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Inhibitory Effects of *Artemisia afra* Extracts on the Bio-Activity of Banana Tyrosinase

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Abstract: Melasma, formerly known as chloasma, is an acquired pigmentary condition, occurring most commonly on the face of humans. This disorder, which is more prevalent in females and darker skin types, is predominantly attributed to Ultraviolet (UV) exposure and hormonal influences. This condition is caused by an over-production of melanin, a pigment that gives color to the skin, hair, and eyes as well as protecting the skin against harmful ultraviolet radiation. The enzyme tyrosinase plays an important role in the biosynthesis of melanin from the amino acid, tyrosine. When this enzyme is inhibited, the bio-production of melanin can be limited and this bio-control of melanin synthesis could be used to manage melasma. Although several tyrosinase inhibitors are known, the search for natural inhibitors continues especially substances that do not negatively affect health. Water and methanol extractions from the stem and leaves of *Artemisia afra* were investigated for their potential inhibitory effect of tyrosinase extracted from the pulp and peel of bananas. The extent of tyrosinase inhibition was concluded while the enzyme was bio-converting L-DOPA into dopachrome with the amount of dopachrome produced determined spectrophotometrically at 475 nm. Both methanol and water extractions from the leaves and stem of *A. afra* caused an inhibition of the tyrosinase activity. It was also confirmed that bananas could be utilized as a tyrosinase resource.

Keywords: Banana Tyrosinase, Tyrosinase Inhibitor, *Artemisia afra*, Water Extraction, Methanol Extraction

Introduction

Melasma is a common acquired skin disorder that presents as a bilateral, blotchy, brownish pigmentation mostly observed in female patients with darker skin phototypes of Asian, African, and Hispanic descent. The susceptibility to this condition is caused by high exposure to ultraviolet radiation or because of living in intertropical countries (Espósito *et al*., 2022a). In recent years the incidence of melasma has increased in geographical areas with higher air pollution due to climate change (Espósito *et al*., 2022b). In certain global populations, the prevalence of melasma ranges from 1,5-33% (Ogbechie-Godec and Elbuluk, 2017) while in higher-risk populations it could be as high as 50% (Rathore *et al*., 2011).

Melasma is associated with the overactivity of melanocytes leading to an excess formation of melanin in the dermis and epidermis (Grimes *et al*., 2005) with regions of the human body most affected by this condition including the face, especially the forehead, cheeks, and upper lip although non-facial sites such as the forearms can also be affected, although less commonly (Bak *et al*., 2009). Due to the high rates of melasma recurrence and incomplete resolution the effective management of this condition remains a therapeutic challenge with first-line treatment options advised as sun protection and topical therapy. The principle of local therapies would be for the anti-melasma agent to contain a chemical substance that could inhibit the activity of the enzyme tyrosinase which is responsible for melanin production through melanogenesis (Lai *et al*., 2017). The effectiveness of topical therapy can be determined by a substance such as hydroquinone which inhibits the action of tyrosinase, an enzyme responsible for the bio-conversion of tyrosine into melanin (Austin *et al*., 2019). Other chemical agents used to manage melasma include vitamin A derivatives or retinoids such as tretinoin, tazarotene, and trifarotene (Gupta *et al*., 2006) as well as azelaic acid (Austin *et al*., 2019; Jutley *et al*., 2014).

Melanin is a natural skin pigment protecting the body against the harmful effects of ultraviolet radiation with the over-production thereof causing melasma which in most cases hurts the quality of life (Balik, *et al*., 2020; Kagha and

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Goldman, 2020). The search for substances especially natural compounds that could inhibit tyrosinase activity is topical and aligned with it is the process of identifying tyrosinase resources that could be used as modeling agents to investigate the potential inhibitory effect of tyrosinase activity. Tyrosinase is mostly isolated from mushrooms (Haghbeen *et al*., 2004; Vanitha and Soundhari, 2017) and when purified it can be used to investigate not only the catalytic properties of this enzyme but can also be utilized as a substrate to determine the potential inhibitory effect of agents acting on this enzyme. *In vitro*, tyrosinase can bio-convert Levo-DOPA (L-DOPA) into dopachrome of which the concentration can be determined spectrophotometrically and be used to conclude the inhibition or activation of the tyrosinase enzyme.

Although mushrooms are a major source of tyrosinase this enzymatic activity is widely distributed and can be obtained from different fungi such as *Agaricus fungi* (Strothkamp *et al*., 1976) *Aspergillus oryzae* and *Lentinula boryana* (Faria *et al*., 2007). The enzyme is also present in bacterial sources such as *Symbiobacterium thermophilum*, *Pseudomonas maltophiliia* (Claus and Decker, 2006), and *Verrucomicrobium spinosum* (Matoba *et al*., 2006) as well as in apple (Janovitz-Klapp *et al*., 1989) sunflower seed (Raymond *et al*., 1993) and *Solanum melongena* (Lee *et al*., 1997). The browning of fruit, fungi, and vegetables as well as the hyperpigmentation of human skin are two undesirable conditions with the tyrosinase enzyme responsible for both phenomena. While many scientific reports describe chemical agents that could act as inhibitors of the tyrosinase activity the search for more effective inhibitors continues. Bananas (Sarkar *et al*., 2022) are described as a source of tyrosinase enzyme while various species of the *Artemisia* plant such as *Artemisia annua* (Acquaviva *et al*., 2023), *Artemisia capillaris* (Tabassum, *et al*., 2016) and *Artemisia absinthium* (Yagi, *et al*., 2024) are showing tyrosinase inhibitory effects.

During the current investigation, banana tyrosinase was exposed to extracts from *A. afra* to determine the possible inhibitory effect of these extracts on the tyrosinase enzyme while bio-converting L-Dopa into dopachrome.

Materials and Methods

Research Design

The inhibition of tyrosinase activity is a crucial step in the management of melasma and the potential inhibitory effect of extracts from the plant *A. afra* on banana tyrosinase was determined. A fixed mass of banana peel and pulp were mixed separately with a phosphate buffer to extract tyrosinase activity. Water and methanol extraction from the stem and leaves of *A. afra* were incubated with the banana-extracted tyrosinase while this enzyme was bio-converting L-DOPA into dopachrome. The inhibitory effect of the *Artemisia* extracts was determined by comparing the extent of L-DOPA conversion into dopachrome by banana tyrosinase in the presence of the *Artemisia* extracts to the extent of L-DOPA conversion in the absence of the *Artemisia* extracts.

Tyrosinase Extraction from Banana Peel and Pulp

Bananas were separated into the pulp (103, 31 g) and peel (59,11 g) and homogenized with 30 mL of a 0,05 M phosphate buffer, pH 4,5. The different homogenates were transferred into a centrifuge tube and centrifuged for 15 min at 4500 rpm whereafter the supernatants were removed and filtered through a Whatman no 1 filter paper. The filtrates were collected in glass flasks and stored at 4°C.

Extraction of Phytochemicals from Artemisia afra with Water and Methanol

The *Artemisia* plant was collected from the Limpopo province in South Africa and kept for one week at room temperature in a well-ventilated room to dry whereafter it was separated into the stem and leaves with each section ground into a fine powder with a blender. These powder samples (10 g) of the stem and leaves were mixed separately with methanol (400 mL) and water (400 mL) in sealed glass beakers and shaken for 24 h at 180 rpm whereafter the solvents of each filtrate were removed by rotary evaporation. The methanol extractions were further dried by exposing them to flowing air while the water extracts were freeze-dried. The dried samples were kept separately in closed glass containers at room temperature.

Preparation of a L-DOPA Solution and Incubation with Banana Tyrosinase

L-DOPA was used as the substrate for tyrosinase activity with a stock solution prepared in the phosphate buffer at a concentration of 0,4 $mg.mL^{-1}$ and kept at 4°C. The banana tyrosinase activity was determined by mixing a volume of each tyrosinase solution (extract from peel and pulp) with the L-DOPA solution in a cuvette placed in a spectrophotometer. The incubation was performed at 25°C and during each incubation period, the L-DOPA conversion into dopachrome was followed periodically at 475 nm using the method described by Shin *et al*. (2023).

Assay for the Tyrosinase Inhibitory Effect of Artemisia Extracts

Before determining the effect of the *Artemesia* extracts on tyrosinase activity the dried methanol and water extracts were dissolved in 1 mL of methanol and 1 mL of water, respectively. Samples of these solutions were mixed with the banana-extracted tyrosinase while bio-converting L-DOPA into dopachrome. The control incubation, as well as all the tests, were performed in triplicate in a 4.00 mL cuvette placed in a spectrophotometer with absorbance readings taken every 10 sec during a 7-min incubation period according to the method described by Shin *et al*. (2023). The composition of the various incubations is described below.

Control Incubations

L-DOPA solution (2400 uL), banana pulp tyrosinase (100 uL), and phosphate buffer (100 uL).

L-DOPA solution (2400 uL), banana peel tyrosinase (100 uL), and phosphate buffer (100 uL).

Test Incubations

L-DOPA solution (2400 uL), banana pulp tyrosinase (100 uL), and water extract of *A. afra* leaf (100 uL).

L-DOPA solution (2400 uL), banana pulp tyrosinase (100 uL), and water extract of *A. afra* stem (100 uL).

L-DOPA solution (2400 uL), banana pulp tyrosinase (100 uL), and methanol extract of *A. afra* stem (100 uL).

L-DOPA solution (2400 uL), banana peel tyrosinase (100 uL), and water extract of *A. afra* stem (100 uL).

Calculation of Tyrosinase Activity Inhibition

The relative activity of the tyrosinase enzyme was determined in terms of the amount of dopachrome produced from L-DOPA as measured at 475 nm according to the method described by Shin *et al*. (2023). When measured after each incubation period, the difference in the absorbance readings indicated to what extent the tyrosinase enzyme was inhibited. The percentage inhibition of the tyrosinase enzyme was calculated as follows:

$$Percentage\ Inhibition = \frac{\begin{array}{c}Absorbance\ reading\ in\ the\\ absence\ of\ Artemisia\ minus\\ absorbance\ reading\ in\ the\\ presence\ of\ Artemisia\end{array}}{\begin{array}{c}Absorbance\ reading\ in\ the\\ absence\ of\ Artemisia\end{array}} \times 100$$

Results and Discussion

Phytomedicines are becoming one of the most important aspects of the global health system and the usage of medicinal plants is increasing as reflected by the development of this industry. In many cases, the bioactive compounds isolated from medicinal plants are regarded as alternatives for disease prevention and treatment with water and methanol amongst the most common solvents used to extract phytochemicals from plant materials. These solvents were also used to extract phytochemicals from the stem and leaves of *A. afra* as described by Altemimi *et al*. (2017). The inhibitory effect analyses of *A. afra*-extracted phytochemicals on banana tyrosinase activity are a novel approach and Fig. (1) reflects the effect of water-extracted compounds from the leaves of *A. afra* on the bioconversion of L-DOPA into dopachrome by banana pulp tyrosinase. The change in the number of 475 nm absorbance units reflects dopachrome formation from L-DOPA during a 400-sec incubation period. When performed in the absence of *A. afra* extract an average increased rate of $6{,}5.10^{-4}$ absorbance units per second of tyrosinase activity was calculated while in the presence of this *Artemisia* extract the tyrosinase activity was majorly inhibited resulting in the average rate of 1.10^{-4} absorbance units per second of tyrosinase activity. From these results, it was calculated that the average inhibited enzymatic rate decreased 6,5 times (83% inhibition) whilst the instantaneous inhibition after 10 sec was calculated as a value of 29% with a 56% instantaneous inhibition after 400 s of incubation. The instantaneous reaction rate at each incubation interval is reflected in Fig. (1) with an initial high reaction rate at 10 sec of incubation which decreased during each incubation period until the end of the incubation period. This decreasing rate of dopachrome formation from L-DOPA supports the reaction principle that the amount of L-DOPA (substrate) will decrease as the reaction continues resulting in a lower reaction rate. In the presence of the *A. afra* water-extraction, the average reaction rate was lower than the control rate indicating the banana pulp tyrosinase was inhibited by phytochemicals extracted with water from the leaves of the *A. afra* plant.

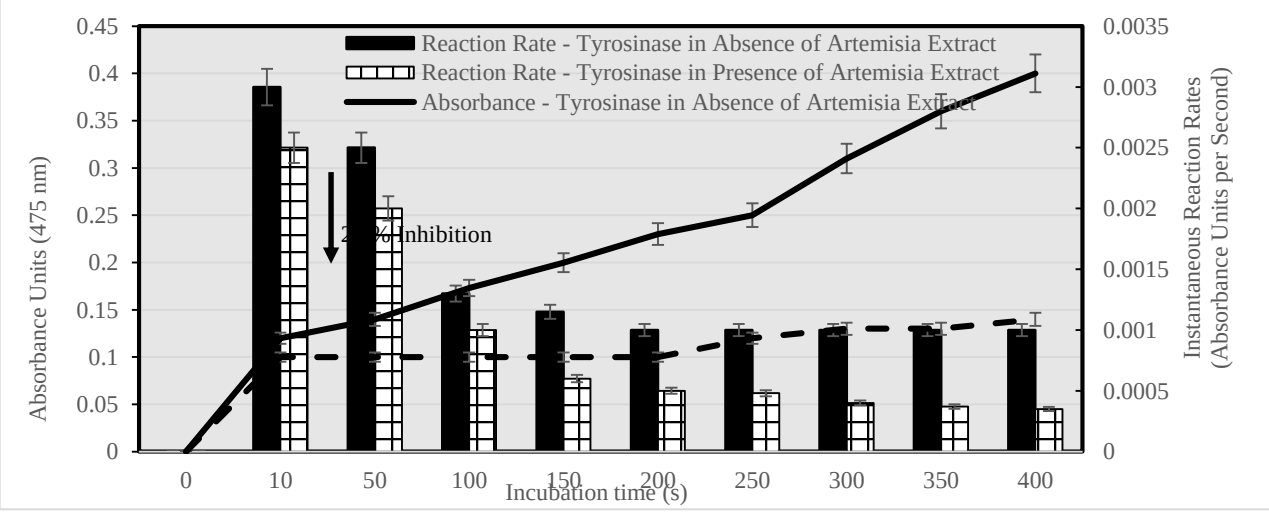

Fig. 1: Inhibitory effect of water-extracted phytochemicals from the leaves of *Artemisia afra* on banana pulp tyrosinase while bio-converting L-DOPA into dopachrome

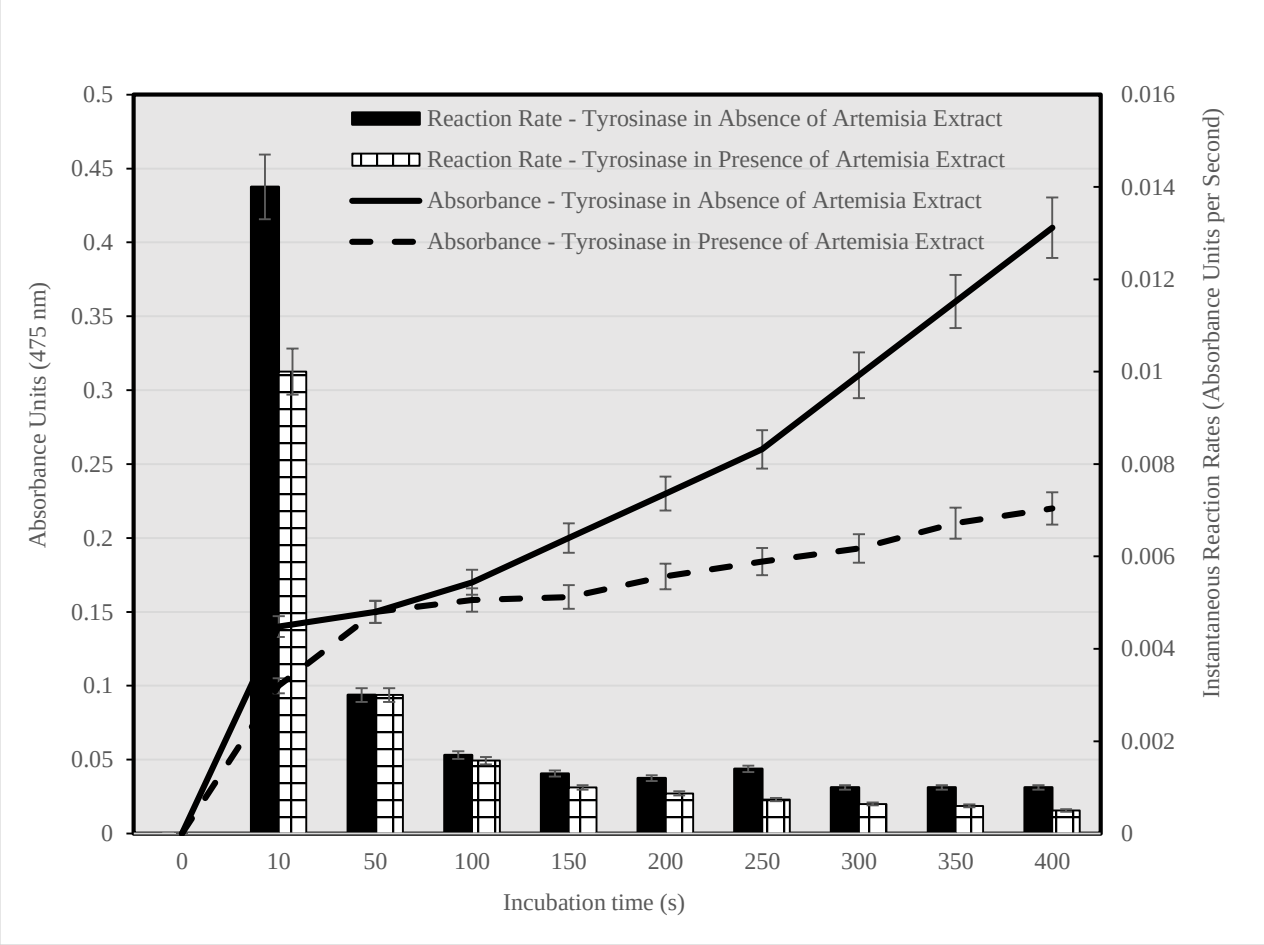

Fig. 2: Inhibitory effect of water-extracted phytochemicals from the stem of *Artemisia afra* on banana pulp tyrosinase while bio-converting L-DOPA into dopachrome

The time-dependent bioconversion of L-Dopa by the banana peel tyrosinase enzyme in the presence of a water extract from the stem of *A. afra* is reflected in Fig. (2). The increase in dopachrome formation by the tyrosinase enzyme (control) occurred at a rate of 0,00067 absorbance units per sec of incubation. In the presence of the *A. afra* stem extract the rate of dopachrome production decreased to 0,000238 absorbance units per sec of tyrosinase activity. This decrease in the average rate of L-DOPA conversion into dopachrome due to the phytochemicals in the stem of *A. afra* and extracted with water represents a 63% inhibition of the tyrosinase activity.

During the incubation period, the instantaneous reaction rates as determined at the various incubation times showed a decrease for both control and the inhibited tyrosinase action. It was also observed that the bio-conversion rate at each incubation time was lower than the control value. The relative reaction rates also decreased with increasing incubation time which supports the notion that the amount of L-DOPA decreased during the incubation period. The extent of inhibition caused by the water extraction from the stem of *A. afra* is, however, less than the extent of inhibition observed when L-DOPA was treated with phytochemicals extracted with water from the leaves of the same plant.

Figure (3) represents the effect of phytochemicals extracted from the stem of *A. afra* with methanol on the bio-activity of banana pulp tyrosinase. The control incubation reflecting the formation of dopachrome showed a similar time-dependent trend that is reflected in Figs. (1-2), when water was the extraction solvent. The average activity was calculated as 0.00067 absorbance units per sec of tyrosinase incubation. Opposite to the inhibition pattern obtained when the tyrosinase was exposed to water-based extractions from *A. afra*, the time-dependent inhibition observed with the methanol extractions showed a decline pattern with the absorbance units decreased from a value of 0,1-0,014 after 400 sec of inhibition at a negative rate of 0,0002 absorbance units per sec of incubation. The degree of inhibition increased from 28% after 10 s of incubation to 97% at the end of the incubation period. The inhibition of tyrosinase activity by methanol-extracted phytochemicals from the stem of *A. afra* is also demonstrated by the increasing instantaneous inhibition of tyrosinase as experienced during the prolonged incubation time.

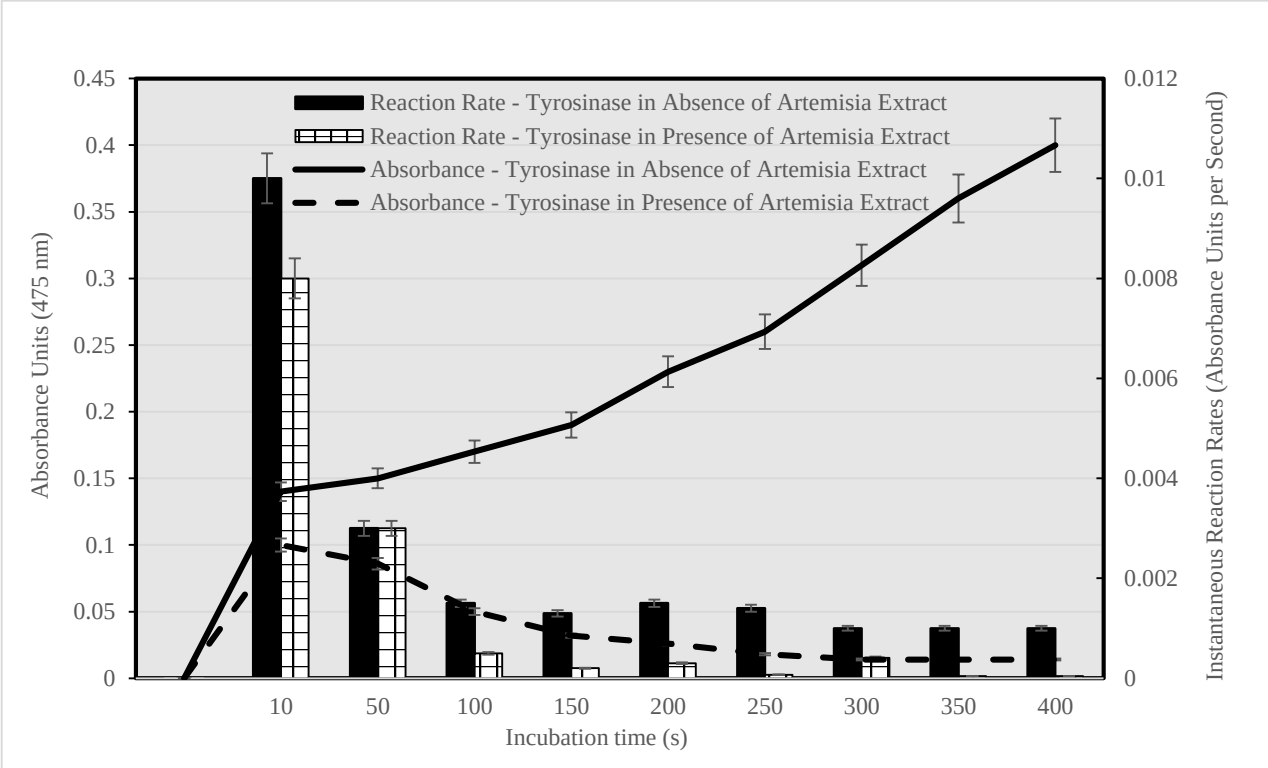

Fig. 3: Inhibitory effect of methanol-extracted phytochemicals from the stem of *Artemisia afra* on banana pulp tyrosinase while bio-converting L-DOPA into dopachrome

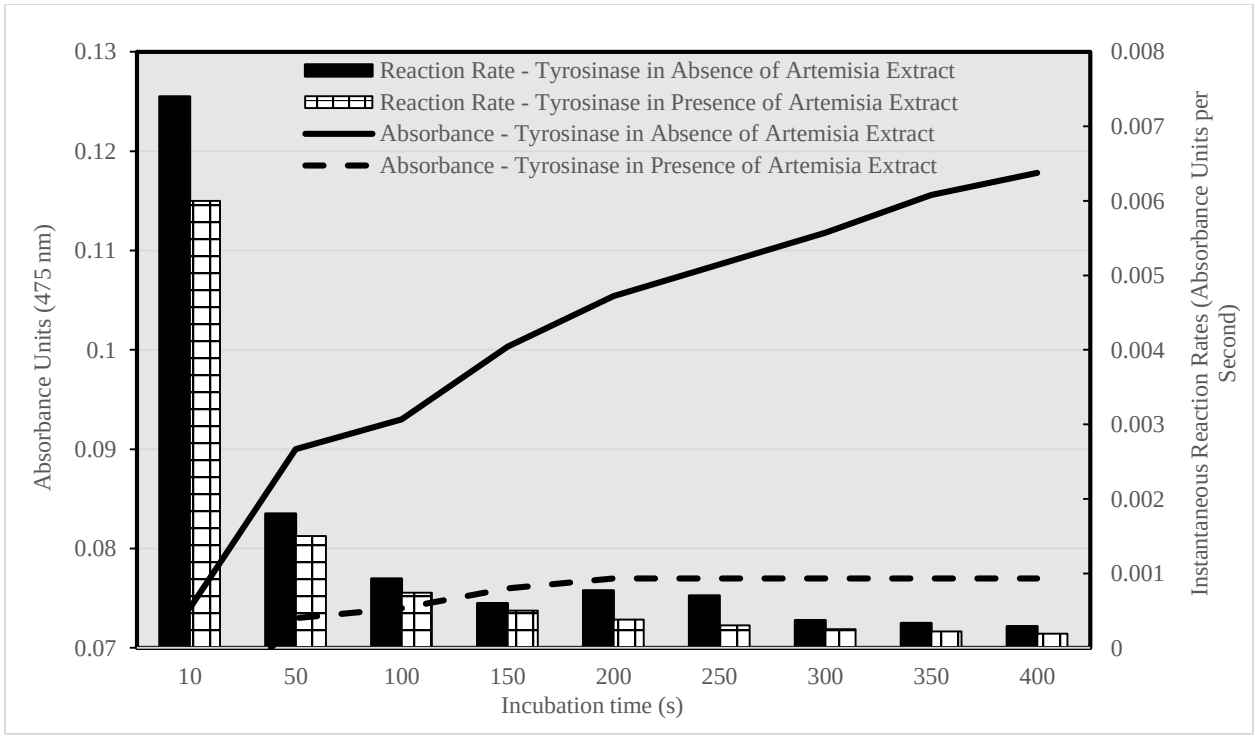

Fig. 4: Inhibitory effect of water-extracted phytochemicals from the stem of *A. afra* on banana peel tyrosinase while bio-converting L-DOPA into dopachrome

Figure (4) illustrates the inhibitory effect of phytochemicals extracted with water from the stem of *A. afra* on the bioaction of banana peel tyrosinase while bio-converting L-DOPA into dopachrome. During this investigation, the control tyrosinase activity showed an increased formation of dopachrome at a rate of 0.00011 absorbance units per sec of the tyrosinase activity. In the presence of the *Artemisia* extract the rate of dopachrome formation was lower at $1,10^{-5}$ absorbance units per sec of tyrosinase activity and this lower rate of dopachrome formation represents a 1,5 times inhibition of the tyrosinase activity by phytochemicals extracted with water from the stem of *A. afra*.

Tyrosinase is a copper-containing enzyme that plays an important role in the biosynthesis of melanin with its implications in many physiological pathways such as skin pigmentation, wound healing, and immune responses to pathogens (Nunes and Vogel, 2018). Melanin is a pigment that serves to shield the skin from ultraviolet radiation and dysregulation of tyrosinase activity and excessive melanin production and deposition can lead to hyperpigmentation and skin melanoma (Logesh *et al*., 2023). As a result of these skin conditions tyrosinase inhibitors are frequently used in fields such as dermatology, cosmetics, and pharmaceuticals because of their efficacy in regulating skin-related conditions such as hyperpigmentation (Rathee *et al*., 2021). Natural tyrosinase inhibitors are often preferred over their synthetic counterparts due to the potential adverse effects associated with synthetic tyrosinase inhibitors. Adverse effects include skin irritation, allergic reactions, and other undesirable responses (Roulier *et al*., 2020).

Natural products from plants are widely used as cosmetic or cosmeceutical ingredients because of their capability to slow down the intrinsic skin aging processes and to contrast the extrinsic ones. Plant's anti-aging properties are generally attributed to their anti-oxidant metabolites, which minimize free radical activity and protect skin against solar radiation (Sahu *et al*., 2013). Examples of plants that show tyrosinase inhibitory activity include *Cassia fistula* and *Gynura procumbens* (Rosiana *et al*., 2024), evening primrose (*Oenothera biennis* L) (Wang *et al*., 2024a), *Centaurium spicatum* (Alruhaimi *et al*., 2024) and *Olea eurpoaea* L. (Wang *et al*., 2024b). Fruits have also been described for their tyrosinase inhibitory properties and these include *Morus nigra* fruits (Koyu *et al*., 2018) and fruits from *Sarcopoterium spinosum* (Piras *et al*., 2017), apples (Chai *et al*., 2024), as well as fruits from *Citrus hystrix* and *C. maxima* (Abirami *et al*., 2014).

The browning of intact and fresh-cut fruits is one of the features with a negative effect on the fruit industry and this colorizing process is divided into enzymatic browning and non-enzymatic browning with the enzyme tyrosinase mostly responsible for the enzymatic process (Singh *et al*., 2018). Different fruits could thus act as a resource for the tyrosinase enzyme and when extracted this enzyme can be used as the substrate to investigate the effect of potential tyrosinase inhibitors during *in-vitro* studies of tyrosinase bio-converting L-DOPA into dopachrome. Most banana peels tend to change dark after a while and since tyrosinase has been identified in the peels of this fruit it could be used to isolate the tyrosinase enzyme for further investigation as a substrate to investigate possible tyrosinase inhibitors (Ademakinwa and Agunbiade, 2022). The genus *Artemisia* consists of about 500 species occurring around the world with several important drugs discovered from this genus such as artemisinin the well-known anti-malarial drug that was isolated from the Chinese herb *Artemisia annua* (Liu *et al*., 2009). *A. afra* is one of the most popular and commonly used herbal medicines in southern Africa and is employed for the treatment of a variety of ailments such as coughs, gout, asthma, diabetes, and heart inflammation (Thring and Weitz, 2006). Recent studies have shown that aqueous powder suspensions of *A. afra* are not active against malaria in mice (Walz *et al*., 2024), that it reduces the bacterial load in a Salmonella-infected rat model (Amoussa *et al*., 2023) and shows strong bactericidal activity against *Mycobacterium tuberculosis* (Martini *et al*., 2020).

The inhibition of tyrosinase activity is a common strategy to inhibit melanin formation which if produced in excess is responsible for the skin disorder known as melasma. For many people who are suffering from this condition, it is important to apply a skin-whitening agent to limit the skin-darkening effect of melasma. Currently, the most effective agent used to control this condition is managed with commercial whitening products such as hydroquinone, kojic acid, and arbutin whose long-term use could affect human health (Tu *et al*., 2012). To limit the possible negative health effects caused by these agents the search for natural tyrosinase inhibitors is topical with certain phytochemicals already proven to be good tyrosinase inhibitors such as resveratrol from *Vitis vinifera* L. (Zeng *et al*., 2021) and galangin extracted from *Alpinia officinarum* (Chung *et al*., 2018). Although mushrooms are known as a good resource of tyrosinase activity the search for alternative tyrosinase resources such as bananas is also continuing and these tyrosinase enzymes can be used to investigate the tyrosinase inhibitory profiles of phytochemicals obtained from plant materials such as *A. afra*.

Phytomedicines are becoming as important as antibiotics and chemical medicines and to further optimize the efficiency of these substances it would be important to especially focus on different extraction procedures ensuring a high yield of the phytochemicals. Although the *in vitro* studies of the current phytomedicines look promising, more investigations must be conducted to conclude the pharmacokinetics as pharmacodynamics of the *A. afra* phytochemicals when applied to manage melasma.

Conclusion

Although not life-threatening, melasma has been shown to impact the quality of life and self-esteem of those affected. A procedure to manage this condition could be done by inhibiting the catalytic action of tyrosinase which is responsible for the production of melanin, the major chemical component of melasma. Phytochemicals extracted

with water and methanol from the stem and leaves of *A. afra* proved to be tyrosinase inhibitors and simultaneously it was concluded that bananas could serve as a resource of the tyrosinase enzyme.

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Author's Contributions

The laboratory work was performed by the first author and both authors participated in the entire process of the article preparation.

Ethics

This article is original and contains unpublished data. The authors declare no conflict of interest regarding the publication of this paper. The authors declare no ethical issues will arise after the work has been published.

References

Abirami, A., Nagarani, G., & Siddhuraju, P. (2014). *In vitro* Antioxidant, Anti-Diabetic, Cholinesterase and Tyrosinase Inhibitory Potential of Fresh Juice from Citrus Hystrix and C. Maxima Fruits. *Food Science and Human Wellness*, *3*(1), 16–25. https://doi.org/10.1016/j.fshw.2014.02.001

Acquaviva, A., Nilofar, Bouyahya, A., Zengin, G., Di Simone, S. C., Recinella, L., Leone, S., Brunetti, L., Uba, A. I., Cakilcioğlu, U., Polat, R., Darendelioglu, E., Menghini, L., Ferrante, C., Libero, M. L., Orlando, G., & Chiavaroli, A. (2023). Chemical Characterization of Different Extracts from Artemisia annua and Their Antioxidant, Enzyme Inhibitory and Anti-Inflammatory Properties. *Chemistry and Biodiversity*, *20*(8), e202300547. https://doi.org/10.1002/cbdv.202300547

Ademakinwa, A. N., & Agunbiade, M. O. (2022). Banana Peel Wastes as a Source of Tyrosinase Useful in the Production of l-DOPA. *Sustainable Chemistry and Pharmacy*, *30*, 100853. https://doi.org/10.1016/j.scp.2022.100853

Austin, E., Julie K., N., & Jagdeo, J. (2019). Topical Treatments for Melasma: A Systematic Review of Randomized Controlled Trials. *Journal of Drugs in Dermatology*, *18*(11), 1156.

Alruhaimi, R. S., Mahmoud, A. M., Elbagory, I., Ahmeda, A. F., El-Bassuony, A. A., Lamsabhi, A. M., & Kamel, E. M. (2024). Unveiling the Tyrosinase Inhibitory Potential of Phenolics from Centaurium Spicatum: Bridging in Silico and *in vitro* Perspectives. *Bioorganic Chemistry*, *147*, 107397. https://doi.org/10.1016/j.bioorg.2024.107397

Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D., & Lightfoot, D. (2017). Phytochemicals: Extraction, Isolation and Identification of Bioactive Compounds from Plant Extracts. *Plants*, *6*(4), 42. https://doi.org/10.3390/plants6040042

Amoussa, A.M.O., Lagnika, L., Jullia, V. & Chassagne, F. (2023). Anti-*Salmonella* Activity of Plant Species in Benin Republic: *Artemisia Afra* and *Detarium Senegalense* with Promising *in vitro* and *in vivo* Activities. *Biomedicine and Pharmacotherapy*, *158*, 114119. https://doi.org/10.1016/j.biopha.2022.114119

Bak, H., Lee, H. J., Chang, S.-E., Choi, J.-H., Kim, M. N., & Kim, B. J. (2009). Increased Expression of Nerve Growth Factor Receptor and Neural Endopeptidase in the Lesional Skin of Melasma. *Dermatologic Surgery*, *35*(8), 1244–1250. https://doi.org/10.1111/j.1524-4725.2009.01219.x

Balik, Z. B., Balik, A. R., Yüksel, S., Karaosmanoğlu, N., & Ekşioğlu, H. M. (2020). Evaluation of Demographic Features, Clinical Characteristics and Quality of Life in Melasma Patients as Compared to the Control Group. *Turkiye Klinikleri Journal of Dermatology*, *30*(3), 81–87. https://doi.org/10.5336/dermato.2020-78570

Chai, W.-M., Bai, Q., Pan, Q., Wang, L., & Zhu, D. (2024). 6,7-Bis-(2-Methoxyethoxy)-4(3H)-Guinazolinone as a Novel Inhibitor of Tyrosinase and Potential Anti-Browning Agent of Fresh-Cut Apples. *Journal of Bioscience and Bioengineering*, *137*(3), 165–172. https://doi.org/10.1016/j.jbiosc.2023.12.006

Chung, K. W., Jeong, H. O., Lee, E. K., Kim, S. J., Chun, P., Chung, H. Y., & Moon, H. R. (2018). Evaluation of Antimelanogenic Activity and Mechanism of Galangin in Silico and in Vivo. *Biological and Pharmaceutical Bulletin*, *41*(1), 73–79. https://doi.org/10.1248/bpb.b17-00597

Claus, H., & Decker, H. (2006). Bacterial Tyrosinases. *Systematic and Applied Microbiology*, *29*(1), 3–14. https://doi.org/10.1016/j.syapm.2005.07.012

Espósito, A. C. C., Cassiano, D. P., da Silva, C. N., Lima, P. B., Dias, J. A. F., Hassun, K., Bagatin, E., Miot, L. D. B., & Miot, H. A. (2022a). Update on Melasma—Part I: Pathogenesis. *Dermatology and Therapy*, *12*(9), 1967–1988. https://doi.org/10.1007/s13555-022-00779-x

Espósito, M. C. C., Espósito, A. C. C., Lemos, F. K., Jorge, M. F. S., D'Elia, M. P. B., & Miot, H. A. (2022b). Melasma: Trends in Worldwide Internet Searches (2000-2019). *Indian Journal of Dermatology, Venereology and Leprology*, *88*(5), 675–677. https://doi.org/10.25259/ijdvl_845_2021

Faria, R. O. de, Moure, V. R., Balmant, W., Lopes de Almeida Amazonas, M. A., Krieger, N., & Mitchell, D. A. (2007). *The Tyrosinase Produced by Lentinula boryana Pegler Suffers Substrate Inhibition by L - DOPA*. Food Technology and Biotechnology.

Grimes, P. E., Yamada, N., & Bhawan, J. (2005). Light Microscopic, Immunohistochemical and Ultrastructural Alterations in Patients with Melasma. *The American Journal of Dermatopathology*, *27*(2), 96–101. https://doi.org/10.1097/01.dad.0000154419.18653.2e

Gupta, A. K., Gover, M. D., Nouri, K., & Taylor, S. (2006). The Treatment of Melasma: A Review of Clinical Trials. *Journal of the American Academy of Dermatology*, *55*(6), 1048–1065. https://doi.org/10.1016/j.jaad.2006.02.009

Haghbeen, B. K., Ferdous, R. J., Karkhaneh, A. A., & Borojerdi, S. S. (2004). Purification of Tyrosinase from Edible Mushroom. *Iranian Journal of Biotechnology*, *2*(3), 189–194.

Janovitz-Klapp, A., Richard, F., & Nicolas, J. (1989). Polyphenoloxidase from Apple, Partial Purification and Some Properties. *Phytochemistry*, *28*(11), 2903–2907. https://doi.org/10.1016/0031-9422(89)80250-x

Jutley, G. S., Rajaratnam, R., Halpern, J., Salim, A., & Emmett, C. (2014). Systematic Review of Randomized Controlled Trials on Interventions for Melasma: An Abridged Cochrane Review. *Journal of the American Academy of Dermatology*, *70*(2), 369–373. https://doi.org/10.1016/j.jaad.2013.07.044

Kagha, K., Fabi, S., & Goldman, M. (2020). Melasma's Impact on Quality of Life. *Journal of Drugs in Dermatology*, *19*(2), 184–187. https://doi.org/10.36849/jdd.2020.4663

Koyu, H., Kazan, A., Demir, S., Haznedaroglu, M. Z., & Yesil-Celiktas, O. (2018). Optimization of Microwave Assisted Extraction of Morus Nigra L. Fruits Maximizing Tyrosinase Inhibitory Activity with Isolation of Bioactive Constituents. *Food Chemistry*, *248*, 183–191. https://doi.org/10.1016/j.foodchem.2017.12.049

Lai, X., Wichers, H. J., Soler-Lopez, M., & Dijkstra, B. W. (2017). Structure of Human Tyrosinase Related Protein 1 Reveals a Binuclear Zinc Active Site Important for Melanogenesis. *Angewandte Chemie International Edition*, *56*(33), 9812–9815. https://doi.org/10.1002/anie.201704616

Lee, J., Kong, K.-H., & Cho, S. (1997). Purification and Characterization of Tyrosinase from Solanum Melongena. *Journal of Biochemistry and Molecular Biology*, *30*, 150–156.

Liu, N. Q., Van der Kooy, F., & Verpoorte, R. (2009). Artemisia afra: A Potential Flagship for African Medicinal Plants? *South African Journal of Botany*, *75*(2), 185–195. https://doi.org/10.1016/j.sajb.2008.11.001

Logesh, R., Prasad, S. R., Chipurupalli, S., Robinson, N., & Mohankumar, S. K. (2023). Natural Tyrosinase Enzyme Inhibitors: A Path from Melanin to Melanoma and its Reported Pharmacological Activities. *Biochimica et Biophysica Acta (BBA) - Reviews on Cancer*, *1878*(6), 188968. https://doi.org/10.1016/j.bbcan.2023.188968

Martini, M. C., Zhang, T., Williams, J. T., Abramovitch, R. B., Weathers, P. J., & Shell, S. S. (2020). Artemisia annua and Artemisia afra Extracts Exhibit Strong Bactericidal Activity Against Mycobacterium Tuberculosis. *Journal of Ethnopharmacology*, *262*, 113191. https://doi.org/10.1016/j.jep.2020.113191

Matoba, Y., Kumagai, T., Yamamoto, A., Yoshitsu, H., & Sugiyama, M. (2006). Crystallographic Evidence That the Dinuclear Copper Center of Tyrosinase Is Flexible During Catalysis. *Journal of Biological Chemistry*, *281*(13), 8981–8990. https://doi.org/10.1074/jbc.m509785200

Nunes, C. S., & Vogel, K. (2018). Chapter 20 - Tyrosinases—Physiology, Pathophysiology and Applications. *Enzymes in Human and Animal Nutrition*, 403–412. https://doi.org/10.1016/b978-0-12-805419-2.00020-4

Ogbechie-Godec, O. A., & Elbuluk, N. (2017). Melasma: An Up-to-Date Comprehensive Review. *Dermatology and Therapy*, *7*(3), 305–318. https://doi.org/10.1007/s13555-017-0194-1

Piras, A., Era, B., Di Petrillo, A., González Paramás, A. M., Maxia, A., Maccioni, A., Porcedda, S., Falconieri, D., & Rosa, A. (2017). Evaluation of Antioxidant and Tyrosinase Inhibitory Activities of the Extracts of Sarcopoterium spinosum (L.) Spach Fruits. *Natural Product Research*, *31*(24), 2900–2904. https://doi.org/10.1080/14786419.2017.1297994

Rathee, P., Kumar, S., Kumar, D., Kumari, B., & Yadav, S. S. (2021). Skin Hyperpigmentation and its Treatment with Herbs: An Alternative Method. *Future Journal of Pharmaceutical Sciences*, *7*(1), 132. https://doi.org/10.1186/s43094-021-00284-6

Raymond, J., Rakariyatham, N., & Azanza, J. L. (1993). Purification and Some Properties of Polyphenoloxidase from Sunflower Seeds. *Phytochemistry*, *34*(4), 927–931. https://doi.org/10.1016/s0031-9422(00)90689-7

Rathore, S. P., Gupta, S., & Gupta, V. (2011). Pattern and Prevalence of Physiological Cutaneous Changes in Pregnancy: A Study of 2000 Antenatal Women. *Indian Journal of Dermatology, Venereology and Leprology*, *77*(3), 402–402. https://doi.org/10.4103/0378-6323.79741

Rosiana, V., Taurhesia, S., & Djamil, R. (2024). Synergistic Effect on Tyrosinase Inhibition of Cassia Fistula Flower with Gynura procumbens Leaf Extracts. *South African Journal of Botany*, *170*, 172–176. https://doi.org/10.1016/j.sajb.2024.05.033

Roulier, B., Pérès, B., & Haudecoeur, R. (2020). Advances in the Design of Genuine Human Tyrosinase Inhibitors for Targeting Melanogenesis and Related Pigmentations. *Journal of Medicinal Chemistry*, *63*(22), 13428–13443. https://doi.org/10.1021/acs.jmedchem.0c00994

Sahu, R. K., Roy, A., Matlam, M., Deshmukh, V. K., Dwivedi, J., & Jha, A. K. (2013). Review on Skin Aging and Compilation of Scientific Validated Medicinal Plants, Prominence to Flourish a Better Research Reconnoiters in Herbal Cosmetic. *Research Journal of Medicinal Plant*, *7*(1), 1–22. https://doi.org/10.3923/rjmp.2013.1.22

Sarkar, T., Shil, A., Singha, D. K., & Chakraborty, M. (2022). Role of Tyrosinase Extraction from Banana (Musa acuminata). *International Journal of Research Publication and Reviews*, *3*(1), 1300–1303.

Shin, M., Truong, V.-L., Lee, M., Kim, D., Kim, M. S., Cho, H., Jung, Y. H., Yang, J., Jeong, W. S., & Kim, Y. Hoon. (2023). Investigation of Phenyllactic Acid as a Potent Tyrosinase Inhibitor Produced by Probiotics. *Current Research in Food Science*, *6*, 100413. https://doi.org/10.1016/j.crfs.2022.100413

Singh, B., Suri, K., Shevkani, K., Kaur, A., Kaur, A., & Singh, N. (2018). Enzymatic Browning of Fruit and Vegetables: A Review. *Enzymes in Food Technology: Improvements and Innovations*, 63–78. https://doi.org/10.1007/978-981-13-1933-4_4

Strothkamp, K. G., Jolley, R. L., & Mason, H. S. (1976). Quaternary Structure of Mushroom Tyrosinase. *Biochemical and Biophysical Research Communications*, *70*(2), 519–524. https://doi.org/10.1016/0006-291x(76)91077-9

Thring, T. S. A., & Weitz, F. M. (2006). Medicinal Plant use in the Bredasdorp/Elim Region of the Southern Overberg in the Western Cape Province of South Africa. *Journal of Ethnopharmacology*, *103*(2), 261–275. https://doi.org/10.1016/j.jep.2005.08.013

Tabassum, N., Lee, J.-H., Yim, S.-H., Batkhuu, G. J., Jung, D.-W., & Williams, D. R. (2016). Isolation of 4,5-O-Dicaffeoylquinic Acid as a Pigmentation Inhibitor Occurring in Artemisia capillaris Thunberg and its Validation in Vivo. *Evidence-Based Complementary and Alternative Medicine*, *2016*(1), 1–11. https://doi.org/10.1155/2016/7823541

Tu, C., Lin, M., Lu, S., Qi, X., Zhang, R., & Zhang, Y. (2012). Curcumin Inhibits Melanogenesis in Human Melanocytes. *Phytotherapy Research*, *26*(2), 174–179. https://doi.org/10.1002/ptr.3517

Vanitha, M., & Soundhari, C. (2017). Isolation and Characterisation of Mushroom Tyrosinase and Screening of Herbal Extracts for Anti Tyrosinase Activity. *International Journal of ChemTech Research*, *10*(9), 1156–1167.

Walz, A., Lehmann, U., Duthaler, U., Mäser, P. & Wittlin, S. (2024). *In vivo* Antimalarial efficacy of *Artemisia afra* Powder Suspensions. *Phytomedicine*, 129, 155644. https://doi.org/10.1016/j.phymed.2024.155644

Wang, H., Chen, J., Hu, J., Si, J., Xie, Y., Wei, J., Liu, Y., & Pei, D. (2024a). Tyrosinase Inhibitor Screened from Olea Europaea L. Leaves: Identification, molecular Docking Analysis and Molecular Mechanisms. *Industrial Crops and Products*, *210*, 118112. https://doi.org/10.1016/j.indcrop.2024.118112

Wang, Z., Cui, M., Wang, H., Ma, L., Han, Y., Han, D., & Yan, H. (2024b). Identification of Tyrosinase Inhibitors in Defatted Seeds of Evening Primrose (Oenothera biennis L.) by Affinity-Labeled Molecular Networking. *Food Research International*, *180*, 114097. https://doi.org/10.1016/j.foodres.2024.114097

Yagi, S., Zengin, G., Ibrahime Sinan, K., Yavuz Paksoy, M., Cziáky, Z., Jekő, J., & Dall'Acqua, S. (2024). Analyzing the Chemical Constituents and in vitro Biological Effects of Extracts Artemisia absinthium L. Extracts Obtained Using Different Methodologies and Solvents. *Chemistry and Biodiversity*, *21*(8), e202400893. https://doi.org/10.1002/cbdv.202400893

Zeng, H.-J., Li, Q.-Y., Ma, J., Yang, R., & Qu, L.-B. (2021). A Comparative Study on the Effects of Resveratrol and Oxyresveratrol Against Tyrosinase Activity and Their Inhibitory Mechanism. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *251*, 119405. https://doi.org/10.1016/j.saa.2020.119405