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**RESEARCH ARTICLE**

# Kinetic Behaviour of Skin Pigmentation Inhibitory Activity of some Selected Nigerian Medicinal Plants and Other Related Antioxidant Properties

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**Abstract:** ***Background*:** Over exposure to ultra violet (UV) radiation is one of the most significant external stress-inducing factors resulting into occurrence of skin pigmentation among others in human body. The biological implication of such disorders is not only limited to premature skin aging and cancer, but also resulted into economic loss of perishable agricultural products.

***Methods*:** Methanol extracts of ten (10) medicinal plants with skin health traditional history were selected for this study. The biological profile of the extracts was assessed in an *in-vitro* system using colorimetric assays: tyrosinase inhibition, ferric-ion reducing antioxidant power (FRAP), trolox equivalent absorbance capacity (TEAC) and Fe II-induced microsomal lipid peroxidation.

***Results*:** Representative of asteraceae such as *Laggera pterodonta* (S3); *Ageratum conyzoides* (S4) and *Chromolaena odorata* (S5), while *Euphorbia convoluloides* (S8) were found to possess good anti-tyrosinase activity ($IC_{50}$ = 177.50; 125.08; 167.58; 161.92) µg/ml respectively, in which the rate of formation of dopachrome proceeded via pseudo second order kinetic using the Largergren model. Other notable inhibition of oxidative stress displayed by the methanol extracts includes S7 (FRAP = 1905.12 ± 2.85 µM AAE/g); S1 & S6 (TEAC = 2163.48 ± 2.80; 1044.35 ± 28.99) µM TE/g, while S7 & S9 showed highest inhibitory activities on Fe II-induced microsomal lipid peroxidation ($IC_{50}$ = 33.625; 35.125) µg/ml, respectively in competitive manner to that of the commercial anti-oxidant EGCG ($IC_{50}$ = 36.250 µg/ml).

***Conclusion*:** The outcome of the biological properties of the selected plant extracts in this study suggested the existence of relationship between the traditional claims and the scientific data therein.

**Keywords:** Skin pigmentation, oxidative stress, agricultural products, asteraceae, euphorbiaceae, colorimetric, kinetics, biological activities.

## 1. INTRODUCTION

The ultra violet radiation (UVR) of the visible light spectrum has been reported to have primary implication in the degenerating action of tyrosinase enzyme during the physiological process of melanogenesis [1]. During this process, reactive oxygen species (ROS) and hydrogen peroxide ($H_2O_2$) which create oxidative stress in melanocytes are thereby resulted in the formation of melanin pigment in human skin and other parts as a result of over-accumulation of ROS from physiological response of human body against various environmental stresses [2]. Skin diseases including but not limited to acne, contact dermatitis, urticaria, hyperpigmentary, and cancer are primarily caused by longtime exposure to this UVR, as well as varying degree of human activities and individual lifestyles [2].

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Sequel to the above, hyperpigmentation occurs because the body produces too much dark macromolecules called melanin by melanocytes and thereafter deposited such in the epidermis by keratinocytes [3]. Melanin plays a vital role as photo protective agent against the harmful effects of UVR, and also essential for the regulation of colour of the skin. However, over accumulation of melanin in specific parts of the skin, eye and hair result in the formation of undesirable and abnormal secretion of a dark macromolecular pigment called skin hyperpigmentation [3, 4].

The process of hyperpigmentation is triggered by tyrosinase, a monophenol monooxygenase (EC 1.14.18.1) multifunctional enzyme widely distributed in nature with two copper-containing ions coordinated with histidine residue in the active site [5]. Tyrosinase enzyme catalyzes the first two stages during the process of melanogenesis using molecular oxygen [6]. The first stage involves the hydroxylation of L-tyrosine to L-3,4–dihydroxyphenylalamine (L-DOPA) by monophenolase action, while the second stage involves subsequent oxidation of L-DOPA to DOPA-quinone by diphe-

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nolase action which is considered as a rate-limiting enzyme for controlling the production of melanin pigment [7, 8]. Tyrosinase is also responsible for speeding enzymatic browning reactions in perishable agricultural products and shortening their lifespan, thereby deteriorates the colour clarity of plant-derived products and creating economic loss [6].

Certain ROS scavengers and inhibitors of ROS generation inhibit UV-induced melanogenesis and antioxidants and are such widely incorporated into cosmetic products to treat various skin problems, such as depigmentation of hyperpigmented spots [9]. Hence, antioxidants and free radical scavengers also play an important role in protecting human skin from these harmful effects by UVR. Over the years, compounds such as aloesin, arbutin, kojic acid and glabridin have been used instead of hydroquinone to help prevent skin darkening. However, these compounds have undesirable side effects and can be weakly active. An example for this claim is carcinogenic effect demonstrated by kojic acid [10], skin irritation, mutagenic effects to mammalian cells and cytotoxicity to melanocytes demonstrated by hydroquinone [11, 12], and poor skin penetrations and stability demonstrated by aloesin and glabridin [13], while other anti-browning compounds such as sulphites and ascorbic acid resulted into depreciation in nutritional quality of food with unwanted side effects [14].

Since the accumulation of excessive epidermal pigmentation leads to various dermatological disorders, tyrosinase inhibitors have become increasingly important in medication, cosmetics and agricultural sector to prevent hyperpigmentation through the inhibition of enzymatic oxidation as the rate limiting enzyme for regulating the production of this undesirable menace [15, 16]. Development of effective skin depigmenting agents with anti-oxidative capacity is a promising strategy to prevent or improve skin against premature or early damage due to over exposure to UVR. Thus, it is important to develop new depigmenting agents as inhibitors of melanin formation that are derived from natural sources, which will lessen the likelihood of unrelated cytotoxicity or other side effects posed by anti-browning compounds earlier discovered.

Asteraceae and Euphorbiaceae are widely used traditionally in the treatment of skin related ailments such as eczema, dermatitis and skin injury [17; 18]. The practical claim of such is *Gutenbergia nigritana* as a remedy for skin infections [19], *Launaea taraxacifolia* as antioxidant [20, 21], while Euphorbia generally demonstrated skin infection treatment [22]. Although, quite a number of scientific investigations have been carried out to validate the traditional uses of medicinal plants generally, there seems to be no traceable scientific report on the possible skin depigmenting and anti-browning activity on the medicinal plants selected for the study.

Based on the traditional claims from different localities where the plants selected for this study were found and scanty scientific data of their potential as skin depigmentation properties, the objectives of this study was to assess the anti-tyrosinase activity and the rate of proceeding for the formation of melanin in an *in vitro* system. Since skin pigmentation is implicated as a mediator of cellular oxidative stress, antioxidant activity through direct free radical scavenging capacity and reducing antioxidant power were as well evaluated.

## 2. MATERIALS AND METHODS

### 2.1. Chemicals and Reagents

Standards (purity > 99.0%) of kojic acid, EGCG (epigallocatechin gallate), trolox (6-hydroxyl-2, 5, 7, 8- tetramethylchroman-2-carboxylic acid), and other reagents including ABTS (2,2-azino-bis (3-ethylbenzo thiazoline-6-sulphonic acid) diammonium salt), potassium peroxodisulphate ($K_2S_2O_8$), AAPH (2,2-Azobis (2-methylpropionamidine) dihydrochloride, perchloric acid, TPTZ (2,4,6-tri[2-pyridyl]-S-triazine, iron (III) chloride hexahydrate ($FeCl_3.6H_2O$), hydrogen peroxide, potassium chloride (KCl), iron (II) sulphate ($FeSO_4$) were secured from Sigma-Aldrich, Inc. (Cape Town, South Africa). All antioxidant assays including FRAP, TEAC, lipid peroxidation, and tyrosinase inhibition were measured using Multiskan spectrum Plate Reader (Thermo Electron Corporation, MA, USA).

### 2.2. Plant Materials

The plant materials used in this study were wildly collected in May 2017, from their respective natural habitat (within the coordinates 7°37′16′ N 5°13′17′ E) from Ekiti State, South Western Nigeria. Voucher specimens were identified at the Ekiti State University Herbarium, Ado-Ekiti, Ekiti State, Nigeria, by Mr. Omotayo, with plant details and their respective herbarium numbers presented in Table **1**.

### 2.3. Preparation of Plant Extracts

5.0 g of the dried powder of each plant material was put in a 200 ml conical flask and mixed with methanol 10 % m/v). These were extracted at room temperature with constant shaking for 48 hours according to the method described [23] with slight modifications. The resultant supernatant was vacuum filtered with Whatman No. 1 filter paper and evaporated to dryness using a rotary evaporator (Buchi, Germany) with water bath temperature set at 40 $^0$C. Dry extracts were stored at -4 $^0$C and dissolved in dimethyl sulfoxide (DMSO) to a concentration of 1mg/ml just before use.

### 2.4. Determination of Tyrosinase Inhibition

This assay was performed using spectrophotometric method as previously described [24]. Samples were dissolved in dimethyl sulphoxide (DMSO) to a stock solution of 1 mg/ml, and further dilutions were done with 50 mM sodium phosphate buffer (pH 6.5) for all working solutions. Kojic acid was used as positive control. In the wells of a 96-well plate, 70 µl of each plant extracts working solution was combined with 30 $\mu$l of tyrosinase from mushroom (500 Units/ml in sodium phosphate buffer) in triplicate. After incubation at room temperature for 5 minutes, 110 $\mu$l of substrate (2 mM L-Tyrosine) was added to each well. Final effective concentrations of the extract samples ranged from 83.34 – 333.33 µg/ml (333.33, 166.67 & 83.34 µg/ml). Absorbance of the wells were then determined at 492 nm with Multiskan Plate Reader through order of reaction of the formation of dopachrome measured at 5 minutes interval for 30 minutes. End point spectrophotometric measurement was

also carried out after 30 minutes of incubation to determine the half maximal inhibitory concentration ($IC_{50}$). All the experiments were repeated in triplicate. The percentage of tyrosinase inhibition was calculated as follows:

Tyrosinase inhibition (%) = [(A - B) - (C - D)]/ (A - B) × 100 (1)

Where A is the absorbance of the control with the enzyme, B is the absorbance of the control without the enzyme, C is the absorbance of the test sample with the enzyme and D is the absorbance of the test sample without the enzyme.

### 2.5. Determination of Ferric-Ion Reducing Antioxidant Power (FRAP) Assay

The FRAP assay was carried out in accordance to the method described previously [25]. In a 96-well plate, 10 µl of the stock solution (1 mg/ml w/v) of each of the methanol crude extracts were mixed with 300 µl FRAP reagent. The FRAP reagent was prepared by mixing (10:1:1, v/v/v) of acetate buffer (300 mM, pH 3.6), TPTZ (10 mM in 40 mM HCl) and $FeCl_3.6H_2O$ (20 mM). Incubation commenced at room temperature for 30 minutes, and the plate was read at a wavelength of 593 nm. L-Ascorbic acid (Sigma Aldrich, South Africa) was used as a standard with concentrations varying between 0 and 1000 µM. Further dilutions were done to the samples that were highly concentrated and such dilution factors were recorded and used for calculations of the affected samples. Data obtained were analysed using programmed FRAP template and the results were expressed as µM ascorbic acid equivalents per milligram dry weight (µM AAE/g) of the test samples.

### 2.6. Trolox Equivalent Absorbance Capacity (TEAC) Assay

The total antioxidant activity of the test sample was measured using previously described methods [26]. The stock solutions which contain 7 mM ABTS and 140 mM $K_2S_2O_8$ was prepared and kept at -4 $^oC$. The working solution was then prepared by adding 88 µl $K_2S_2O_8$ solution to 5 ml ABTS solution. The two solutions were mixed and allowed to react for 24 hours at room temperature in the dark. Trolox was used as the standard with concentrations ranging between 0 and 500 µM. After 24 hours, the ABTS mix solution was diluted with ethanol to read a start-up absorbance (control) of approximately 2.0 (±0.1). The stock solution (1 mg/ml) of the test samples (25 µl) were allowed to react with 300 µl ABTS in the dark at room temperature for 30 minutes. The absorbance was read at 734 nm at 25 $^oC$ in the Plate Reader. Data obtained were analysed using programmed TEAC template and the results were expressed as µM trolox equivalents per milligram dry weight (µM TE/g) of the test samples.

### 2.7. Inhibition of Fe (II)-Induced Microsomal Lipid Peroxidation Assay

The lipid peroxidation assay was carried out in accordance to the method described previously [27] with a few modifications. Rat liver microsomes were isolated from S9 Rats using sepharose column eluted with 0.01 M potassium phosphate buffer; pH 7.4, supplemented with 1.15 % KCl at 5 °C. The reaction mixture contained microsomes from S9 Rats (1mg of protein/ml in 0.01 M potassium phosphate buffer; pH 7.4, supplemented with 1.15 % KCl). The positive control includes microsomes, buffer and iron (II) sulphate, in the absence of the samples to be tested. The sample stock solutions were prepared in DMSO (1mg/ml, w/v). The working sample solutions were prepared in 0.01 M potassium phosphate buffer pH 7.4, supplemented with 1.15 % KCl diluted to 100, 50 and 10 µg/ml concentrations. 100 µl of each sample (working solutions) were dissolved in potassium phosphate buffer and pre-incubated with 500 µl microsomes at 37 $^oC$ for 30 minutes in a shaking water bath. 200 µl of KCl-buffer were added to the mixture, followed by 200 µl of a 2.5 mM $FeSO_4$ solution and incubated at 37 $^oC$ for 1 hour in a shaking water bath. The reaction was terminated with 10 % TCA solution (1 ml) containing 125 µl BHT (0.01 %) and 1 mM EDTA. Samples were centrifuged at 2000 rpm for 15 minutes, and 1 ml of each supernatant was mixed with 1 ml of 0.67 % TBA solution. The reaction mixture was then incubated in a water bath at 90 $^oC$ for 20 minutes and the absorbance wewaseasured at 532 nm using plate reader. The percentage inhibition of TBARS formation relative to the positive control was calculated by:

$$[(A_{control} - A_{sample)/} A_{control} \text{ X } 100)] \quad (2)$$

### 2.8. Kinetic Mechanism in the Formation of Dopachrome

The Largergren models (pseudo first and pseudo second order models), proposed in 1898, and described [28, 29] were used for the inhibition data. The original pseudo first order kinetic model is represented in equation 3

$$\frac{dq_t}{dt} = K_1(q_e - q_t) \quad (3)$$

Where $k_1$ is the rate constant of pseudo first-order kinetic model; $q_{e,}$ is concentration at the equilibrium phase.

Pseudo-second order kinetic model is expressed in equation 4 as follows;

$$\frac{dq_t}{dt} = K_2(q_e - q_t)^2 \quad (4)$$

Where $k_2$ is the rate constant of pseudo second-order kinetic model; $q_{e,}$ is the concentration at the equilibrium phase.

The rate constant $k_1$ and the concentration $q_e$ at the equilibrium phase can be easily obtained from the slope and the intercept of plot *log* ($q_e$-$q_t$) versus *t* (Fig. **2**), respectively. While the rate constant $k_2$, concentration $q_e$ can be deduced from the plots of t/qt versus time, t.

### 2.9. Data Analysis

Graph pad prism version 5.0 was used for the analyses of tyrosinase and LPO activities for the estimation of their $IC_{50}$. ANOVA was used for comparison of mean values and $p <$ 0.5 considered to be statistically significant. The data were as well subjected to kinetic analyses using pseudo-first and pseudo-second order kinetic models.

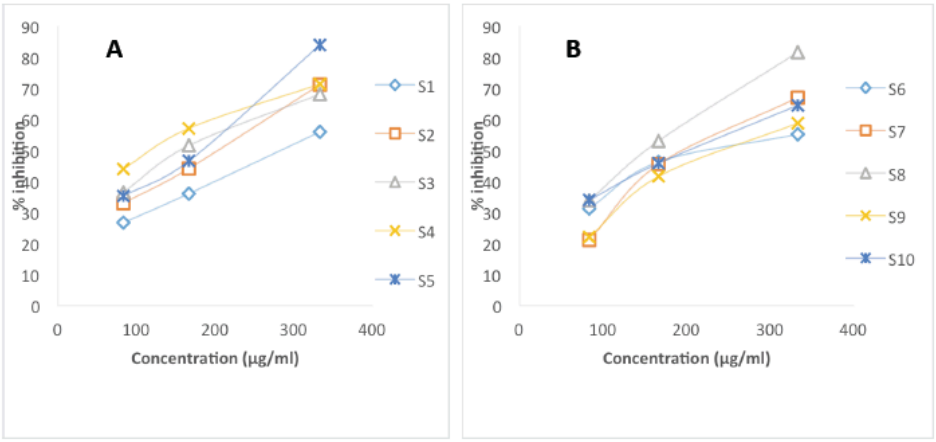
## 3. RESULTS AND DISCUSSION

The study was carried out to document scientific information on varying degree of traditional uses of asteraceae and euphorbiaceae family as skin diseases treatment. Such

**Table 1. Samples authentication and % yield.**

| Plant Code | Plant Name | Family | Herbarium No. | % yield |
|---|---|---|---|---|
| S1 | *Gutenbergia nigritana* [Benth] Oliv. & Hiern | Asteraceae | UHAE2018/012 | 2.36 |
| S2 | *Lounaca taraxacifolia* Amin MS ex C. Jeffrey syn. *lactuca* | | UHAE2018/015 | 1.92 |
| S3 | *Laggera pterodonta* [DC] Sch. Bip | | UHAE2018/014 | 6.51 |
| S4 | *Ageratum conyzoides* Linn | | UHAE2018/007 | 3.52 |
| S5 | *Chromolaena odorata* [Linn] Kings & Robinson syn. *Eupatorium odoratum* | | UHAE2018/008 | 2.5 |
| S6 | *Acalypha wikesiana* Mull. Arg | Euphorbiaceae | UHAE2018/006 | 2.74 |
| S7 | *Erythrococa chevalieri* [Baill] Frain syn. *Erythrococa anomala* [Baill] | | UHAE2018/011 | 1.51 |
| S8 | *Euphorbia convoluloides* Hoctst ex Benth | | UHAE2018/009 | 4.34 |
| S9 | *Euphorbia lateriflora* Schum & Thonn | | UHAE2018/010 | 2.71 |
| S10 | *Jatropha curcas* Linn | | UHAE2018/013 | 7.44 |

S1-S5: Asteraceae; S6-S10: Euphorbiaceae




*Kojic acid (standard drug) demonstrated full inhibition at different concentrations of the samples used.

**Fig. (1).** % inhibition recorded for the reaction mixture of L-tyrosine and tyrosinase in 50 mM PBS (pH 6.5) in the presence of Asteraceae (S1-S5, Fig. **A**) and Euphorbiaceae (S6-S10, Fig. **B**) as inhibitors at different concentrations.

conventional uses of the plants belonging to these two families include treatment of skin related ailments [17-19], while pharmacological importance includes wide application as antioxidant [20, 21]. The % yield recorded (between the ranges of 1.51 – 7.45) in Table **1** from the representative of the two families can be used as pivot information on the economic viability of the selected plants.

The % inhibition of the test samples (S1-S10) against the activity of tyrosinase enzyme presented in Fig. (**1**) demonstrated a dose-dependent manner between the three different concentrations (333.33, 166.67 & 83.34 µg/ml). Asteraceae (S2-S5) were found to demonstrate mild anti-tyrosinase activity within the range of $IC_{50}$ 125.08 – 198.75 µg/ml when compared to that of euphorbiaceae with only S8 $IC_{50}$ inhibition 161.92 µg/ml in Table **3** was observed. The results therefore nominated asteraceae with more anti-tyrosinase power than euphorbiaceae among the representative plants selected for this study. Hence the information arose from the study can tentatively support the importance of the two families in the traditional usage to treat skin infections.

### 3.1. Kinetic Mechanism in the Formation of Dopachrome

In order to suppress the production of melanin, it is important to suppress the action of tyrosinase through inhibition [6]. However, skin depigmenting agents so far discovered aside hydroquinone work very slowly which do not give sufficient improvement in such efficacy. The Largergren model, proposed in 1898 states that the first order kinetic equation and can be represented as follows:

The pseudo-first order parameters, $q_e$, $k_1$ and $R^2$, as extrapolated from the slopes and intercepts of (Fig. **2**) are presented in Table **2**. While the pseudo-second order parameters, $q_e$, $k_2$, *and* $R^2$ as obtained from the slopes and intercepts of (Fig. **3**) are also presented in Table **2**.

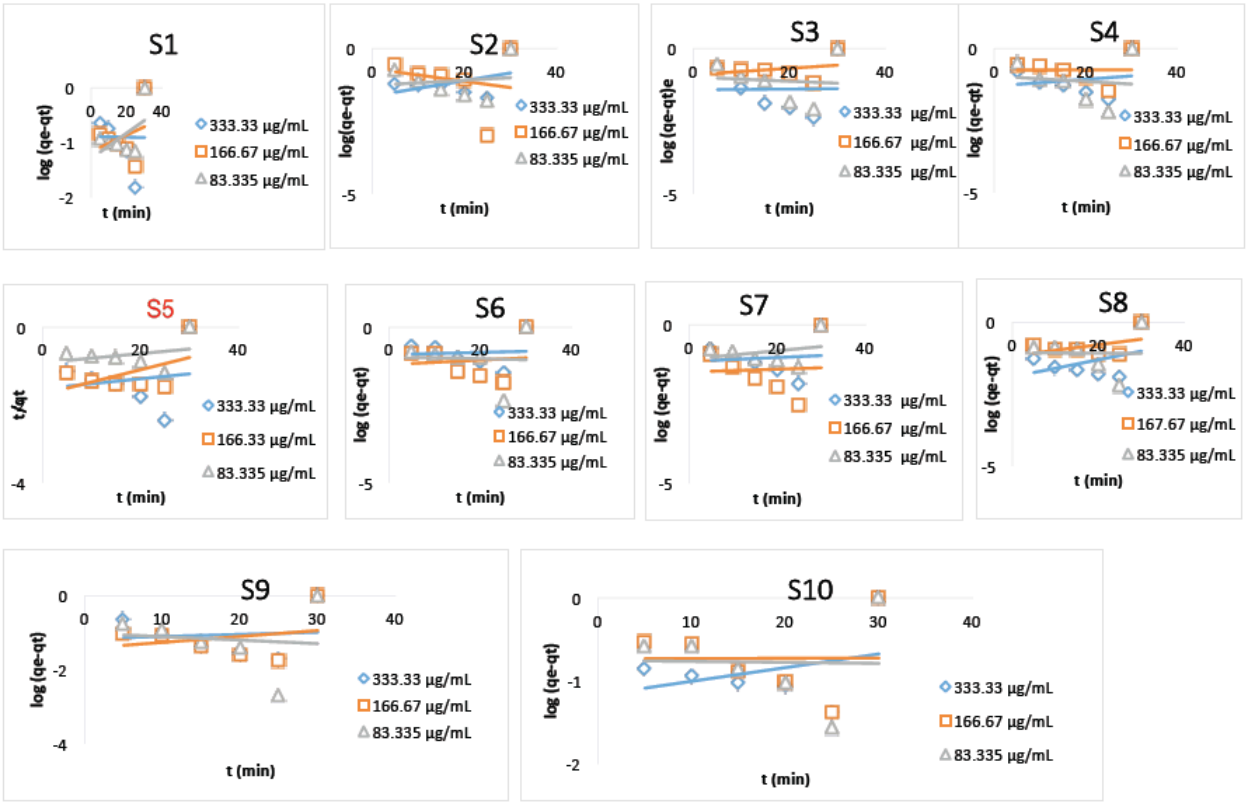



**Fig. (2).** Pseudo first order plots for the formation of dopachrome.

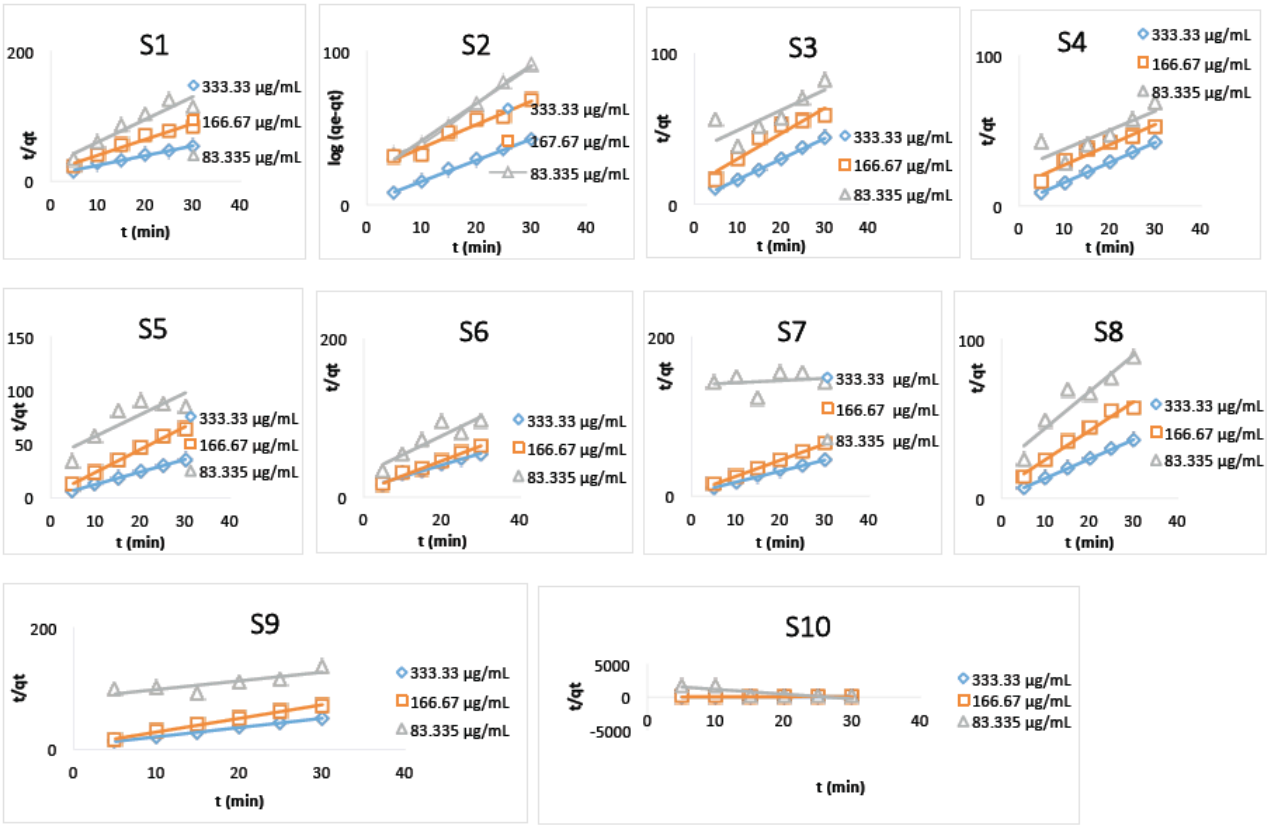



**Fig. (3).** Pseudo second order plots for the formation of dopachrome.

**Table 2. Order of reaction of the formation of dopachrome.**

| | | | Pseudo-First Order | | | Pseudo-second Order | | |
|---|---|---|---|---|---|---|---|---|
| **Sample** | **Conc. µg/mL** | **$Q_{e\ EXP}$** | **$Q_{e\ EST}$** | **$K_1$** | **$R^2$** | **$Q_{e\ EST}$** | **$K_2$** | **$R^2$** |
| S1 | 333.33 | 0.559 | 0.1309 | 0.0014 | 0.0001 | 0.6698 | 0.1565 | 0.9889 |
| | 166.67 | 0.361 | 0.0684 | -0.0352 | 0.0868 | 0.4110 | 0.1555 | 0.9606 |
| | 83.335 | 0.267 | 0.0514 | -0.0534 | 0.2432 | 0.2871 | 0.1369 | 0.8858 |
| S2 | 333.33 | 0.712 | 0.0120 | 3.8644 | 0.1678 | 0.7241 | 1.0200 | 0.9982 |
| | 166.67 | 0.441 | 0.2011 | 0.0497 | 0.0394 | 0.6565 | 0.0701 | 0.9573 |
| | 83.335 | 0.333 | 0.0538 | -0.0205 | 0.016 | 0.4104 | 0.1410 | 0.9909 |
| S3 | 333.33 | 0.68 | 0.0379 | -0.0028 | 0.0002 | 0.7316 | 0.5102 | 0.9973 |
| | 166.67 | 0.515 | 0.1264 | -0.2487 | 0.0661 | 0.5960 | 0.1256 | 0.9132 |
| | 83.335 | 0.365 | 0.1036 | 0.01612 | 0.007 | 0.7455 | 0.0378 | 0.6709 |
| S4 | 333.33 | 0.713 | 0.0496 | -0.0267 | 0.0297 | 0.7498 | 0.5615 | 0.9996 |
| | 166.67 | 0.571 | 0.1773 | -0.0009 | 0.0001 | 0.7413 | 0.0970 | 0.9439 |
| | 83.335 | 0.440 | 0.1097 | 0.02141 | 0.0113 | 0.7868 | 0.0505 | 0.7228 |
| S5 | 333.33 | 0.839 | 0.0262 | -0.0293 | 0.022 | 0.8584 | 1.2932 | 0.9998 |
| | 166.67 | 0.465 | 0.0177 | -0.0728 | 0.2546 | 0.4688 | 0.8400 | 0.9957 |
| | 83.335 | 0.353 | 0.1218 | -0.0270 | 0.0806 | 0.4946 | 0.0542 | 0.723 |
| S6 | 333.33 | 0.551 | 0.1315 | -0.0078 | 0.004 | 0.6758 | 0.1310 | 0.9746 |
| | 166.67 | 0.463 | 0.0625 | -0.0166 | 0.0108 | 0.5289 | 0.2263 | 0.9872 |
| | 83.335 | 0.313 | 0.1114 | 0.0071 | 0.0014 | 0.4175 | 0.0800 | 0.8213 |
| S7 | 333.33 | 0.669 | 0.0709 | -0.0143 | 0.0084 | 0.7204 | 0.3911 | 0.9992 |
| | 166.67 | 0.455 | 0.0331 | -0.0099 | 0.0021 | 0.4892 | 0.4792 | 0.9996 |
| | 83.335 | 0.210 | 0.0835 | -0.0305 | 0.0737 | 3.6245 | 0.0020 | 0.0448 |
| S8 | 333.33 | 0.815 | 0.0127 | -0.0686 | 0.1545 | 0.8227 | 2.0942 | 0.9998 |
| | 166.67 | 0.530 | 0.0686 | -0.0445 | 0.185 | 0.5502 | 0.2872 | 0.9729 |
| | 83.335 | 0.338 | 0.0887 | 0.0023 | 0.0001 | 0.4358 | 0.1068 | 0.9003 |
| S9 | 333.33 | 0.587 | 0.0700 | 0.0057 | -1.1562 | 0.6586 | 0.2758 | 0.9981 |
| | 166.67 | 0.416 | 0.0381 | 0.0160 | -1.4192 | 0.4471 | 0.3620 | 0.9964 |
| | 83.335 | 0.220 | 0.0979 | -0.0094 | -1.0090 | 0.7040 | 0.0169 | 0.701 |
| S10 | 333.33 | 0.645 | 0.0680 | -0.038 | 0.1087 | 0.6752 | 0.3680 | 0.9880 |
| | 166.67 | 0.458 | 0.1858 | -0.0007 | 0.00003 | 0.8915 | 0.0344 | 0.7380 |
| | 83.335 | 0.265 | 0.1776 | 0.0028 | 0.0005 | -0.0141 | -0.0379 | 0.6996 |

Comparing the applicability of pseudo first order and pseudo second order in the formation of dopachrome across the ten plant inhibitor samples (S1-S10). The values of correlation coefficient ($R^2$) for pseudo-first order kinetic model were tending to zero, this means that the experimental data failed to fit into the kinetic model.

The pseudo-second-order plots gave higher $R^2$ values than the pseudo-first-order, as the obtained values all tended to unity (Table **2**). However, it is not enough to conclude that a particular model is obeyed as a result of high $R^2$ values tending to 1, it is important to compare the closeness of amount calculated ($q_{e\ CAL}$) with experimental value ($q_{e\ EXP}$) [30]. As presented in Table **2**, the values of $q_{e\ CAL}$ from the pseudo-second-order kinetic model were close to $qe_{\ EXP}$ than that of the pseudo-first-order kinetic model. It can, therefore, be concluded that pseudo second order kinetic model had better applicability in the formation of dopachrome.

**Table 3. Total antioxidant capacities and end point determination of anti-tyrosinase activity.**

| Sample | FRAP (μM AAE/g) | TEAC (μM TE/g) | LPO $IC_{50}$ (μg/ml) | TYR ($IC_{50}$ (μg/ml) |
|---|---|---|---|---|
| S1 | 88.79 ± 3.27 | 2163.48 ± 2.80 | >100.00 | >200.00 |
| S2 | 176.48 ± 0.71 | nd | >100.00 | 198.75 |
| S3 | 955.11 ± 17.68 | 62.90 ± 0.38 | >100.00 | 177.50 |
| S4 | 246.82 ± 3.82 | nd | 98.875 | 125.08 |
| S5 | 79.26 ± 5.25 | nd | >100.00 | 167.58 |
| S6 | 25.53 ± 3.94 | 1044.35 ± 28.99 | 68.125 | >200.00 |
| S7 | 1905.12 ± 2.85 | 578.16 ± 4.07 | 33.625 | >200.00 |
| S8 | 263.49 ± 3.01 | 50.23 ± 0.38 | 84.625 | 161.92 |
| S9 | 20.49 ± 1.95 | 306.03 ± 0.65 | 35.125 | >200.00 |
| S10 | 77.65 ± 3.38 | nd | >100.00 | >200.00 |
| EGCG | 3294 ± 2.77 | 9687 ± 3.04 | 36.250 | NA |
| KJA | NA | NA | NA | 12.01 |

nd: not detected; EGCG: Epigallocatechin gallate; NA: not applicable; LPO: Fe (II)-induced microsomal lipid peroxidation; TYR: anti-tyrosinase activity; S1-S10: test samples. For all $IC_{50}$ values, $p<0.5$.

### 3.2. Total Antioxidant Capacities

Free radicals are known to play a definite role in a wide variety of pathological manifestations. Antioxidants fight against free radicals and protect us from various diseases. They exert their action either by scavenging the reactive oxygen species or protecting the antioxidant defence mechanisms [31-33]. The electron donation ability of natural products can be measured.

The protective role of representative family of asteraceae and euphorbiaceae and that of commercial antioxidant EGCG on Fenton's radicals (hydroxyl) using Fe (II)-induced microsome was studied at different concentrations. The $IC_{50}$ concentration values for the S7, S9 (33.625 & 35.125 μg/ml) are in competitive manner to that of EGCG (36.250 μg/ml) in Table **3**. The result of LPO corroborate to that of the previous study on Acalypha sp. found to demonstrate significant activity [31]. Such activity includes *A. wilkensiana* as anti-oxidant [34], Acalypha species generally used by a traditional healer in the treatment of malaria, gastrointestinal and dermatological disorders [35], as well as an antioxidant [36].

It is well known that antioxidants and free radical scavengers play an important role in protecting human skin from the harmful effects of ultra violet rays of sunlight, and they may be used to treat skin problems [37]. These results were consistent with the previous studies that reported that samples with high antioxidant activity can be associated with significant anti-tyrosinase activity, as both play important roles in preventing free radical-related skin damage [38-40].

## CONCLUSION

The study demonstrates that both asteraceae and euphorbiaceae displayed varying degree of preventing skin hyperpigmentation and other related oxidative stress typified by the TEAC, FRAP and LPO assays gave credence to their efficacy thereby confirming the assumed ethnomedicinal relationship between the two plant families. It may be collectively stated that most of the representative family of asteraceae display moderate anti-tyrosinase activity, while that of euphorbaceae serve as good sources of lipid peroxidation preventer. It can, therefore, be concluded that pseudo second order kinetic model had better applicability in the formation of dopachrome. The plants selected for the study can be a healthy source of natural anti-tyrosinase and other related inhibitors of oxidative stress.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

## HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are the basis of this research.

## CONSENT FOR PUBLICATION

Not applicable.

## CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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## REFERENCES

[1] Regazzeti, C.; Sormani, L.; Debayle, D.; Bernerd, F.; Tulic, M.K.; De Donatis, G.M.; Chignon-Sicard, B.; Rocchi, S.; Passeron, T. Melanocytes sense blue light and regulate pigmentation through Opsin-3. *J. Investigative Dermatol.,* **2018**, 138, 171-178.

[2] Kim, L-S.; Yoon, S-J.; Park, Y-J.; Lee, H-B. Inhibitory effect of ephedrannins A and B from roots of *Ephedra sinica* STAPF on melanogenesis. *Biochim. Biophys. Acta*, **2015**, 1850, 1389-1396.

[3] Wangthong, S.; Tonsiripakdee, l.; Monhaphol, T.; Nonthabenjawan, R.; Wanichwecharungruang, P.S. TLC developing technique for tyrosinase inhibitor detection. *Biomed. Chromatogr*., **2007**, 21, 94-100.

[4] Chompo, J.; Upadhyay, A.; Fukuta, M.; Tawata, S. Effect of Alpinia zerumbet components on antioxidant and skin disease-related enzymes. *BMC Complement. Altern. Med.,* **2012**, 12, 106-114.

[5] Ferro, S.; De Luca, L.; Germano, M.P.; Buemi, M.R.; Lelo, L.; Certo, G.; Kanteev, M.; Fishman, A.; Rapisarda, A.; Gitto, R. Chemical exploration of 4-(4-fluorobenzyl)piperidine fragment for the development of new tyrosinase inhibitors*. Eur. J. Med. Chem.,* **2017**, 125, 992-1001.

[6] Chang, T-S. An update review of tyrosinase inhibitors. *Int. J. Mol. Sci*., **2009**, 10, 2440-2475.

[7] Summers, B. A lightening tour of skin-brightening options. Pharm. Cosmetic Rev., **2006**, 33(6), 29–33.

[8] Mapunya, M.B.; Nikolova, R.V.; Lall, N. Melanogenesis and anti-tyrosinase activity of selected South African plants. *Evidence Based Complement. Altern. Med.*, **2012**, 2012, 1155-1161.

[9] Kim, M.; Shin, S.; Lee, J-A.; Park, D.; Lee, J.; Jung, E. Inhibition of melanogenesis by Gaillardia aristata flower extract. *BMC Complement. Altern. Med.*, **2015**, 15, 449.

[10] Fuyuno, I. Spotlight turns on cosmetics for Asian skin. Nature, **2004**, 432, 938-1038.

[11] Curto, E.V.; Kwong, C.; Hermersdorfer, H.; Glatt, H.; Santis, C.; Virador, V.; Hearing, V.J.; Dooley, T.P. Inhibitors of mammalian melanocyte tyrosinase: *in vitro* comparisons of alkyl esters of gentisic acid with other putative inhibitors. *Biochem Pharmacol.*, **1999**, 57, 663–672.

[12] Parvez, S.; Kang, M.; Chung, H.S.; Cho, C.; Hong, M.C.; Shin, M.K.; Bae, H. Survey and mechanism of skin depigmenting and lightening agents*. Phytother. Res*., **2006**, 20, 921-934.

[13] Hermanns, J.F.; Pierard-Franchimont, C.; Pierard, G.E**.** Skin colour assessment in safety testing of cosmetics. An overview. *Int'l. J. Cosmet*., **2000**, 22, 67-71.

[14] Biswas, R.; Mukherjee, P.K.; Dalai, M.K.; Mandal, P.K. Tyrosinase inhibitory potential of purpurin in *Rubia cordifolia*-A bioactivity guided approach*. Ind. Crops Prod.*, **2015**, 74, 319-326.

[15] Moon, J-Y.; Yim, E-Y.; Song, G.; Lee, N.H.; Hyun, C-G. Screening of elastase and tyrosinase inhibitory activity from Jeju Island plants. *EurAsian J. Biosci.,* **2010**, 4, 41-53.

[16] Huang, X.H.; Chen, Q.X.; Wang, Q.; Song, K.K.; Wang, J.; Sha, L.; Guan, X. Inhibition of the activity of mushroom tyrosinase by alkylbenzoic acids. *Food. Chem.,* **2006**, 94, 1-6.

[17] Pang, Y.; Wang, D.; Fan, Z.; Chen, X.; Yu, F.; Hu, X.; Wang, K.; Yuan, L. *Blumea balsamifera- A phytochemical and pharmacological review. Molecules*, **2014**, 19(7), 9453-9477.

[18] Popoola, O.K.; Marnewick, J.L.; Rautenbach, F.; Ameer, F.; Iwuoha, E.I.; Hussein, A.A. Inhibition of oxidative stress and skin aging-related enzymes by prenylated chalcones and other flavonoids from *Helichrysum teretifolium*. *Molecules*, **2015**, 20, 7143-7155.

[19] Ahmadu, A.A.; Tarimaledei, P.; Onanuga, A**.** *Triterpenoids from Gutenbergia nigritana (Benth) Oliv and Hiern. Afri. J. Trad. Complement. Altern. Med.*, **2013**, 10, 405-409.

[20] Adetutu, A.; Olorunnisola, O.S.; Oyewo, E.B. Phytochemical composition, antioxidant properties and antibacterial activities of five West-African green leafy vegetables. *Canadian J. Pure & App. Sci.*, **2013**, 7, 2357-2362.

[21] Owoeye, O.; Arinola, G.O. A Vegetable, *Launaea taraxacifolia*, Mitigated mercuric chloride alteration of the microanatomy of Rat brain. *J. Dietary Suppl.*, **2017**, 14, 613-625.

[22] Donkor, A-M.; Oduro-Mensah, D.; Fiazorh, M. Extracts of *Euphobia hirta* Linn and *Physalis angulata* L. and their amalgamation demonstrate potency against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *Int'l. J. Pharm. Pharmaceutical Sci.,* **2016**, 8, 322-326.

[23] Iloki-Assanga, S.B.; Lewis-Lujan, L.M.; Lara-Espinoza, C.L.; Gil-Salido, A.A.; Fernandez-Angulo, D.; Rubio-Pino, J.L.; Haines, D.O. Solvent effects on phytochemical constituent profiles and antioxidant activities, using four different extraction formulations for analysis of *Bucida buceras* L. and *Phoradendron californicum*. *BMC Res. Notes*, **2015**, 8, 396.

[24] Vardhan, A.K.; Pandey, B. Screening of plant parts for anti-tyrosinase activity by tyrosinase assay using mushroom tyrosinase. *Indian J. Sci. Res.*, **2014**, 4, 134-139.

[25] Benzie, I.F.F.; Strain, J.J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP Assay. *Anal. Biochem.*, **1996**, 238, 70-76.

[26] Pellegrini, N.; Re, R.; Yang, M.; Rice-Evans, C.A. Screening of dietary carotenoid rich fruit extracts for antioxidant activities applying ABTS radical cation decolorisation assay. *Methods Enzymol.*, **1999**, 299, 379-389.

[27] Snijman, P.W.; Joubert, E.; Ferreira, D.; Li, X.; Ding, Y.; Green, I.R.; Gelderblom, W.C.A. Antioxidant activity of the dihydrochalcones aspalathin and nothofagin and their corresponding flavones in relation to other rooibos (*Aspalathus linearis*) flavonoids, epigallocatechin gallate, and trolox. *J. Agric. Chem.*, **2009**, 57, 6678-6684.

[28] Ho, Y.S.; McKay, G. The sorption of Pb (II) ions on peat. *Water Res*., **1999**, 33, 578-584.

[29] Demirbas, E., Kobya, M., Senturk, E., Ozkan, T. *Water SA*, **2004**, 30, 533-539.

[30] Sivakami, M.S.; Thandapani, G.; Jayachandran, V.; Hee-Seok, J.; Se-Kwon, K.; Sudha, P.N**.** Preparation and characterization of nano chistosan for treatment waste water. *Int. J. Biol. Macromol.*, **2013**, 57, 204-212.

[31] Méité, S.I.; Bagré, B.; Bahi, G.; N'guessan, J.D.; Djaman, A.J. Antioxidant activity of water extract of *Mareya micrantha* (Benth.) Mull. Arg. (Euphorbiaceae). *European J. Biomed. Pharmaceutical Sci.,* **2017**, 4, 5-11.

[32] Satya P.M.; Suman J.; Narendra, K.; Srinivas, K.; Srilakshmi B.J.; Lakshmi C.M.; Krishma S.A. Phytochemical and Pharmacological evaluation of Euphorbiaceae family plant leaves-*Acalypha indica* L., *Croton bonplandianum* Baill. *Mintage J. Pharmaceutical Med. Sci.,* **2015**, 4, 17-22.

[33] Ravi, S.; Shanmugam, B.; Subbaiah, G.V.; Prasad, S.H.; Reddy, K.S. Identification of food preservative, stress relief compounds by GC–MS and HR-LC/Q-TOF/MS; evaluation of antioxidant activity of *Acalypha indica* leaves methanolic extract (*in vitro*) and polyphenolic fraction (*in vivo*). *J. Food Sci. Technol.*, **2017**, 54, 1585-1596.

[34] Anokwuru, C.P.; Sinisi, A.; Samie, A.; Taglialatela-Scafati, O. Antibacterial and antioxidant constituents of Acalypha wilkensiana. *Nat. Prod. Res.,* **2014**, 29, 1180-1183.

[35] Udobang, J.A.; Nwafor, P.A.; Okokon, J.E. Analgesic and antimalarial activities of crude leaf extract and fractions of *Acalypha wilkensiana*. *J. Ethnopharmacol.*, **2010**, 127, 373-378.

[36] Ma, Q.; Wei, R.; Liu, W.; Sang, Z.; Guo, X. Cytotoxic Anthraquinones from the Aerial Parts of *Acalypha australis*. *Chem. Nat. Compounds,* **2017**, 53, 949-952.

[37] Jemli, E.I.; Kamal, R.; Marmouzi, I.; Zerrouki, A.; Cherrah, Y.; Alaoui, K. Radical scavenging activity and ferric reducing ability of *Junniperus thurifera* (L.); *J. oxycedrus* (L.); *J. phoenicea* (L.); and *Tetraclinis articulata* (L.). *Adv. Pharm. Sci.,* **2016**, .

[38] Ko, H.H.; Chang, W.L.; Lu, T.M. Anti-tyrosinase and antioxidant effects of ent-kaurane diterpenes from leaves of *Broussoneta papyrifera*. *J. Nat. Prod.*, **2008**, 71, 1930-1933.

[39] Baurin, N.; Arnoult, E.; Scior, T.; Do, Q.T.; Bernard, P. Preliminary screening of some tropical plants for anti-tyrosinase activity. *J. Ethnopharmacol.,* **2002**, 82, 155-158.

[40] Kim, Y.J.; Uyama, H. Tyrosinase inhibitors from natural and synthetic sources: structure, inhibition mechanism and perspective for the future. *Cellular Mol. Life Sci*., **2005**, 1707-1723.

[41] Prior, R.L.; Hoang, H.; Gu, L.; Wu, X.; Bacchiocca, M.; Howard, L.; Hampschwoodill, M.; Huang, D.; Ou, B.; Jacob, R. Assays for hydrophilic antioxidant capacity (ORACFL). *J. Agric. Food Chem.*, **2003**, 51, 3273-3279.

[42] Cao, G.; Prior, R.L. Measurement of oxygen radical absorbance capacity in biological samples. *Methods Enzymol.*, **1998**, 22, 749-760.