International Journal of Science and Research (IJSR)
ISSN: 2319-7064
Impact Factor 2024: 7.101

# An Experimental Evaluation of the Tyrosinase-Inhibiting Properties of *Talinum triangulare* and *Physalis Angulata* Extracts for Use as Adjuvants in Melanoma Prevention

*Running Title:* Exploring Tyrosinase Inhibition: Insights from Talinum Triangulare and Physalis Angulata Extracts

**Marcia Cristina Campos de Oliveira[1], Mariana de Abreu Soares[2]**

[1]Department of Chemical Organic, University Federal Rural do Rio de Janeiro, Rio de Janeiro, Brasil (Corresponding Author)

[2]Department of Chemical Organic, University Federal Rural do Rio de Janeiro, Rio de Janeiro, Brasil

**Abstract:** ***Melanoma is a common cancer in the Western world, with increasing incidence rates. Sun exposure is widely regarded as the primary risk factor for melanoma. The prognosis for patients with malignant melanoma varies significantly between countries, but public awareness campaigns promoting early detection have led to a substantial reduction in mortality rates. However, current therapies for melanoma are often hampered by melanin-mediated resistance, reducing their overall effectiveness. Suppressing tyrosinase activity and reducing melanogenesis using tyrosinase inhibitors is a promising strategy for sensitizing melanoma cells and enhancing the efficacy of adjuvant therapy. This experimental study investigates the tyrosinase inhibitory activity of extracts from Talinum triangulare and Physalis angulata, two plant species known for their medicinal properties. In vitro assays were used to test several extracts and fractions for their ability to reduce tyrosinase activity, which is a key enzyme involved in melanin synthesis. The methanolic-aqueous extract of T. triangulare demonstrated complete inhibition at the maximum tested concentration of 5.0 mg/mL and an $IC_{50}$ value of 1.8 mg/mL. Aqueous extracts obtained by decoction also exhibited consistent inhibitory effects. P. angulata showed moderate inhibition; however, purification resulted in fractions with improved $IC_{50}$ values. These results suggest that both plants, especially T. triangulare, may have therapeutic potential in adjuvant melanoma treatments by reducing melanin-mediated drug resistance.***

**Keywords:** Tyrosinase inhibition, melanoma, Talinum triangulare, Physalis angulata, bioactive plant extracts

## 1. Introduction

Melanoma is a prevalent cancer in the Western world, with a rising incidence. Sun exposure remains the primary risk factor. Although public awareness campaigns promoting early detection have led to reduced mortality rates, the prognosis for patients with malignant melanoma can vary greatly from region to region. Early-stage melanoma patients often respond well to treatment, however, the 5-year survival rate for advanced-stage melanoma is only 16% [1, 2, 3]. The significant health threat posed by melanoma has driven the development of targeted therapies [4, 5]. Despite progress, these treatments have limited efficacy in advanced stages and are often accompanied by severe side effects.

The introduction of new therapeutic approaches, including targeted and immunotherapies, has improved treatment outcomes, opening a new era for managing this aggressive cancer [6]. Melanin suppression mediated by tyrosinase inhibitors represents a promising, low-toxicity strategy to sensitize melanoma to conventional treatments. Given the global burden of melanoma and the limitations of current treatments, identifying plant-based tyrosinase inhibitors is a promising strategy for increasing therapeutic efficacy while reducing toxicity.

### 1.1 Tyrosinase enzyme (EC 1.14.18.1)

Tyrosinase, also known as phenoloxidase, is a copper-containing metalloenzyme widely distributed in plants, microorganisms, and animals. This enzyme catalyzes two distinct reactions: the ortho-hydroxylation of monophenols to ortho-diphenols and the oxidation of ortho-diphenols to the corresponding ortho-quinones. The initial reaction for melanin formation involves the hydroxylation of the substrate L-tyrosine to dihydroxyphenylalanine (DOPA), with the release of a water molecule, catalyzed by tyrosinase. Tyrosinase is also involved in the oxidation of DOPA to DOPA quinone and in the oxidation of 5,6-dihydroxyindole to indole-5,6-quinone. Notably, tyrosinase regulates the rate-limiting steps of melanogenesis, making it the main molecular determinant of melanin production [7].

Due to its crucial role in melanogenesis, tyrosinase has attracted significant interest in medicine and the cosmetics industry. In humans, melanin is responsible for pigmentation in the eyes, hair, and skin, providing protection against ultraviolet radiation. However, tyrosinase hyperactivity or overexpression can lead to hyperpigmentation disorders and has been linked to melanoma development. Inhibiting tyrosinase activity can reduce melanin production, potentially preventing melanoma formation [8].

Natural products have emerged as important tyrosinase inhibitors, gaining attention for their therapeutic and aesthetic applications. These inhibitors are valued for their structural diversity, biocompatibility, and relatively low toxicity compared to synthetic alternatives [9].

### 1.2 *Talinum triangulare and Physalis angulata*

Plant extracts are rich sources of natural tyrosinase inhibitors, including potent examples from Green Tea, Licorice Root, Coriander, and various Amazonian and South African plants, which often contain active compounds like flavonoids, phenolic acids, and polyphenols [10].

*Talinum triangulare (Jacq.) Willd.*, a perennial herb belonging to the Talinaceae family, is a traditional leafy vegetable used by communities for ethnomedicinal and ethnoculinary preparations. The literature survey shows that it has been traditionally useful in the treatment of several diseases, such as anaemia, diabetes, measles, and ulcers and the preparation of various traditional foods [11]. A study showed that the stems of *T. triangulare* contain phenolic compounds with high antioxidant properties, making them a promising source of bioactive antioxidants. Furthermore, the methanol/water extract of *T. triangulare* could be used as a natural antioxidant additive in food and pharmaceutical products. Screening the plant extracts using the DPPH free radical method proved effective in selecting those with antioxidant activity. These extracts are rich in antioxidants that have remarkable free radical scavenging properties. [12]. A phytochemical analysis reveals a wide variety of bioactive compounds. Phytochemical studies of its leaves and stems reveal a diversity of metabolites, including carotenoids, steroids, and porphyrins. Notable compounds include β-carotene, lutein, ten different pheophytins, campesterol, sitosterol, stigmasterol, and scotenol, among others [13].

*Physalis angulata*, a species belonging to the Solanaceae family, is widely distributed throughout subtropical regions. Its extracts or infusions are used in folk medicine across various countries to treat a range of diseases, including asthma, malaria, and hepatitis. Phytochemical studies have identified the presence of steroids (physalins and physagulins) and flavonoids in this species [14].

The presence of bioactive compounds such as pheophytins, steroids, and pheophytins in *T. triangulare* and *P. angulata* suggests that these plants are promising sources of natural tyrosinase inhibitors. The distinct metabolic profiles suggest the presence of synergistic mechanisms that enhance tyrosinase inhibition. The diverse phytochemical profiles of these species support their potential use in developing novel, accessible, and cost-effective treatments for hyperpigmentation disorders and melanoma, leveraging known bioactive compounds like pheophytins and flavonoids.

The aim of this study is to investigate the in vitro tyrosinase inhibitory effects of *Talinum triangulare* and *Physalis angulata* extracts in order to explore their potential use in adjuvant therapies for melanoma.

## 2. Result and Discussion

*Talinum triangulare* was collected in the municipality of Seropédica, Rio de Janeiro, Brazil, and the leaves, stems, and roots were separated. The leaves and stems were dried and ground in a domestic blender before being subjected to maceration in an 80:20 MeOH: $H_2O$ solvent system. The extract was concentrated using a rotary evaporator to obtain the dry extracts, TTCMA and TTFMA. TTFMA was then subjected to liquid-liquid partitioning with ethyl acetate to yield the aqueous and organic fractions (TTFAc). Methanolic extracts were also prepared from the root (TTRM), stem (TTCM), and leaves (TTFM). Aqueous extracts were obtained from the stem and leaves using hot extraction methods (infusion and decoction) and cold maceration.

*Physalis angulata* was obtained from an experimental cultivation grown by producers in southern Brazil. Extracts in ethanol: water (80:20) were prepared from the aerial parts and fresh stem by maceration (PAHE). PAHE was subjected to liquid-liquid partitioning with ethyl acetate to yield two fractions: one in ethyl acetate (PAFAc) and one hydroethanolic (PAFHE). The PAFHE fraction was then subjected to column chromatography using a solvent gradient (chloroform, chloroform: methanol and methanol) to produce 17 fractions.

The tyrosinase activity was evaluated using the spectrophotometric method, measuring the formation of dopachrome from the oxidation of L-DOPA by the tyrosinase enzyme. The ability of each extract and its fractions to inhibit tyrosinase activity was evaluated using L-DOPA as a substrate, according to the assay protocol described by Soares et al. (2017). Extract and fraction solutions were prepared in DMSO (10 mg/mL). Different volumes of the solution were added to a reaction medium containing tyrosinase (50–70 units), EDTA (0.022 mmol/L), L-DOPA (0.17 mmol/L), and PBS (50 mmol/L, pH 6.8) at room temperature. After 30 minutes, the reaction was analyzed using a Shimadzu UV-VIS spectrophotometer (model Mini 1240, Japan) at 475 nm. Percent inhibition was calculated according to equation 1.

**Equation 1:** % inhibition or activation = $\{[(B_{30} - B_0) - (Am_{30} - Am_0)] / (B_{30} - B_0)\} \times 100$ (1) where $\mathbf{B_0}$ = absorbance of L-DOPA + tyrosinase at t = 0 min, $\mathbf{B_{30}}$ = absorbance of L-DOPA + tyrosinase at t = 30 min, $\mathbf{A_0}$= absorbance of L-DOPA + tyrosinase + inhibitor/activator at t = 0 min, and $\mathbf{A_{30}}$ = absorbance of L-DOPA + tyrosinase + inhibitor/activator at t = 30 min.

### 2.1 Inhibition of tyrosinase activity by *Talinum triangulare* and *Physalis angulata* extracts and fractions

An evaluation was first carried out with all the *Talinum triangulare* and *Physalis angulata* extracts and fractions obtained at a concentration of 5.0 mg/mL, so that the inhibitory activities of the extracts and fractions could be compared. The results are shown in Table 1.

**Table 1:** Tyrosinase Inhibitory Activity of *Talinum triangulare* and *Physalis angulata* extracts and fractions (5.0 $mg/mL^{-1}$) below

| Extract and Fraction | Tyrosinase inhibition (%) |
|---|---|
| TTFMA | 100 |
| TTFM | 99 |
| TTFAc | 98 |
| TTCMA | 80 |
| TTCM | 72 |
| TTRM | 90 |
| PAHE | 98 |
| PAFAc | 100 |
| PAFHE | 96 |

The extracts and fractions of *Talinum triangulare* exhibited significant tyrosinase inhibition, ranging from 70 to 100% at a concentration of 5.0 mg/mL. Notably, the methanol-water leaf extract (TTFMA) demonstrated complete inhibition (100%), with an $IC_{50}$ value of 1.8 mg/mL **(Figure 1)**. $IC_{50}$ determination for the other fractions was not pursued due to insufficient sample quantity. Encouraged by the TTFMA results, we prepared aqueous extracts from fresh leaves using decoction, which showed significant inhibition: 1.3 mg/mL (66%), 1.16 mg/mL (53%), 0.83 mg/mL (43%), 0.66 mg/mL (35%), 0.5 and 0.33 mg/mL (9.5%). This extract had an $IC_{50}$ of 1.2 mg/mL and maintained consistent inhibition, as observed in the kinetic graph **(Figure 2, 3)**.

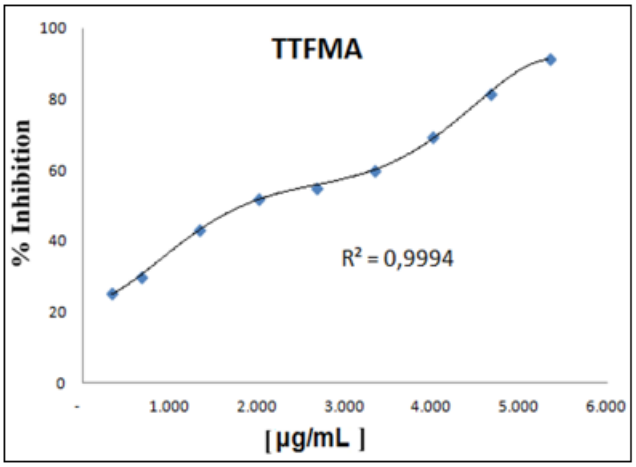


**Figure 1:** The tyrosinase inhibition curve of the methanol-water extract of *Talinum triangulare* (TTFMA) shows the percentage inhibition as a function of concentration. $IC_{50}$ = 1.8 mg/mL.

Encouraged by the TTFMA results, we prepared aqueous extracts from fresh leaves using decoction, which showed significant inhibition: 1.3 mg/mL (66%), 1.16 mg/mL (53%), 0.83 mg/mL (43%), 0.66 mg/mL (35%), 0.5 and 0.33 mg/mL (9.5%). This extract had an $IC_{50}$ of 1.2 $mg.mL^{-1}$ (Figure 2) and maintained consistent inhibition, as observed in the kinetic graph (Figure 3).

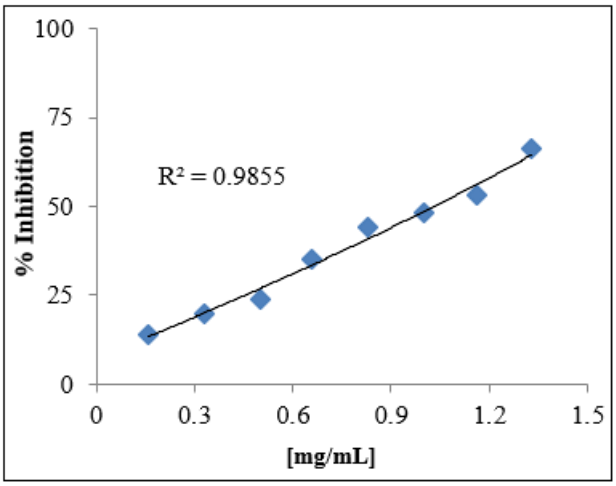


**Figure 2:** The tyrosinase inhibition curve of the aqueous extract of *Talinum triangulare*, prepared by decoction, shows the percentage inhibition as a function of concentration. $IC_{50}$ = 1.2 mg/mL

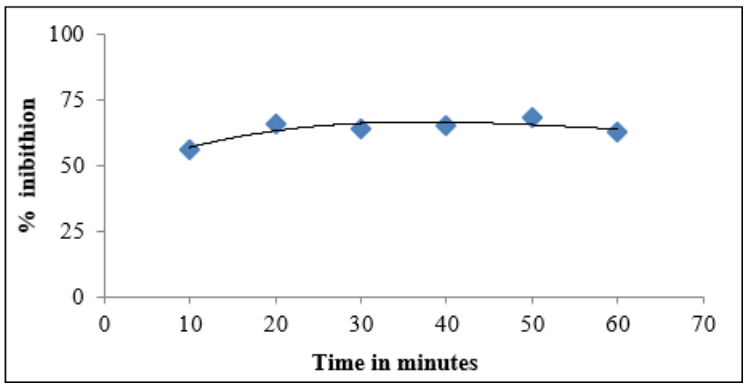


**Figure 3:** Kinetic graph of tyrosinase inhibition by the aqueous extract of *Talinum triangulare* at 1.1 mg/mL: inhibition profile over time.

In contrast, *P. angulata* extracts had higher $IC_{50}$ values (2.95–3.20 mg/mL), indicating lower inhibitory potency compared to *T. triangulare* **(Figure 4)**. The most polar fraction PAFHA of *P. angulata* was subjected to open column chromatography using a solvent gradient of chloroform, chloroform: methanol, and methanol. This yielded 17 fractions. Notably, the four fractions obtained by eluting the column with ethanol showed improved $IC_{50}$ values of 0.98–1.33 mg/mL **(Figure 5)** indicating enhanced inhibitory potency against tyrosinase. Further purification steps were required to achieve this enhancement.

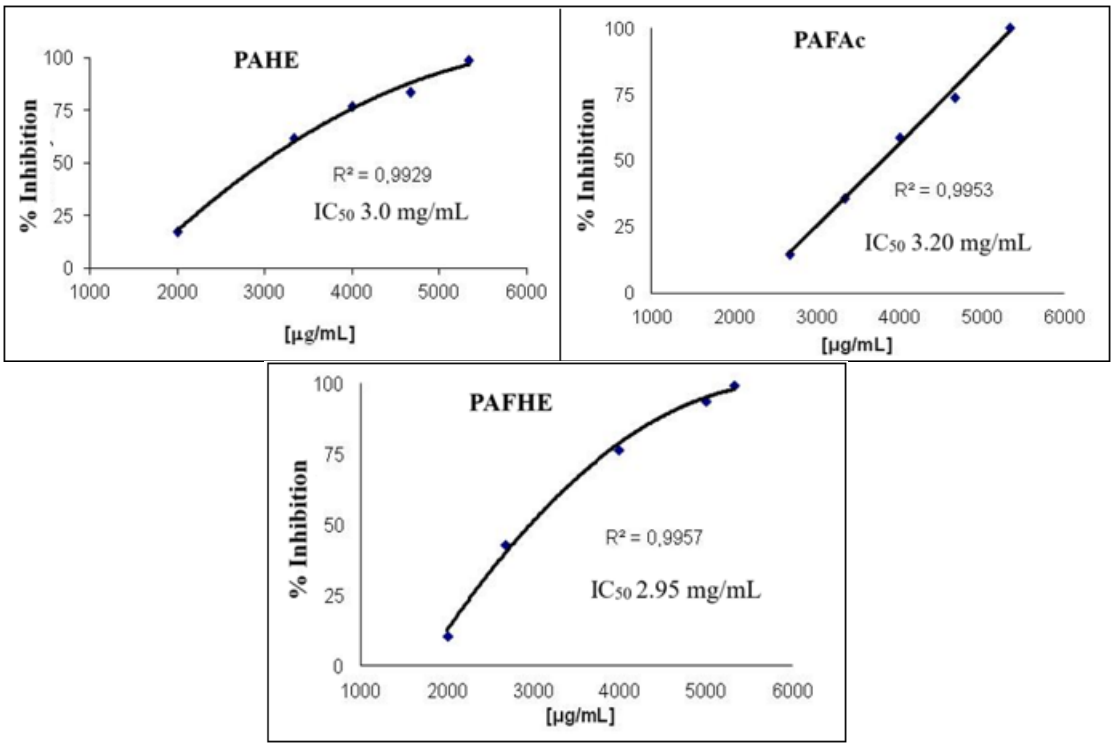


**Figure 4.** Tyrosinase inhibitory activity of *P. paniculatum* extract and fractions. $IC_{50}$ values are indicated.

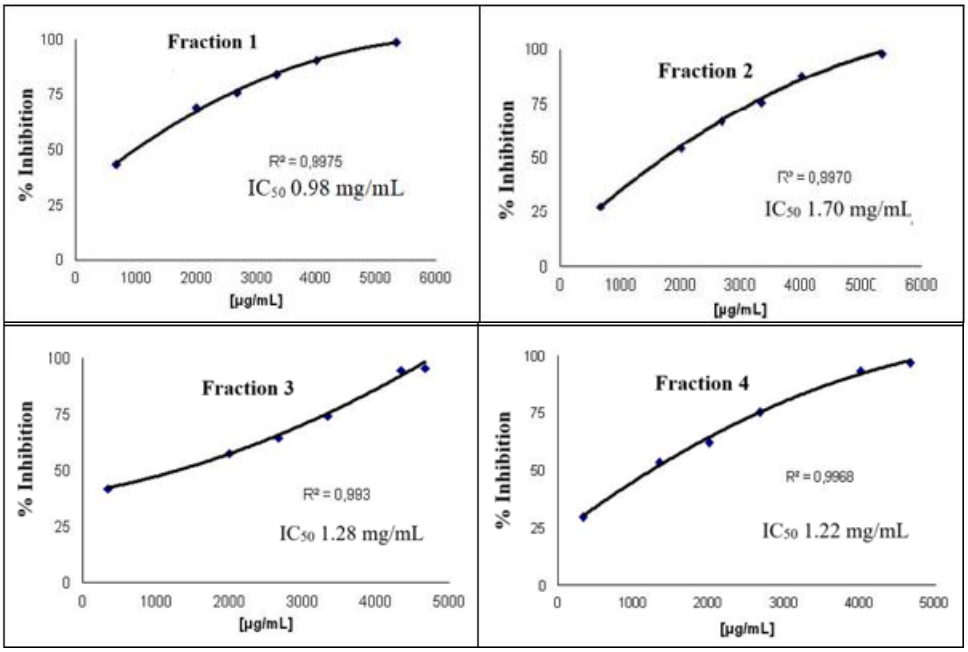


**Figure 5:** Tyrosinase inhibitory activity of Fractions 1-4 obtained from PAFHA fraction of *P. angulata*. $IC_{50}$ values are indicated in each graph.

## 3. Conclusion

In conclusion, *T. triangulare* (rich in pheophytins and saponins) demonstrated potent tyrosinase inhibition with $IC_{50}$ 1.8 mg/mL to methanol-water extract, outperforming *P. angulata*, which required purification to achieve comparable activity, obtaining $IC_{50}$ values from 0.98 to 1.70 mg/mL. The distinct metabolite profiles of these plants, including physalins and alkaloids in *P. angulata*, suggest synergistic mechanisms of action. These findings highlight the therapeutic potential of these species in melanoma adjuvant therapy, warranting further investigation to isolate bioactive compounds and assess in vivo efficacy.

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