

Antioxidant and Tyrosinase Inhibiting Activities of Ethylacetate, Methanolic and Aqueous Extracts of *Basella alba* Leaves

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Abstract

Basella alba is a leafy green plant commonly cultivated and consumed as vegetable in many Asian and tropical African communities. The plant has been scientifically proven to possess a number of beneficial effects including antioxidant effect. This study was aimed at investigating comparatively, the flavonoids content, the antioxidant and antityrosinase activities of ethylacetate, methanol and aqueous extract of *Basella alba* leaves and establish a possible relationship among these parameters. Two flavonoids, namely flavanol and flavonol were quantitatively measured in the extracts and antioxidant capacities assessed using TEAC, ORAC and FRAP assays. The result showed that ethylacetate extract contains much higher concentrations of flavanol and flavonol when compared to both methanol and aqueous extracts which contain negligible amounts of the two flavonoids. Values of antioxidant capacities were as follows; Aqueous (TEAC = 439.90 ± 0.07 $\mu\text{mol TE/g}$, ORAC = 1384.40 ± 90.65 $\mu\text{mol TE/g}$, FRAP = 48.17 ± 0.00 $\mu\text{mol AAE/g}$), ethylacetate (TEAC = 61.30 ± 0.07 $\mu\text{mol TE/g}$, ORAC = 483.09 ± 35.00 $\mu\text{mol TE/g}$, FRAP = 117.99 ± 0.01 $\mu\text{mol AAE/g}$) and methanol (TEAC = 44.20 ± 0.03 $\mu\text{mol TE/g}$, ORAC = 1286.25 ± 9.00 $\mu\text{mol TE/g}$, FRAP = 46.64 ± 0.00 $\mu\text{mol AAE/g}$). All three extracts had inhibitory activity against tyrosinase at effective concentrations of 193.58 ± 0.02 , 203.33 ± 0.02 and 222.83 ± 0.03 $\mu\text{g/mL}$ for aqueous, methanol and ethylacetate extracts respectively. It was concluded from the results that the aqueous extract of *Basella alba* leaves produced the best antioxidant and antityrosinase activities and the two actions may be directly related.

Keywords: Aqueous Extracts; Antioxidant; Antityrosinase; Anti-inflammatory

Abbreviations: ATP: Adenosine Triphosphate; ROS: Reactive Oxidative Species; SOD: Superoxide Dismutase; GPx: Glutathione Peroxidase; CX: Cardiac Xenobiotics

Introduction

Basella alba (Family: *Basellaceae*) is a climbing perennial herb with deep-green, heart-shaped leaves and bears fleshy dark bluish fruits [1]. It is commonly cultivated and consumed as vegetable in many Asian and tropical African communities. *Basella alba* have being used traditionally for its diverse health benefits and have been scientifically proven to possess anti-inflammatory, analgesic, antioxidant, antifungal, anticancer, androgenic and red cell membrane-stabilizing properties among others [2-4]. Of particular importance to this study is the possibility of a correlation between the antioxidant property of the plant and its reported use in skin care by Bangladeshians for the prevention of freckles. This presumption is based on the background knowledge that oxidative stress can contribute significantly to skin aging by stimulating the activities of skin-damaging enzymes like tyrosinase [5].

Tyrosinase (Polyphenol oxidase) is a copper-containing endogenous enzyme which plays a role in the initial steps in melanin biosynthesis that result in the conversion of L-tyrosine to dopaquinone via consecutive hydroxylation and oxidation reactions [6,7]. The enzyme is widely distributed in microorganisms, animals and plants. Due to inhibition of enzymatic oxidation, anti-tyrosinase agents have become increasingly significant in cosmetics, medicine as well as in the agricultural sector for the prevention of hyperpigmentation and elongation of the life span of perishable agricultural products [8-11]. Kojic acid, arbutin and hydroquinones are substances with proven tyrosinase inhibiting activities, but their use in humans have been limited due to the deleterious effects on mammalian cells [6]. This necessitated the investigation of many natural substances with antityrosinase activities including *Basella alba*, most of which are medicinal plants with high composition of phenolic compounds like flavonoids [5]. Flavonoids are said to be the most abundant phenolic compounds present in all parts of most plants and are capable of exerting potent antioxidant activities in biological systems both in vivo and in vitro [12]. Over 6000

biologically active flavonoids have been identified and of these, flavonols appears to be the most important especially in offering protection against oxidative stress and ultraviolet radiation [13].

The antioxidant activity of plant extracts can be assessed using a wide range of biochemical assays which in essence measures the quantity and potency of antioxidant phytochemicals in the extracted material. A large number of these methods of assessment including trolox equivalent antioxidant capacity (TEAC) and ferric reducing antioxidant power (FRAP) are based on colorimetric reactions [14]. The TEAC method assesses the ability of compounds to scavenge ABTS (2,2'-azinobis-(3-ethylbenzothiazoline)-6-sulfonic acid) radical i.e (ABTS^{•+}) which has a blue-green colour and a TEAC value given by comparing colour reduction induced by the compound to that induced by a vitamin E analogue, Trolox [14]. FRAP relies on the ability of antioxidants to reduce complex ferric ion-TPTZ (2,4,6-tri(2-pyridyl)-1,3,5-triazine) which creates a very intense navy blue colored solution whose absorbance gives an estimate of the amount of iron reduced and correlates with the amount of antioxidants present [15]. The oxygen radical absorption capacity (ORAC) assay measures the extent of fluorescence loss from fluorescein as the antioxidants scavenge for peroxy radicals induced by 2,2'-azobis-(2-amidino-propane) dihydrochloride (AAPH) [15]. The extent of loss of fluorescence is an indication of the amount of antioxidants present in the substance being assayed.

The use of different solvents for the extraction of bioactive phytochemicals is traditional to science and the extraction yield is largely dependent on polarity of the solvent and extraction conditions [16]. Expectedly, the nature and amount of bioactive substances and antioxidant capacity of any particular plant extract varies with the solvent of extraction. In this present study, *Basella alba* leaves were extracted using three common solvents, ethylacetate (EtOAc), methanol (MeOH) and water (aqueous) with the aim of comparing the antioxidant and antityrosinase capacities of the three types of extract. Additionally, the study is expected to establish whether or not there is a correlation between the antioxidant power and the tyrosinase inhibiting abilities of the extract types.

Materials and Methods

Plant Material

Basella alba was harvested fresh from humid locations in Osun state, western Nigeria and used for this study. A sample of this was deposited at the Botany Department of the University of Ibadan, Nigeria with the voucher number UIH-22391, following identification and authentication. The leaves were separated from the stem, washed to remove dust and other particles and then air-dried at room temperature. The dried leaf was blended into fine powder using a household electric blender (LOGIK® China).

Preparation of Plant Extracts

EtOAc and MeOH extracts were prepared by placing 100g of the dry powder of *Basella alba* leaves in a 2L conical flask and then mixed with 1L of organic solvents, ethanol and methanol respectively. These were extracted at room temperature with constant shaking for 48 hours according to the method described by Iloki-Assanga, et al. with slight modifications [17]. The resultant supernatants were filtered and evaporated to dryness using a rotary evaporator (Heidolph 2, Germany) with water bath temperature set at 40°C. The aqueous extract was prepared by extracting 100g of the leaf powder in 1L of distilled water as described by Iloki-Assanga, et al. [17]. Dry extracts were stored at -4°C and dissolved in dimethyl sulfoxide (DMSO) to a concentration of 1µg/ml prior to use.

Determination of Flavonoids

Two subclasses of flavonoids; flavanols and flavonols were analysed in the extracts. Flavanol content was determined using the method described by McMurrough and McDowell [18]. The assay was based on the reaction between 4-dimethylaminocinnamaldehyde (DMACA) and flavanols to form a characteristic light blue colour that was measured spectrophotometrically at 640nm. Catechin hydrate in methanol served as stock standard. Flavonol was determined according to the method described by Delcour and Janssens, using Quercetin as the standard for measuring flavonols at 360nm. All reagents used in both assays were prepared fresh on the day of the assay [19].

Analysis of Antioxidant Capacities

Three different methods were used to analyze the Antioxidant capacities of the extracts; TEAC was

determined according to the method described earlier by Re, et al. [20]. The ABTS radical cation scavenging ability of each extract was determined spectrophotometrically at an absorbance of 734nm at 25°C after 30minutes of incubation with ABTS mixture at room temperature. TEAC values were given as micromoles Trolox equivalents per gram (µM TE/g).

Determination of FRAP was done by a redox-linked colorimetric method as described by Benzie and Strain [21]. Briefly, extract samples were added to FRAP reagent in a 96-well plate and incubated at 37°C for 30minutes. Absorbance was recorded at 593nm and the change in absorbance measured after 30minutes of incubation was directly related to the electron donating antioxidant in the reaction mixture. L-Ascorbic acid was used as a standard and the results were expressed as micromole ascorbic acid equivalents per gram (µM AAE/g) of extract.

ORAC was determined according to the method of CaO and Prior which measures antioxidant's scavenging activities against the peroxy radical of 2,2'-azobis (2-aminopropane) dihydrochloride (AAPH) at 37°C [22]. The test samples were added to fluorescein and loss of fluorescence corresponded to the extent of decomposition of the radical which is a measure of the antioxidant power of the sample. ORAC values were expressed as micromoles Trolox equivalents (µM TE) per gram of extract.

Determination of Tyrosinase Inhibiting Activity

The tyrosinase enzyme assay was performed according to the methods described by Chompo, et al. and Vardhan, et al. [23,24]. Extract samples were constituted to a stock concentration of 1 mg/ml by dissolution with DMSO, and further dilutions were then done. Kojic acid as control drug, tyrosinase (500 Units/mL in sodium phosphate buffer) and substrate (2mM L-Tyrosine) were used for the assay as modified in earlier study by Popoola et al., 2015. Enzyme activity was determined by reading the absorbance at 490 nm and percentage tyrosinase inhibition calculated using the formula:

$$\text{Tyrosinase inhibition (\%)} = \frac{[(A - B) - (C - D)]}{(A - B)} \times 100$$

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

Data Analysis

All data from the assays of antioxidant parameters were analysed and the final results calculated using Microsoft Excel (Version 2010). Results were expressed as mean \pm standard deviation of triplicate values per sample. Graphpad Prism version 5.0 was used for the analysis of tyrosinase activities and calculation of IC_{50} . ANOVA was used for comparison of mean values and $p < 0.05$ considered to be statistically significant.

Results

Flavonoids Concentration and Antioxidant Capacities of Extracts

The flavonoids content and antioxidant capacities of each of the extract types are presented in Table 1. The

ethylacetate extract of *Basella alba* leaves contains much higher concentrations of flavanol ($13.44 \pm 0.03 \mu\text{mol/g}$) and flavonol ($175.54 \pm 0.01 \mu\text{mol/g}$) when compared to both methanolic and aqueous extracts which contain negligible amounts of the two flavonoids. Incidentally, FRAP value was also highest in the ethylacetate fraction ($117.99 \pm 0.01 \mu\text{mol AAE/g}$) as compared to methanolic ($46.64 \pm 0.00 \mu\text{mol AAE/g}$) and aqueous ($48.17 \pm 0.00 \mu\text{mol AAE/g}$) leaf extracts of the plant. However, the highest values for TEAC ($439.90 \pm 0.07 \mu\text{mol TE/g}$) and ORAC ($1384.40 \pm 90.65 \mu\text{mol TE/g}$) were recorded in the aqueous extract when compared to values for methanolic (TEAC; $44.20 \pm 0.03 \mu\text{mol TE/g}$, ORAC; $1286.25 \pm 9.00 \mu\text{mol TE/g}$) and ethylacetate (TEAC; $61.30 \pm 0.07 \mu\text{mol TE/g}$, ORAC; $483.09 \pm 35.00 \mu\text{mol TE/g}$) extracts.

Extraction solvent	Flavanol ($\mu\text{mol/g}$)	Flavonol ($\mu\text{mol/g}$)	TEAC ($\mu\text{mol TE/g}$)	FRAP ($\mu\text{mol AAE/g}$)	ORAC ($\mu\text{mol TE/g}$)
EtOAC	13.44 ± 0.03	175.54 ± 0.01	61.30 ± 0.07	117.99 ± 0.01	483.09 ± 35.00
MeOH	0.28 ± 0.00	89.70 ± 0.02	44.20 ± 0.03	46.64 ± 0.00	1286.25 ± 9.00
Aq.	0.19 ± 0.00	79.67 ± 0.01	439.90 ± 0.07	48.17 ± 0.00	1384.40 ± 90.65

Table 1: Flavonoids content and antioxidant capacities of three different extracts of *Basella alba* leaves.

$\mu\text{mol/g}$ = Micromole per gram; $\mu\text{mol TE/g}$ = Micromole trolox equivalent per gram; $\mu\text{mol AAE/g}$ = Micromole amino acid equivalent per gram; EtOAC = Ethylacetate; MeOH = Methanol; Aq. =Aqueous

Tyrosinase Inhibiting Activity of Extracts

The effects of *Basella alba* extracts on mushroom tyrosinase activity using L-Tyrosine as substrate in an in-vitro system is reported in Table 2. The results indicated that all extracts have a direct inhibitory activity against mushroom tyrosinase at effective concentrations of 193.58 ± 0.02 ; 203.33 ± 0.02 & $222.83 \pm 0.03 \mu\text{g/mL}$ for aqueous, methanol and ethylacetate extracts respectively. These antityrosinase activities of the three extracts were significantly ($p < 0.01$) weaker when compared to that of standard substance, kojic acid at IC_{50} of $7.33 \pm 1.78 \mu\text{g/mL}$.

Fraction	Antityrosinase $IC_{50} \pm SD$
EtOAC	$222.83 \pm 0.03^*$
MeOH	$203.33 \pm 0.02^*$
Aq.	$193.58 \pm 0.02^*$
Kojic acid	7.33 ± 1.78

Table 2: Tyrosinase inhibiting activity of extracts.

* $P < 0.01$ vs. Kojic acid; EtOAC = Ethylacetate; MeOH = Methanol; Aq. =Aqueous

Discussion

The findings in this study suggest that the aqueous extract of *Basella alba* leaves exhibit stronger antioxidant properties when compared to methanolic and ethylacetate fractions, despite containing less flavonol and flavanol than the ethylacetate fraction. A significant correlation is said to exist between the total phenolic content and antioxidant activities of *Basella alba*, which suggests that there may be some phenolic compounds other than flavonol and flavanol that are extractable in aqueous, but not in ethylacetate or methanolic fractions [25]. This may be responsible for the much higher TEAC and ORAC values for the aqueous fraction and further studies to investigate this should involve isolation of active compounds in each extract type for more specific assays. On the other hand, the FRAP reading was observed to be much lower in aqueous compared the ethylacetate fractions, which further confirms the inconsistency that is known to characterize the measurement of total antioxidant capacities and is partly because each assay measures different components of total antioxidant capacity [26-28]. Furthermore, TEAC

and ORAC readings are believed to be more representative of the antioxidant capacities of the various extract types since these assays directly measure the ability of the extracts to inhibit oxidation, while FRAP is an indirect assay that measures the ability of the extracts to reduce iron complexes [29].

The results further show that all of the extracts from the different solvents displayed similar anti-tyrosinase activity within the range of 193.58-222.83 µg/mL. Therefore, the plant *Basella alba* can be a potential source of further investigation for new anti-tyrosinase agents. The expected active constituent from the quantitative determination of the chemical composition (Table 1) is primarily made up of flavanols in ethylacetate while flavonols are abundant in all the three types of extract. This indicates that the compounds responsible for the observed biological activities are highly extractable in both moderately polar (ethylacetate) and polar (methanol and water) solvents. Although the outcome of this investigation gave moderate antityrosinase activities, this study suggested that *Basella alba* could play an important role in the regulation of tyrosinase activity and could possibly act as a natural inhibitor of browning reaction and formation of freckles in the skin.

Conclusion

The findings from this study led to the conclusion that aqueous extraction of *Basella alba* leaves produced the best yield with regards to antioxidant as well as tyrosinase inhibiting effects of the plant. This also suggests a direct relationship between antioxidant and tyrosinase inhibiting activities of *Basella alba*. Additionally, a significant part of the antioxidant activities of *Basella alba* can be attributed to components other than flavonol and flavanol and the plant can be a healthy source of natural antityrosinase compounds.

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Conflict of Interest

The authors declare that there is no conflict of interest with regard to publishing any aspect of this study

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