

Original Research Article

Antityrosinase activities of *Thespesia populnea* bark and *Phyllanthus emblica* fruit

ABSTRACT

Aims: Currently there is enormous demand for skin-lightening agents, creating a quest for identification of new substances. Tyrosinase has become a main drug target for reducing skin pigmentation. The objective of this study was to detect tyrosinase inhibitory effects of eight medicinal plants that are used to treat skin conditions.

Study design: Experimental.

Place and Duration of Study: Department of Biochemistry, Faculty of Medicine, University of Peradeniya, Sri Lanka, from June 2016 to December 2016.

Methodology: Tyrosinase inhibitory effects of methanol extracts of eight plant species that are used to treat various skin conditions were measured using 3,4-Dihydroxy-L-phenylalanine (L-DOPA) as the substrate. Kojic acid was used as the positive control. IC₅₀ values of extracts with high activity were measured using a range of concentrations. Statistical significance of the differences between the inhibitory effects was calculated using ANOVA.

Results: *Thespesia populnea* bark showed the highest inhibition among the tested, with an IC₅₀ of 190 µg/mL. *Phyllanthus emblica* fruit also showed higher inhibitory effect with IC₅₀ value of 251 µg/mL. IC₅₀ value of kojic acid (3.4 µg/mL) against tyrosinase was significantly lower than that of *P. emblica* and *T. populnea*. Even though *Santalum album* stem and *Vernonia cinerea* plant showed 36.3 and 32.8% inhibitions respectively at 0.5 mg/mL, these effects did not show a dose-dependent increase. Extracts of *Coscinium fenestratum*, *Persea Americana*, *Vigna radiate* and *Vigna unguiculata* showed only lower inhibitory effects on tyrosinase.

Conclusion: Current study reveals significantly high antityrosinase activity in methanol extracts of *P. emblica* fruit and *T. populnea* bark.

Keywords: Tyrosinase inhibitors, *Thespesia populnea*, *Phyllanthus emblica*

1. INTRODUCTION

Colour of the human skin is mostly determined by the degree of synthesis of dark macromolecular pigment melanin [1]. Melanin protects the skin from variety of detrimental agents such as free radicals and UV radiation [2]. However, increased amount of melanin synthesis occurs as a result of genetic and environmental factors causing pigmentary skin disorders such as melasma, freckles and age spots [3]. Abnormal melanin deposition in the skin has great impact on the demand for cosmetics and has prompted research and development of agents that could interfere with melanin synthesis [1].

Melanogenesis occurs in melanosomes, a specialized organelle found in epidermal melanocytes through a series of enzyme catalysed reactions [4]. Melanogenesis is commenced with the enzyme tyrosinase, which catalyse the rate-limiting step and the

remaining reactions will follow spontaneously [4]. Tyrosinase (polyphenol oxidase) is a copper containing monooxygenase and is located in the membranes of melanosomes. It catalyses two reactions with various phenolic substrates. Two reactions are the hydroxylation of tyrosine to 3,4-dihydroxyphenylalanine (L-DOPA) and oxidation of L-DOPA to DOPA-quinone [5]. Highly reactive quinones polymerize spontaneously to form high-molecular weight pigments [5].

Currently, there is a huge demand across Asia for cosmetics/ cosmeceuticals used in skin-lightening, creating a quest for identification of new substances. As the regulatory enzyme of melanin synthesis, tyrosinase has become a widespread drug target used to minimise skin pigmentation [1] in therapeutics and cosmetics [6]. Well known tyrosinase inhibitors such as arbutin and kojic acid have shown only low inhibitory activity against pigmentation in melanocytes in a clinical trial [7]. Hydroquinone used as a whitening compound in some cosmetics is considered cytotoxic and mutagenic to mammalian cells [8]. Therefore, it is necessary to search for new tyrosinase inhibitors from natural resources that do not have side effects. [9]. More than 200 medicinal plants that are used to enhance the health and beauty of skin are described in Ayurveda as *varṇya* drugs [10].

The objective of this study was to detect tyrosinase inhibitory effects of eight medicinal plants that are used to treat skin conditions.

2. MATERIALS AND METHODS

2.1 Plant material

Parts from eight plants that are used to treat various skin conditions were selected (Table 1). Plants were authenticated by the Deputy Director, National Herbarium, Royal Botanical Garden, Peradeniya. Plant materials were cleaned, dried under shade for approximately one week and powdered using a grinder. Dry powder (10 g) of each plant part was extracted with methanol three times (100 ml) using a sonicator. Filtrate was dried by evaporating methanol below 50°C using a rotary evaporator. Extracts were stored at room temperature until further investigation. Extracts were dissolved in phosphate buffer (pH 6.5) before the assay to make 10 mg/mL extracts. DMSO was used for extracts with poor water solubility to yield 100 mg/mL solutions and then diluted to 10 mg/mL with phosphate buffer.

2.2 Measurement of tyrosinase activity

Tyrosinase activity of the test samples were measured with the method of Vaibhav and Lakshaman [11]. Fifty microliter of 500 U/mL tyrosinase enzyme from mushroom was pre-incubated with phosphate buffer (pH 6.5) and 50 µl of 10 mg/mL plant extracts for 10 min at 25°C. Respective control was prepared in the absence of extracts and the blanks were prepared by adding 50 µl of phosphate buffer instead of tyrosinase. Then 200 µl of 1.78 mM 3,4-Dihydroxy-L-phenylalanine (L-DOPA) was added and mixed. Total volume of the reaction mixture was 1 mL. Reaction mixtures were incubated for 10 min at 25°C and exactly after 10 min incubation, absorbance was read at 475 nm. Kojic acid was used as the positive control. Each measurement was taken in duplicate and on three separate occasions.

Inhibitory effects on tyrosinase activity were calculated using following formula. IC₅₀ values of extracts with high activity were measured using a range of extract concentrations.

Percentage inhibitory effect =
$$\frac{(\text{Control} - \text{Control blank}) - (\text{Test} - \text{Test blank})}{\text{Control} - \text{Control blank}} \times 100$$

(Control - Control blank)

Table 1: Plants and parts used in the study

Plant	Family	Common name	Part
<i>Coscinium fenestratum</i>	<i>Menispermaceae</i>	<i>Vanival</i>	Stem
<i>Persea americana</i>	<i>Lauraceae</i>	<i>Avacado</i>	Fruit pulp
<i>Phyllanthus emblica</i>	<i>Phyllanthaceae</i>	<i>Nelli</i>	Fruit
<i>Santalum album</i>	<i>Santalaceae</i>	<i>Sudu Handun</i>	Bark
<i>Thespesia populnea</i>	<i>Malvaceae</i>	<i>Gansooriya</i>	Bark
<i>Vernonia cinerea</i>	<i>Compositae</i>	<i>Monarakudumbiya</i>	Whole plant
<i>Vigna radiate</i>	<i>Fabaceae</i>	Mung bean	Seeds
<i>Vigna unguiculata</i>	<i>Fabaceae</i>	Cowpea	Seeds

2.3 Statistical analysis

Means and standard deviations were calculated using measurements taken on three separate occasions. Statistical significance of the differences was calculated using ANOVA. $P < 0.01$ was considered as statistically significant.

3. RESULTS AND DISCUSSION

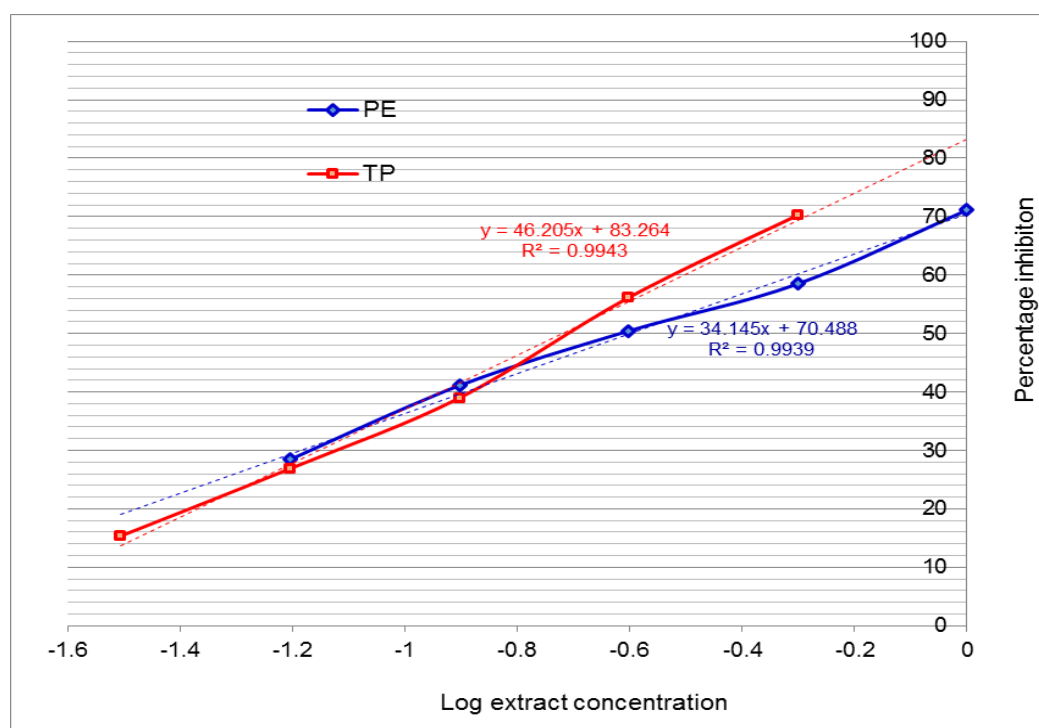
3.1 RESULTS

Percentage inhibitions of the eight methanol extracts on tyrosinase and IC_{50} values of two extracts and positive control are shown in table 2. *T. populnea* showed the highest inhibition among the tested extracts, with an IC_{50} of 190 $\mu\text{g/mL}$ (Table 2 and Fig. 1). *P. emblica* also showed higher inhibitory effect with IC_{50} value of 251 $\mu\text{g/mL}$. These effects were significantly higher than those of the other six plants ($p < 0.002$). Even though *S. album* and *V. cinerea* showed a moderate inhibition at 0.5 mg/mL (36.3 and 32.8% respectively), it was unable to calculate their IC_{50} values as the effects were not dose-dependent (Fig. 2). Extracts of *C. fenestratum*, *P. Americana*, *V. radiate* and *V. unguiculata* showed only lower inhibitory effects on tyrosinase (2.2-14.4%) at 0.5 mg/mL (Table 2). IC_{50} value of kojic acid (3.4 $\mu\text{g/mL}$) against tyrosinase was significantly lower than that of *P. emblica* and *T. populnea* ($p < 0.0008$).

114 **Table 2: Percentage tyrosinase inhibitory effects of plant extracts**
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Plant extract	Mean (%)	SD	IC ₅₀ (µg/mL)
<i>Coscinium fenestratum</i>	10.79	1.80	NA
<i>Persea americana</i>	14.42	4.23	NA
<i>Phyllanthus emblica</i>	53.35*	1.65	251
<i>Santalum album</i>	36.36	4.23	**
<i>Thespesia populnea</i>	70.54*	6.33	190
<i>Vernonia cinerea</i>	32.82	3.31	**
<i>Vigna radiate</i>	2.26	1.14	NA
<i>Vigna unguiculata</i>	7.06	2.61	NA
Kojic acid			3.4***

116 Mean inhibitory effects were obtained at 0.5 mg/mL. *Inhibitory effects of *P. emblica* and *T. populnea*
117 were significantly different from others ($p < 0.002$). ** *S. album* and *V. cinerea* did not show a dose-
118 dependent increase in inhibitory activity. *** IC₅₀ of Kojic acid was significantly lower than that of *P.*
119 *emblica* and *T. populnea* ($p < 0.0008$). NA: IC₅₀ values not available.
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122 **Fig. 1. Tyrosinase inhibitory effects of PE and TP at different concentrations**
123 PE: *Phyllanthus emblica*, TP: *Thespesia populnea*. Concentrations of the extracts used were, 0.03 to
124 0.5 for TP and 0.06 to 1 mg/mL for PE.
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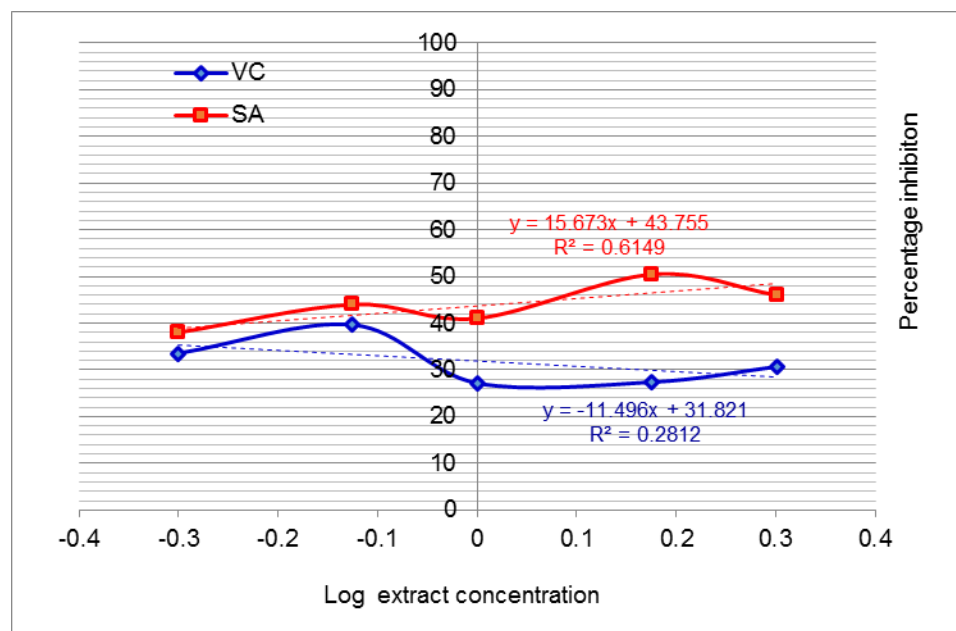


Fig. 2. Tyrosinase inhibitory effects of SA and VC at different concentrations

SA: *Santalum album*, VC: *Vernonia cinerea*. Concentrations of the extracts used were 0.5 to 2.0 mg/mL.

3.2 DISCUSSION

In the present study, parts from eight plants which are used as remedies for various skin ailments or beautifying agents were selected to evaluate for their efficacies against tyrosinase activity.

Phenolic compounds have demonstrated scientific evidence on multiple beneficial effects on skin. They eliminate factors causing skin ageing and skin damage [12]. There is a wide range of evidence to support the antioxidant effects, anti-inflammatory and antimicrobial effects, anti-aging properties, skin renewal properties and stimulatory effects on synthesis of collagen and elastin [12]. Phenolics were shown to suppress melanin production in skin cell cultures [12]. Being substrate (tyrosine) analogues, some phenolics such as resveratrol have demonstrated to be effective in inhibiting tyrosinase [12]. Quercetin exhibited the strongest tyrosinase inhibition (IC_{50} 14.29 μ M) among eight bioactive compounds isolated from *Hypericum laricifolium* [13]. Licuraside, isoliquiritin, and licochalcone A which are flavonoids isolated from *Glycyrrhiza uralensis* and *Glycyrrhiza inflata* were found to be competitive inhibitors of tyrosinase with IC_{50} values of 0.072, 0.038, and 0.026 mM, respectively [14].

P. emblica fruit which is a rich source of phenolic compounds with high antioxidant activity is used in several *varnya* formulations in Ayurveda [15]. A HPLC fraction rich in antioxidants isolated from *P. emblica* fruits has demonstrated skin lightening effects [16]. In another study, a fraction rich in antioxidants and low molecular weight hydrolysable tannins from *P. emblica* fruits inhibited melanin production in melanocyte cultures and *in vivo* [17]. Inhibition of tyrosinase, inhibition of Fe^{2+}/H_2O_2 -induced melanogenesis due to chelation with Fe^{2+} and inhibition of superoxide anion-induced melanogenesis by scavenging superoxide anions were identified as mechanisms of action responsible for skin lightening effects of *P. emblica*

fruits [17]. Ethanol and methanol extracts of *P. emblica* branch extracts demonstrated inhibition of tyrosinase activity with lower IC₅₀ values (247 and 194 µg/mL respectively) than that of ethanol extracts of *P. emblica* fruits (4347 µg/mL) [18]. Ethanol extract of *P. emblica* branch also inhibited tyrosinase mRNA expression in murine melanoma cells [18]. Gallic acid and vanillic acid were the major phenolic compounds identified in those extracts of *P. emblica* branch [18]. Present study supports the previous evidence on tyrosinase inhibitory effects of the *P. emblica* fruit. However, IC₅₀ value obtained against tyrosinase in the current study with methanol extract was much lower (251 µg/mL) than that of values reported for fruit ethanol extract (4347 µg/mL).

S. album oil is used in number of fairness cosmetics. Potent inhibitory effects of sandalwood oil on tyrosinase was revealed with an IC₅₀ of 171 µg/mL. In the same study, strong inhibitory effects of α-santalol, the major constituent of the oil on tyrosinase was identified [19]. The present study reveals that the bark of *S. album* possesses some inhibitory effect on tyrosinase even though the effect was not dose-dependent.

T. populnea bark is used to treat number of skin conditions such as scabies, psoriasis, eczema, ringworm infections [20] and for wound healing [21]. The decoction of the bark is commonly used for the treatment of skin and liver diseases. Bark is known to possess antioxidant, anti-inflammatory activities and hepatoprotective effects [20]. The current study reveals an additional benefit of the *T. populnea* bark extracts with a low IC₅₀ value against tyrosinase (190 µg/mL) suggesting its skin lightening effects. Hepatoprotective effects of phenolic acid rich fraction isolated from *T. populnea* bark were demonstrated in rats against CCl₄, acetaminophen and thioacetamide [22]. When methanol extracts of stem barks of five plants, *Ficus benghalensis*, *F. glomerata*, *F. religiosa*, *F. virens* and *T. populnea* were evaluated, highest DPPH radical scavenging action and highest superoxide scavenging potential were revealed in *T. populnea* (23). Furthermore presence of high amount of tannins and phenolics and good Fe³⁺ reducing power in *T. populnea* were identified [23].

V. cinerea whole plant is shown to have therapeutic effects against chronic skin disorders. This plant has demonstrated wound healing effects, anti-inflammatory effects, immunomodulatory effects, antioxidant effects and antibacterial activity which are beneficial effects with reference to maintenance of skin health [24,25]. The present study reveals some inhibitory effects of *V. cinerea* on tyrosinase. However, the effect was similar to that of *S. album* bark with no dose-dependence on inhibitory effects on tyrosinase.

An ethyl acetate fraction obtained from 70% ethanol extract of *Vigna radiatae* showed highest tyrosinase inhibitory activity when compared with Dichloromethane and n-butanol and residual fractions. Two flavonoids, vitexin and isovitexin, isolated from the ethyl acetate fraction showed inhibitory activities, with IC₅₀ values of 6.3 and 5.6 mg/mL, respectively which are considerably higher values [26]. In the current study, further investigations were not done with *C. fenestratum*, *P. americana*, *V. radiatae* and *V. unguiculata* to assess IC₅₀ value as the inhibitory effects observed at 0.5 mg/mL against tyrosinase were rather low (2.3 to 14.4%).

4. CONCLUSION

Current study reveals significantly high antityrosinase activities in methanol extracts of *P. emblica* fruit and *T. populnea* bark among eight plant extracts investigated. To the best of our knowledge this is the first evidence reported on antityrosinase effect of *T. populnea* bark extracts. Further studies are necessary to validate *in vivo* efficacy and safety of these extracts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

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