- for its antioxidant activity. International Journal of Applied Biology and Pharmaceutical Technology. 2010; 1(3):1364-1368.
15. Singh H, Dixit A, Sharma RA, Sharma A. Estimation of total phenolic, flavonoid content and evaluation of DPPH free radical scavenging potential of *Abutilon indicum* L. International Journal of Pharmaceutical Sciences Review and Research. 2015; 32(1):100-103.
 16. Aminudin NI, Ahmad F, Taher M. *In vitro* antioxidant, cholinesterase and tyrosinase inhibitory activities of *Calophyllum symingtonianum* and *Calophyllum depressinervosum* (Guttiferae). Journal of Coastal Life Medicine. 2015; 3(2):126-131.
 17. Potapovich AI, Kostyuk VA, Kostyuk TV, Luca C, Korkina LG. Effects of pre- and post-treatment with plant polyphenols on human keratinocyte responses to solar UV. Inflammation Research. 2013; 62:773-780.
 18. Soto ML, Falque E, Domínguez H. Relevance of natural phenolics from grape and derivative products in the formulation of cosmetics. Cosmetics. 2015; 2:259-276.
 19. Isaac V, Chiari BG, Miglioli K, Moreira R, Oliveira JRS, Salgado H *et al.* Development of a topical formulation containing *S. lutea* extract: stability, *in vitro* studies and cutaneous permeation. Journal of Applied Pharmaceutical Science. 2012; 2(8):174-179.
 20. Ajibesin KK, Essien EE, Adesanya SA. Antibacterial constituents of the leaves of *Dacryodes edulis*. African Journal of Pharmacy and Pharmacology. 2011; 5(15):1782-1786.
 21. Zofou D, Tematio EL, Ntie-Kang F, Tene M, Ngemenya MN, Tane P *et al.* New Antimalarial hits from *Dacryodes edulis* (Burseraceae) - Part I: isolation, *in vitro* activity, in silico “drug-likeness” and pharmacokinetic profiles. Plos One. 2013; 8(11):1-9.
 22. Hong C, Lee HA, Rhee CH, Choung S, Lee K. Separation of the Antioxidant Compound Quercitrin from *Lindera obtusiloba* Blume and Its Antimelanogenic Effect on B16F10 Melanoma Cells. Biotechnology and Biochemistry. 2013; 77(1):58-64.
 23. Jeong JY, Liu Q, Kim SB, Jo YH, Mo EJ, Yang HH *et al.* Characterization of melanogenesis inhibitory constituents of *Morus alba* leaves and optimization of extraction conditions using response surface methodology. Molecules. 2015; 20(5):8730-8741.
 24. Arct J, Pytkowska K. Flavonoids as components of biologically active cosmeceuticals. Clinics in Dermatology. 2008; 26:347-357.
 25. Aburjai T, Natsheh FM. Plants used in cosmetics. Phytotherapy Research. 2003; 17:87-1000.
 26. Mzid M, Khedir SB, Salem MB, Regaieg W, Rebai T. Antioxidant and antimicrobial activities of ethanol and aqueous extracts from *Urtica urens*. Pharmaceutical Biology. 2017; 55(1):775-781.
 27. Shin SW, Jung E, Kim S, Kim J, Kim E, Lee J *et al.* Antagonizing Effects and Mechanisms of Afzelin against UVB-Induced Cell Damage. Plos One. 2013; 8(4):1-17.
 28. Ekaprasada MT, Nurdin H, Ibrahim S, Dachriyanus. Antioxidant activity of methyl gallate isolated from the leaves of *Toona sureni*. Indonesian Journal of Chemistry. 2009; 9(3):457-460.
 29. Chaudhari PM, Kawade PV, Funne SM. Cosmeceuticals-a review. International Journal of Pharmacy and Technology. 2011; 3(1):774-798.