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**Citation:** Omotoyinbo OV, Awojulu EO and Sanni DM (2020). Phytochemical screening, antioxidant and tyrosinase inhibitory studies of methanol leaf extracts of two tomato varieties. Highlights in BioScience Volume 3. Article ID 20216. doi:10.36462/H.BioSci.20216

**Received:** June 8, 2020

**Accepted:** July 18, 2020

**Published:** August 18, 2020

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**Data Availability Statement:** All relevant data are within the paper and supplementary materials

**Funding:** The authors have no support or funding to report.

**Competing interests:** The authors declare that they have no competing interests.

## Phytochemical screening, antioxidant and tyrosinase inhibitory studies of methanol leaf extracts of two tomato varieties

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### Abstract

This study evaluated the phytochemical compositions, antioxidant properties, chlorophyll content and anti-tyrosinase activity of methanol leaf extracts of two tomato varieties, *Lycopersicon esculentum* (var. Eva F1) and *Lycopersicon esculentum* Mill (var. Hausa). The dried pulverized of the plant's leaves were extracted by decoction and mild agitation. Phytochemicals such as flavonoids, tannin, glycoside, saponin, terpenoid and anthraquinone were present in the extracts of both varieties examined, while alkaloid and phlobatannin were confirmed absent in the extracts. The presence of steroid was observed in var. Eva F1 but absent in var. Hausa. Total phenolic content (TPC) and total flavonoid content (TFC) of var. Eva F1 were  $505.9 \pm 2.61$  mg GAE/ge, and  $35.5 \pm 1.64$  mg RE/ge, while var. Hausa recorded a TPC and TFC value of  $344.3 \pm 2.01$  and  $7.8 \pm 0.15$  mg RE/ge respectively. The chlorophyll content of the extracts were  $6.6 \pm 0.02$  mg/ge (chlorophyll a),  $5.7 \pm 0.05$  mg/ge (chlorophyll b) and  $12.6 \pm 0.14$  mg/ge (total chlorophyll content) for Eva F1 variety, while the chlorophyll contents for var. Hausa were  $7.6 \pm 0.32$  mg.ge (chlorophyll a),  $5.6 \pm 0.06$  mg/ge (chlorophyll b) and  $13.7 \pm 0.14$  mg/ge. Eva F1 and Hausa showed percentage inhibition of 76.3 % and 61.2 % at 400  $\mu$ g/mL. The IC<sub>50</sub> value of var. Eva F1 and var. Hausa were 110  $\mu$ g/mL and 160  $\mu$ g/mL. The inhibition constant ( $K_I$ ) of var. Eva F1 and var. Hausa, were 0.006 and 0.016  $\mu$ g/mL, respectively, and both extracts showed partial competitive inhibition. Hence, this confirms the phytoprotective and tyrosinase inhibitory properties of tomato plant leaves.

**Keywords:** Tyrosinase, Inhibition, kinetics, phytochemicals, antioxidants, *Lycopersicon esculentum*.

### Introduction

Tyrosinase (EC 1.14.18.1) is a metalloenzyme that catalyzes the rate-limiting reactions that are important to melanogenesis. It is classified as an oxidase and involves two distinct reactions, the hydroxylation of a monophenol, and conversion of an o-diphenol to the corresponding o-quinone. The oxidative polymerization of dopaquinone derivatives gives rise to melanin [1]. Melanin is a biopolymer synthesized by melanocyte, within specialized organelles called melanosomes. It is important to prevent UV-induced skin damage by absorbing UV sunlight and removing reactive oxygen species [2]. Although tyrosinase is a relatively important enzyme, abnormal biosynthesis or distribution of melanin may result in several dermatological disorders, such as age-spots, lentigines,

melasma, and inflammatory hyperpigmentation. Due to the rate-limiting step of tyrosinase in the melanin biosynthesis pathway, inhibition of this enzyme have become increasingly important and productive in cosmetics and pharmaceutical industries, where it is used as potent skin-lightening agents for treating skin pigmentation disorders [3,4], and most of the inhibitors characterized from literature are either synthetic compounds or bioactives' obtained from medicinal plants such as polyphenols, flavonoids, aldehydes and their derivatives [5].

*Lycopersicon esculentum* (tomato plant) comes from the *Solanaceae* family and is a good source of phenolic compounds, pigments, antioxidants, and other nutrients when consumed [6]. The extract of tomato leaves has been reported to exhibits antimicrobial and antioxidant properties at a ratio higher than in other parts of the plant [7]. Tomato leaf extract has also been used in much traditional preparation of skin-toning mixtures, while some evidence indicates that it may be a valuable bioactive source, and would seem to be applicable in both medical fields and food industry [7,8].

In this study, the methanol leaf extracts from two different cultivars of tomato (*Lycopersicon esculentum* (var. Eva F1) and *Lycopersicon esculentum* Mill. (var. Hausa) were screened for the presence of Phytochemicals and some antioxidants, while the antityrosinase potential of the extracts was evaluated on mushroom tyrosinase.

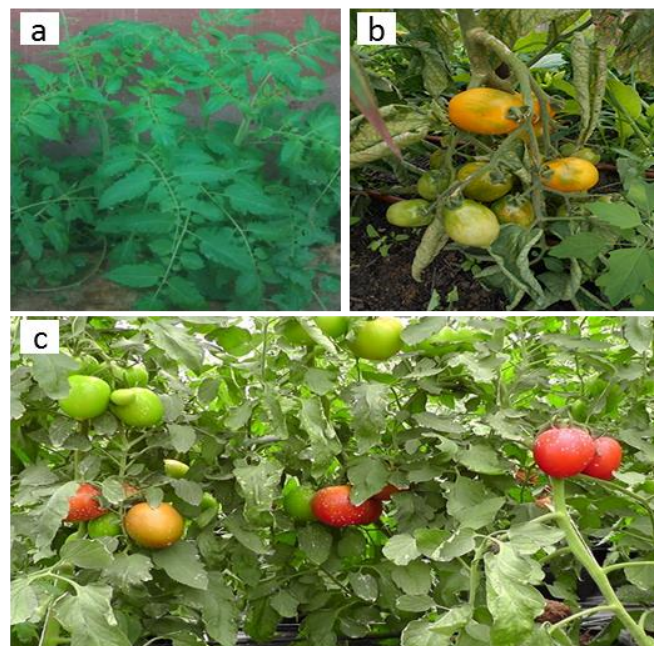
## Materials and Methods

**Reagents:** Mushroom tyrosinase (1.14.18.1), kojic acid, sodium carbonate ( $\text{Na}_2\text{CO}_3$ ), aluminium trichloride ( $\text{AlCl}_3$ ), sodium nitrite ( $\text{NaNO}_2$ ), were obtained from Merck (Sternheim USA). All other chemicals and reagents used were of analytical grade.

**Plant material:** Leaves of *Lycopersicon esculentum* (var. Eva F1) (**Figure 1a, b**) and *Lycopersicon esculentum* Mill. (var. Hausa) (**Figure 1c**) were collected at the Federal University of Technology, Akure greenhouse farm and Muiyiwa Oni Avenue, Akure, Ondo state, respectively. Identification and authentication of samples were carried out at the department of Crop, Soil and Pest Management, Federal University of Technology, Akure.

**Extraction:** The leaves were subjected to drying under a shade at room temperature for 21 days. The dried materials were then pulverized using Kanchan International Blender (China). 90 g of each powdered material was then extracted by decoction at 50 °C for a period of 5 hours using methanol as an extraction solvent. Afterwards, the samples were filtered using a muslin cloth, and subsequently with Whatman filter paper. The residue obtained after filtration was extracted to exhaustion by methanol (absolute). The extraction was repeated three times and filtrates obtained in each stage were combined and concentrated using a rotary

evaporator extractor. Finally, the extracts were air-dried at ambient temperature.



**Figure 1:** Photograph of *L. esculentum* Mill. (var. Hausa) (a) Leaves (b) Fruits and; (c) Photograph of *L. esculentum* (var. Eva F1) leaves and fruits.

**Phytochemical screening:** Screening of the phytochemical constituents in the leaf extract was carried out to identify the constituents using standard phytochemical methods [9,10].

**Test for saponin:** This test was carried out by dissolving 10 mg of extract in water inside a test-tube and the mixture was agitated. Frothing, which persisted on warming was taken as preliminary evidence for the presence of saponin in the leaf extract.

**Test for tannin:** The extract (10 mg) was dissolved in 5 ml of distilled water and then filtered. Thereafter, 1 ml of ferric chloride was added to the filtrate. A blue-black precipitate indicated the presence of tannin.

**Test of phlobatannin:** The solution of the extract in methanol (0.1 mg/ml) was boiled with 1% of hydrochloric acid. Deposition of red precipitate indicated the presence of Phlobatanin in the extract.

**Test for anthraquinone:** About 5 ml of 0.1 mg/ml aqueous solution of the extract was dissolved in 10 ml of benzene solution, the mixture was filtered and 10% ammonia solution was added to the filtrate and then shaken. The formation of an amber coloured solution in the ammonia lower phase confirmed the presence of anthraquinone in the extract.

**Test for glycoside:** The extracts (10 mg) were dissolved in 2 ml of pyridine with 5 drops of 2% sodium nitroprusside and 20% sodium hydroxide added. A yellow colouration at the interface of the mixture confirmed the presence of glycoside.

**Test for steroids:** Exactly 20 ml of ethanoic anhydride were added to 0.5 g of the extract and then filtered before adding 2 ml of concentrated H<sub>2</sub>SO<sub>4</sub> to the filtrate. The presence of steroids was confirmed with a colour change of the solution from violet to blue or green.

**Test for terpenoid:** Exactly 5 ml of 0.1 mg/ml methanol solution of the extracts was mixed with 2 ml chloroform; afterwards another 3 ml of concentrated H<sub>2</sub>SO<sub>4</sub> was carefully added to the solution. The formation of a russet colour at the interface of the mixture confirmed the presence of terpenoids.

**Test for alkaloid:** Exactly 5ml of 1 % aqueous HCl was added to 0.5 g of the extract and placed in a steam water bath while stirring for 2 minutes. The solution was then filtered and 1 ml of the filtrate was treated with a few drops of dragendorff reagent, blue-black turbidity was taken as preliminary evidence for the presence of alkaloid.

### Antioxidant properties

**Total phenolic content:** This was determined according to the method of Harborne and Williams [11], with slight modification. Exactly 0.1 ml of 0.1 mg/ml of the extracts was oxidized with 0.5 ml of 10 % folin-ciocalteu's phenol reagent (v/v) and neutralized by 2.5 ml of 7.5% sodium carbonate. The reaction mixture for each cultivar extract was incubated for 1 hour at room temperature and the absorbance was measured at 700 nm using the same mixture without the sample as blank. The total phenolic content was expressed as mg Gallic Acid Equivalent of extract (mg GAE/g of extract).

**Total flavonoid content:** This was determined according to the method of Bao *et al.* [12] with a slight modification. In a test tube, 0.3 ml of 5 % NaNO<sub>2</sub> was added to 0.1 ml of 0.1 mg/ml extract and the mixture was then incubated at room temperature for 6 minutes. After incubation, 0.6 ml of AlCl<sub>3</sub> (10%) was added and the mixture was further incubated for 5 minutes, then 2 ml of NaOH (1 M) was finally added to the reaction mixture and the absorbance was read at 510 nm using the same mixture without the extract as blank. The result was expressed as mg Rutin equivalent/g of extract (mg RE/ge).

**Chlorophyll content:** The chlorophyll content of the extracts was determined according to the method of Lichtenthaler and Babani [13]. Exactly 0.2 g sample was extracted exhaustively with 80% acetone until the green pigments disappeared. The final mixture was then filtered, and the supernatant combined. The absorbance of the supernatant was measured at 663, 652, 645 and 470 nm using a UV-VIS spectrophotometer. The chlorophyll contents were expressed as mg/g of extract (ge) according to the following equations:

$$\text{Chlorophyll a} = (12.7 \times A_{663} - 2.7 \times A_{645}).$$

$$\text{Chlorophyll b} = (22.9 \times A_{645} - 2.7 \times A_{663}).$$

$$\text{Total chlorophyll} = (27.8 \times A_{652}).$$

**Assay of tyrosinase inhibitory activity:** Tyrosinase inhibitory activity was measured according to the procedure described by Liu *et al.* [14]. Exactly 50 U/ml of tyrosinase was prepared in 50mM sodium phosphate buffer (pH 6.8), 0.35 ml of the leaf extract of various concentrations (3.1, 6.2, 12.5, 25, 50, 100, 200 and 400 µg/ml) was added to 0.15ml of tyrosinase respectively, and the mixture was incubated for 10 minutes at room temperature. Then, 0.55 ml of 12 Mm L-DOPA was added to the existing mixture and further incubated at room temperature for 20 minutes. The reaction mixture without the leaves extract served as control and the change in absorbance was measured at 492 nm against the blank. Kojic acid was used as a standard and all tests were performed in triplicate, and the percentage inhibition of tyrosinase activity was calculated using the formula:

$$\text{Tyrosinase inhibition} = \frac{Abs_{Control} - Abs_{Sample}}{Abs_{Control}} \times 100$$

### Results

The result of phytochemical screening of the methanol leaf extracts of *Lycopersicon esculentum* (var. Eva F1) and *Lycopersicon esculentum* Mill (var. Hausa) (Table 1) shows that the methanol leaf extracts contain saponin, flavonoids, anthraquinone, tannin, steroid, glycoside, and terpenoids, while alkaloid and phlobatanin were confirmed absent, however steroid content was observed in var. Eva F1 but absent in var. Hausa.

**Table 1:** Phytochemical constituents of methanol extract of var. Eva F1 and var. Hausa.

Tomato variety	Phytochemical constituents	
	Var. Eva F1	Var. Hausa
Flavonoid	+	+
Tannin	+	+
Glycoside	+	-
Alkaloid	-	-
Steroid	+	-
Saponin	+	+
Phlobatanin	-	-
Terpenoid	+	+
Anthraquinone	+	+

\*Note: the symbol – indicates the absence of the phytochemical under investigation (hence negative), while + indicates the presence of the phytochemical under investigation (hence positive).

Var. Eva F1 means Eva F1 variety, while Var. Hausa means Hausa variety.

Total phenolics and flavonoids content of the methanol extract of the respective cultivars (Table 2)

reveals that the total phenolic content of the extracts was expressed as gallic acid equivalent (GAE) were  $505.96 \pm 2.57$  and  $344.26 \pm 2.02$  mg GAE/ge, while the total flavonoid content expressed as rutin equivalent (RE) was  $35.55 \pm 1.66$  and  $7.78 \pm 0.15$  for Eva F1 variety and Hausa variety, respectively.

**Table 2:** Total phenol and total flavonoids content of extract of tomato leaf; var. Eva F1 and var. Hausa.

Tomato variety	TPC (mg GAE/ge)	TFC (mg RE/ge)
Var. Eva F1	$505.96 \pm 2.57$	$35.55 \pm 1.66$
Var. Hausa	$344.26 \pm 2.02$	$7.78 \pm 0.15$

Values are expressed as mean  $\pm$  standard deviation (n=3). Note: GAE- Gallic acid equivalent; RE- Rutin equivalent.

The two tomato varieties varied in the chlorophyll contents (Table 3), such that for var. Eva F1 had values of  $6.63 \pm 0.02$ ,  $5.76 \pm 0.05$  and  $12.57 \pm 0.14$  mg/ge for chlorophyll a, chlorophyll b and total chlorophyll content, respectively, var. Hausa had  $7.63 \pm 0.32$ ,  $5.63 \pm 0.06$  and  $13.67 \pm 0.14$  mg/ge. The analysis revealed that all samples had a non-zero standard deviation from the mean values.

**Table 3:** Chlorophyll content of extract of tomato leaf; var. Eva F1 and var. Hausa.

Tomato variety	Chlorophyll a (mg/ge)	Chlorophyll b (mg/ge)	Total chlorophyll (mg/ge)
Var. Eva F1	$6.63 \pm 0.02$	$5.76 \pm 0.05$	$12.57 \pm 0.10$
Var. Hausa	$7.63 \pm 0.32$	$5.63 \pm 0.06$	$13.67 \pm 0.14$

Values are expressed as mean  $\pm$  standard deviation (n=3). Note: mg/ge refers to mg/g of extract.

Inhibitory concentration at 50% (IC<sub>50</sub>) values of the extracts compared with kojic acid (the standard inhibitor) is represented in (Table 4).

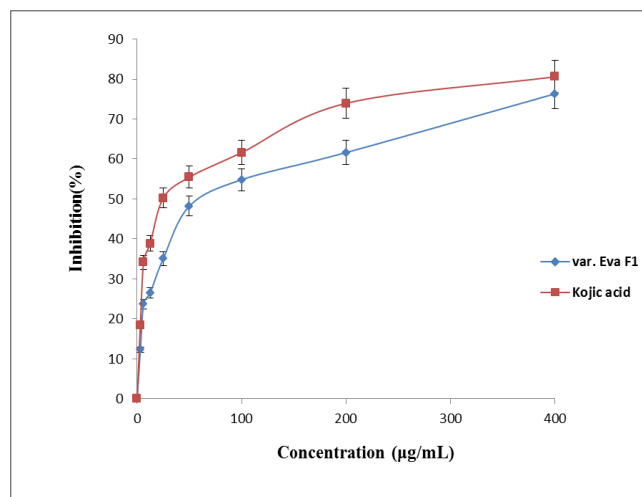
**Table 4:** Michaelis-Menten parameter (K<sub>i</sub>), and IC<sub>50</sub> values of inhibitors.

Tomato variety	IC <sub>50</sub> (µg/ml)	K <sub>i</sub> Value (µg/ml)	Type of inhibition
<i>L. esculentum</i> (var. Eva F1)	65	0.006	Partial competitive
<i>L. Esculentum</i> (var. Hausa)	198	0.016	Partial competitive

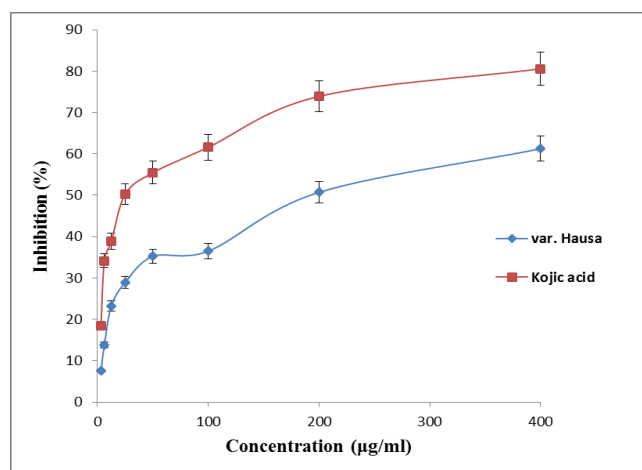
IC<sub>50</sub> value of kojic acid (positive control) is 25 µg/ml.

The extracts had an inhibitory effect on tyrosinase in a dose-dependent manner (Figure 2 and 3). The percentage inhibition of tyrosinase at the maximum test concentration (400 µg/ml) for extracts were 76.30% and 61.24% for Eva F1 and Hausa varieties, respectively while kojic acid had a higher inhibitory power of 80.57%. Further kinetic studies revealed (Figure 4 and 5) the inhibition type of both extracts as competitive inhibition and the inhibition

constant (K<sub>i</sub>) for Eva F1 and Hausa varieties were 0.006 and 0.016 µg/mL, respectively.



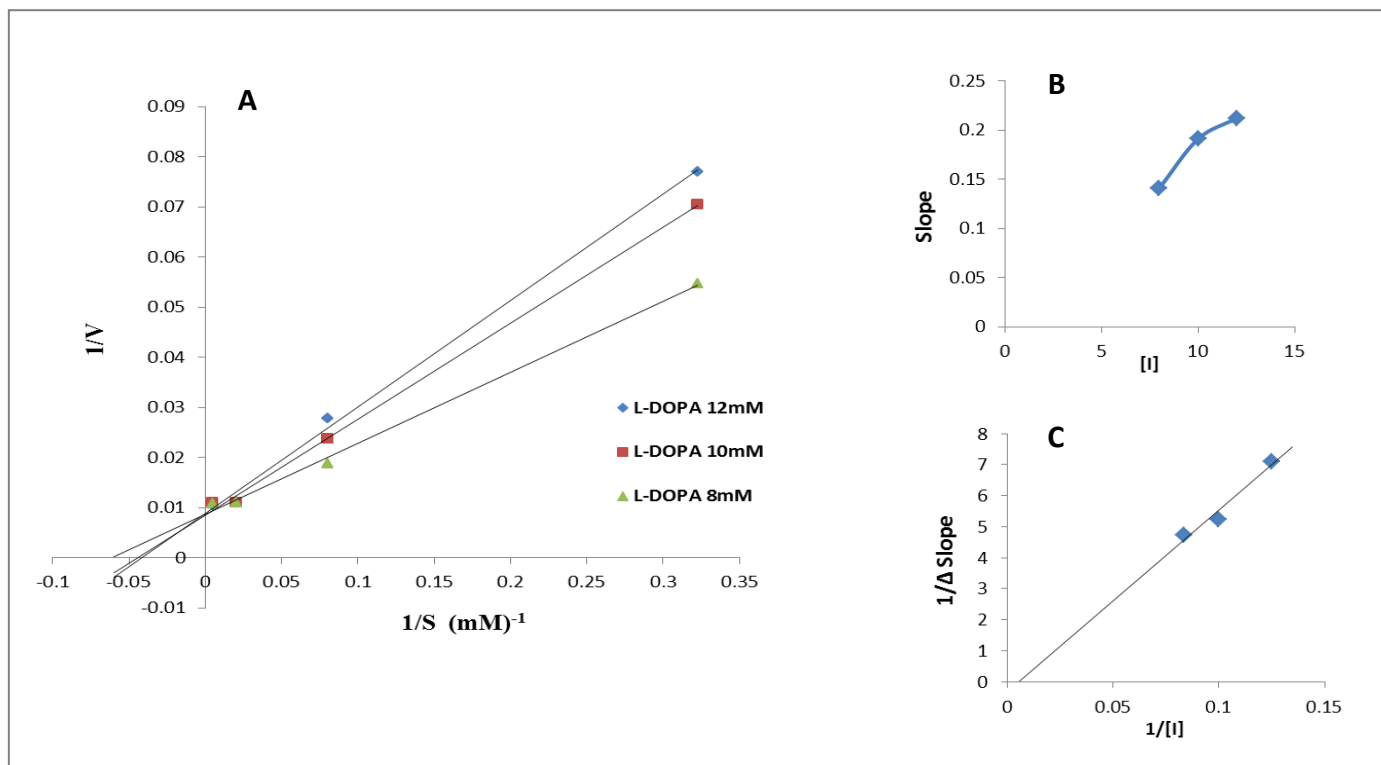
**Figure 2:** The percentage inhibition curve of *L. esculentum* (var. Eva F1) and kojic acid.



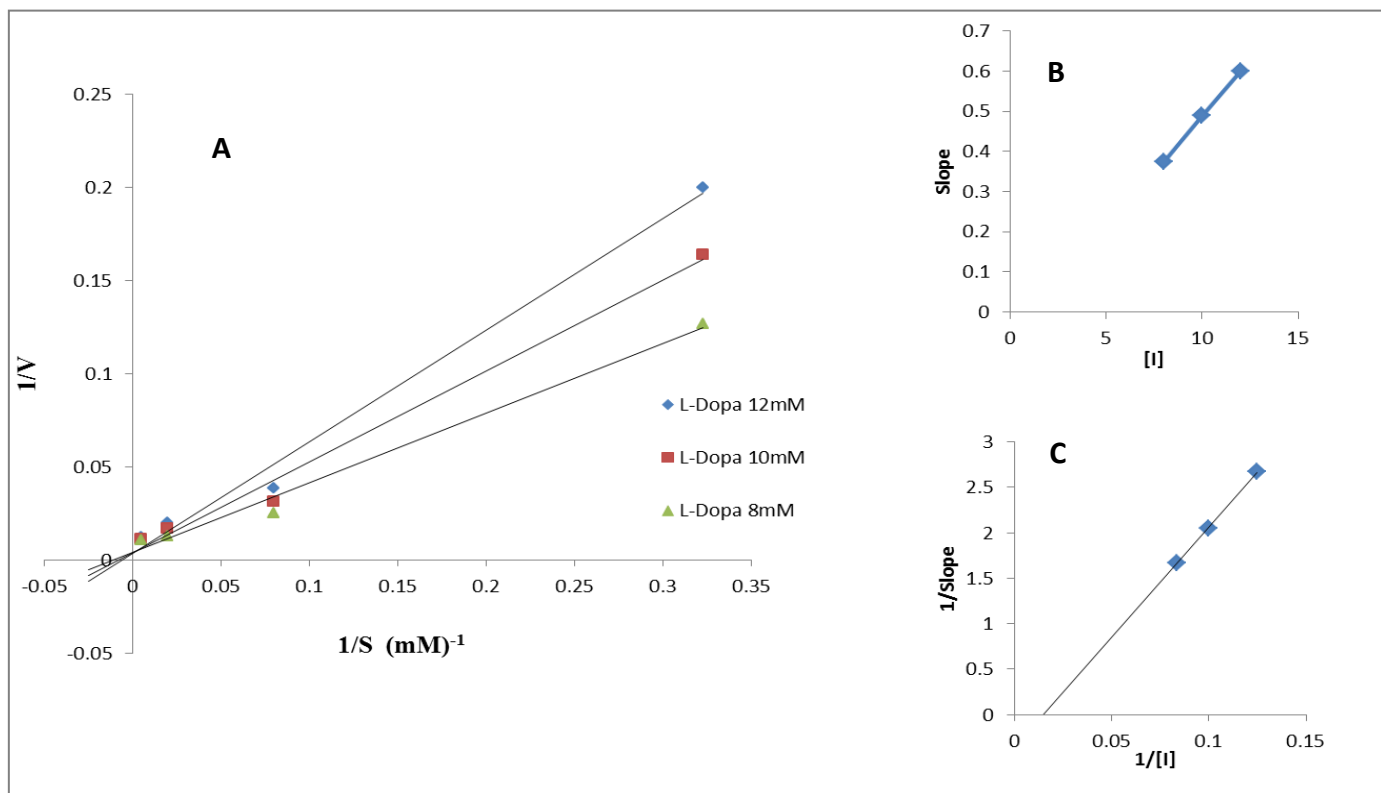
**Figure 3:** The percentage inhibition curve of *L. esculentum* Mill. (var. Hausa) and kojic acid.

### Discussion

In this study, the phytochemical analysis of the methanol leaf extracts of var. Eva F1 and var. Hausa revealed a single variation in plant secondary metabolites examined. It was observed that while the analysis of both varieties of methanol leaves extract confirmed the presence of flavonoids, tannin, glycoside, saponin, terpenoid and anthraquinone, and the absence of alkaloid and phlobatanin, var. Eva F1 contains steroid while var. Hausa extract did not contain the steroids. Manosroi *et al.* [15] reported that phytochemicals found in plant extract depend on the nature of the plant, the solvent system, temperature and time used in the extraction process. The variation in steroid composition could be a result of the mode of cultivation of individual plants, however, the result obtained showed greater similarity in the phytochemical constituent of the extracts of both varieties, and could be an indication of some sort of genetic resemblance.



**Figure 4:** Kinetic properties of *L. esculentum* (var. Eva F1) (A) Lineweaver-Burk plot for the inhibition of var. Eva F1, (B) Re-plot of the slope of the Lineweaver-burk plot versus inhibitor concentration, (C)  $1/\Delta\text{Slope}$  versus  $1/[I]$ .



**Figure 5:** Kinetic properties of *L. esculentum* Mill (var. Hausa) (A) Lineweaver-Burk plot for the inhibition of var. Hausa (B) Replot of the slope of the Lineweaver-burk plot versus inhibitor concentration (C)  $1/\Delta\text{Slope}$  versus  $1/[I]$ .

The observed high TPC and TFC indicate that var. Eva F1 has the best antioxidant properties compared to var. Hausa. Silva-Beltran *et al.* [7] reported that high phenolic content in tomato plants could be as a result of their varied phenolic content and its derivatives, which are essential for the plant's growth and reproduction. Phenolic content present in tomato plants majorly consist of hydroxycinnamic acid and flavonoids and are located mainly in their leaves. The variation observed in TPC and TFC in the two tomato varieties has been affirmed by Silva-Beltran *et al.* [7], who reported that the concentration of flavonoids varied among the plant's cultivars.

Results obtained in this study showed that var. Hausa had the highest content of chlorophyll a, while var. Eva F1 had the highest content of chlorophyll b. However, var. Hausa had the highest total chlorophyll content (TCC). The content of chlorophylls observed in this study was found to be higher than those observed by Silva-Beltran *et al.* [7] for Pitenza and Floradade variety of tomato plants. Lumpkin [16] also reported that the chlorophyll content of tomato plants is strongly influenced by the incidence of light and its concentration increases with exposure to light. Tomato plants synthesize metabolites and pigments such as chlorophyll and carotenoids, that beneficially contributes to consumers nutrition and health [7], while the work of Choi *et al* [8] shows that these Phytochemicals help prevent photooxidation are strongly influenced by the maturity of the plant. In the same light, chlorophyll shows potent antioxidant activity, and it has been suggested that chlorophyll reduces free radicals, acting as a hydrogen ion donor to break the chain reaction resulting to cellular oxidation [17]. The methanol leaf extracts of var. Eva F1 and Hausa inhibited mushroom tyrosinase in a dose-dependent manner. Eva F1 variety revealed higher tyrosinase inhibitory activity of 76.3% at the highest concentration considered (400µg/mL) compared with 61.2% in Hausa variety. Zaveri and Patel [18] reported a percentage inhibition of 58% in tomato fruit (at the highest inhibitor concentration considered), which is lower than the value obtained in the present study. This is quite reasonable, as secondary metabolites are primarily, concentrated in leaf section of plants to serve a defensive role. The IC<sub>50</sub> values obtained for both extracts in this study were higher compared to those obtained by Zaveri and Patel [18] for curry tree and tomato fruit, which could be as a result of the difference in the enzyme units utilized. However, kojic acid (standard inhibitor) recorded an IC<sub>50</sub> of 25 µg/mL. This result points out Eva F1 variety as the most potent tyrosinase inhibitor among the two cultivars were evaluated since low IC<sub>50</sub> values imply that a smaller amount of the extract is needed to reduce the activity of mushroom tyrosinase by half. Kinetic data analysis using Lineweaver-Burk plot, which is also called the double reciprocal plot

reveals that the inhibitory mechanism of both methanol leaf extracts on mushroom tyrosinase was competitive, viz partial competitive inhibition. This correlates with Yang and Quyang [19] conclusion for *Olea* leaf extract analysed by Lineweaver-Burk plot. Furthermore, *L. esculentum* var. Eva F1 had the lowest inhibition constant (K<sub>i</sub>) value of 0.006 µg/mL while var. Hausa had K<sub>i</sub> value of 0.016 µg/mL. This implies that methanol leaf extract of Eva F1 variety has a higher affinity for the active site of mushroom tyrosinase than that of Hausa variety. Also, the K<sub>i</sub> value obtained in this study is considerably lower than that obtained by Yang and Quyang [19] for *Olea europaea* leaf extract, which had a K<sub>i</sub> value of 0.226 mg/mL.

## Conclusion

Although the two varieties of tomato leaf methanol extract showed the presence of most phytochemical evaluated, both varieties tests were negative for alkaloid and phlobatannin content, while var. Hausa also lacked any steroid content. The highest TPC, and TFC were observed for var. Eva F1, while var. Hausa had the highest total chlorophyll content. Eva F1 variety had the best tyrosinase inhibitory activity at the peak concentration considered. This study therefore further establishes the skin whitening and antioxidant properties of tomato leaves as traditionally used in some cultures.

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