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Screening of two medicinal plants for their anti-tyrosinase activity

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Abstract
The present study was designed to appraise the depigmenting ability of two medicinal plants i.e *Asparagus racemosa* and *Holarrhena antidysenterica*. The various solvent extract of medicinal plants were prepared by using decoction method. Tyrosinase enzyme was used as the model system for evaluating the anti-tyrosinase activity of plant extract. The results of the study show that the aqueous extract of *Asparagus racemosa* (57%) exhibited the maximum inhibiting potential of the tyrosinase enzyme. The ethanolic extract of *Holarrhena antidysenterica* (31%) exhibited the minimum inhibiting potential of the tyrosinase enzyme.
Tyrosinase, also known as polyphenol oxidase, is a key enzyme that catalyse synthesis of melanin in plants, microorganism and melanin cell. Melanin biosynthesis inhibitory compound are useful for skin whitening agents used in cosmetics and also remedy for disturbances in hyperpigmentation. Contributing to their roles in tissue remodeling in health and disease.

Keywords: Melanin, hyperpigmentation, depigmentation, *Asparagus racemosa, Holarrhena antidysenterica,* anti tyrosinase

1. Introduction
The skin is the primary defense against invasion by bacteria, viruses, and other toxic elements and is the largest organ exposed to various oxidative insults. Reactive oxygen species (ROS) generated exogenously reacts with various biomolecules present in the skin and they play an important role in the skin disorders. The ultraviolet radiation from the sunlight is the most common exogenous factor and pernicious to the skin. It leads to the alterations in the composition of the skin including the accumulation of elastic fibers, collagen reduction and degeneration and deposition of glycosaminoglycans. Such damage to the skin causes reduction in the elasticity of skin and the linearity of dermal elastic fibers, inducing wrinkling resulting anti-aging appearance of the skin.
The exposure of the skin to ultraviolet radiation also induces the secretion of melanin due to rapid proliferation of melanocytes. The abnormal secretion of melanin leads to hyperpigmentation of the skin. Melanin in the epidermal layers of the skin is produced by a pathway called melanogenesis in which tyrosinase is the important rate limiting enzyme. It catalyses three steps of melanin biosynthesis including the hydroxylation of tyrosine to 3, 4 – dihydroxyphenylalanine (DOPA), Oxidation of DOPA to DOPA quinone and oxidation of 5, 6- dihydroxyindole to indolequinone. So, the enzyme tyrosinase is the key target for finding out the skin lightening agents either from natural or synthetic origin. Many tyrosinase inhibitors are used as skin whitening agents including licorice extract, arbutin, and kojic acid. In recent times, there has been much attention focused on the application of natural plant extracts as skin lightening agents in cosmetic industry. Reactive oxygen species are also implicated in various other disease conditions namely cardiovascular diseases, diabetes mellitus, cancer and neurodegenerative diseases. Medicinal plant such as *Asparagus racemosa, Holarrhena antidysenterica* exhibita wide range of pharmacological activities, and have been shown to have anticancer, anti-inflammatory and anti-aging properties. So, the present investigation was performed to explore the potential skin whitening and antioxidant agents from natural sources because of their effectiveness, lower cost and less adverse effects when compared to synthetic ingredients.

2. Material and Methods

2.1 Medicinal plant

Asparagus racemosus and *Holarrhena antidysenterica* material were procured from commercial vendor in Akola district, Maharashtra and were authenticated by botany department of Shri Shivaji College, Akola. The plant material were dried in shade, grind and the powder were used.

2.2 Plant extract preparation

One gram of *Asparagus racemosa* and *Holarrhena antidysenterica* powder was dissolved in 10ml of water/ethanol. The solution was heated in a boiling water bath at 60 degree centigrade for 60 minutes. The mixture was cooled to room temperature and centrifuged at 6000 rpm for 10 minutes. The supernatant was filtered and the filtrate was collected and used for analysis.

2.3 Preparation of Tyrosinase Solution

100 g fresh tomato was purchased from local market. After decorticated and chopped, the tomato was soaked in 200 mL phosphate buffer solution (0.2 M, pH 7.2), grinded into powder and filtered by paper, the solution was centrifuged at 4000 g × 5 min. The supernatant was suctioned and stored at −4 °C for next use.

2.4 Antityrosinase assay

Tyrosinase (Phenol oxidase activity) which catalyses the transformation of L-tyrosine into L-DOPA by hydroxylation and further into O-dopaquinone by oxidation. Then, through a series of non-enzymatic reaction, O-dopaquinon is rapidly transformed into melanins, which measured at 492 nm in a spectrophotometer. The reaction mixture without the enzyme serves as blank. The reaction mixture with the corresponding solvents (without plant material) serves as control. The percent inhibition of tyrosinase was calculated as follows:

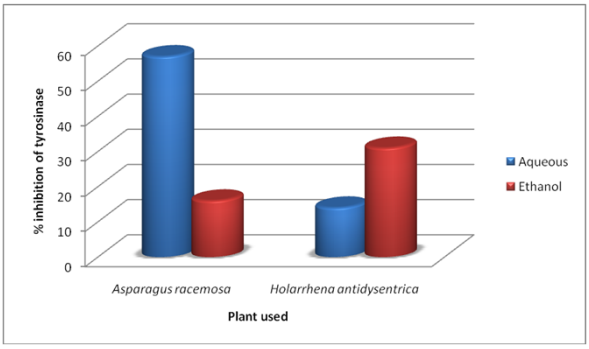
$$\% \text{ Inhibition of tyrosinase} = \frac{\text{OD of Control- OD of Test}}{\text{OD of Control}} \times 100$$

3. Results and Discussion

3.1 Tyrosinase inhibitory activity

Melanogenesis is an important biological phenomenon occurring in the melanocytes to protect the skin from free radical attacks which causes potential cellular injury to the skin. However, the excess secretion of melanin from melanocytes results in hyperpigmentation disorders namely skin darkening. Therefore, the discovery of tyrosinase inhibitors is becoming an urgent concern.

The results of tyrosinase inhibition potential of various solvent extract of two medicinal plant were illustrated in fig. Among the aqueous extract two medicinal plants tested for their tyrosinase inhibiting potential, aqueous extract of *Asparagus racemosus* showed maximum inhibition (57%). As compared to its ethanolic extract of *Holarrhena antidysenterica* (31%) exhibited higher inhibition as compared to aqueous extract.



4. Conclusion

Out of two *Asparagus racemosa and Holarrhena antidysenterica* selected for testing their antityrosinase activity, the aqueous extract of *Asparagus racemosa* possess the strongest inhibition in order to support the skin whitening potential due to tyrosinase inhibitory activity. Among the plants screened, both extract of *Asparagus racemosa* and *Holarrhena antidysenterica*, was found to be superior. The different extract of two different plants *Asparagus racemosa and Holarrhena antidysenterica*. The use of aqueous extract of *Asparagus racemosa* in cosmetic formulation to deliver the skin lightening and anti-aging benefits, once dermal safety is ensured.

5. References

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