Science Progress and Research

Research Article

ISSN: 2635-0955
DOI: https://doi.org/10.52152/spr/2021.149

Extraction, Purification of Tyrosinase and Tyrosinase Inhibitory Activity of Four Medicinal Plants from Nanded District (MS), India

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Submission Date: 12/08/2021 *Acceptance Date: 10/09/2021* *Published Date: 16/09/2021*

ABSTRACT

Tyrosinase has an important role in melanin formation, is responsible for the production of colour pigments of skin, hair, and eye. In the presents study, tyrosinase was isolated from Mushrooms, isolation of enzyme was done by acetone precipitation procedure and precipitation of enzyme was done with ammonium sulphate precipitation method. Plants selected for extraction were *Azadirachta indica* (Neem), *Manikara zapota* (Chiku), *Annona squamosa* (Sitaphal), *and Hibiscus Rosa-sinesis* (China rose). For phytochemical screening Alkaloids-Mayer's Test, Flavonoids (Shinoda Test, Alkaline Reagent Test), sugar (Benedict's reagent Test), Glycosides (Borntrager's Test), Phenolic compounds Test (Ferric chloride Test, Gelatin Test, Lead Acetate Test). Mushroom tyrosinase inhibitory assay was determined by the spectroscopic method. The study shows the tyrosinase inhibitory activity of selected medicinal plants.

Keywords: Mushroom, Tyrosinase inhibitory activity, Melanin, Medicinal Plants.

INTRODUCTION

The formation of melanin is known as melanogenesis and it is well known fact that the melanin protects the skin from damage. Melanin is a biopolymer produced by melanocytes and released and further dispersed in the dermis' basal layer. It shields the skin from the sun's rays. Tyrosinase is useful for the synthesis of pigments from skin, hair, and eye. Aside from its benefits, aberrant melanin synthesis and distribution are responsible for melasma, lentigines, age spots, and post-inflammatory hyperpigmentation, and other dermatological diseases. Recently, due to the development of tyrosinase inhibitors, the research in this field is continuously increasing. L-DOPA (3,4-dihydroxy phenylalanine) drug is prescribed for the treatment of Parkinson's disease. L-DOPA is a substrate for dopamine and can cross the blood-brain barrier. The mushroom tyrosinase (MT) active site is a close protein skeleton and is widely distributed throughout the phylogenetic scale from bacteria to mammals such as mushroom *Agaricus bisporus*. Tyrosinase is an oxygenase oxido-reductase and often referred to as polyphenols oxidase (PPO). It is a copper-containing enzyme that catalyzes the hydroxylation of tyrosine and oxidation of L-DOPA to O-dopaquinone. Tyrosinase has many applications in the field of medical science, the food industry, and industrial biotechnology such as the conversion of o-diphenol

drugs, like L-DOPA and protein cross-linking in food technology. Tyrosinase has a role in the melanin pathway and inhibitors of tyrosinase are useful in medicinal and cosmetics. [1]

The development of excellent tyrosinase inhibitors is critical in the agricultural and food industries to retain the nutritional content of food. [2] The majority of inhibitors are synthetic or derived from higher plants, such as polyphenols, flavonoids, aldehydes, and derivatives. [3] The selectivity and method of inhibition (competitive, non-competitive, or mixed) of synthetic compounds, as well as the chemical composition of compounds has been highly varied. [4] By chelating the active site of the enzymes, certain inhibitors impair both mono- and diphenol activities of tyrosinase. Furthermore, certain substances with fungal origins were discovered to have inhibitory action, such as Kojic acid, which is the best researched inhibitor of tyrosinase and is frequently used as a positive control in a variety of studies to compare the inhibitory potency of different inhibitors. Therefore, the present study was designed to assess the tyrosinase inhibitory activity from natural resources.

MATERIAL AND METHODOLOGY

Plant Collection and Study area

Mushrooms were used as source tyrosinase and purchased from a local market of Vishnupuri (Nanded). . L-dopa (3, 4-dihydroxy-L-phenylalanine), tyrosine, and all other chemicals were purchased from Hi-Media Lab. Ltd. Mumbai. The plant material was collected from the field of the Nanded region, shed dried, and finely powdered for extraction. *Azadirachta indica* (Neem), *Manikara zapota* (Chiku), *Annona squamosal* (Sitaphal), and *Hibiscus rosa-sinesis* (China rose) were selected for plant extraction.

Extraction of plant material

20g of plant powder was taken and added in the 100ml Hexane, Chloroform, Acetone, Methanol, Ethanol, and water respectively in a flask. The powder was kept for extraction at room temperature with gentle stirring overnight. After extraction, filtered through Whatman filter paper. All the above extract was dried and used for phytochemical screening and tyrosinase inhibition.

Phytochemical screening (Qualitative test)

For photochemical screening (qualitative test) for plant material carried out with Alkaloids-Mayer's test, Flavonoids (Shinoda test, Alkaline reagent test), sugar (Benedict's reagent test), Glycosides (Borntrager's test), Phenolic compounds test (Ferric chloride test, Gelatin test, Lead acetate test).

Mushroom tyrosinase extraction

Mushroom was taken after washing, and finely cut it into small pieces. The small pieces of Mushroom were ground with pre-cooled mortar and pestle. Tyrosinase isolated by acetone precipitation procedure. [5]

Purification of enzyme

Precipitation of enzyme was carried out with ammonium sulphate precipitation by the previously reported method. [6, 7]

Mushroom tyrosinase inhibitory assay

Tyrosinase activity is determined by the procedure described by Vanni et al.[8]. Figure 1 and 2 shows maximum inhibition in ethanolic extract 81% and methanolic extract 80% from the same plant *Manikara Zapota* as compared to all the other extracts. *Annona squamosa* was followed by *Manikara* and the remaining two plant extracts do not show considerable activity. Antioxidant activity was checked by the method. [9]

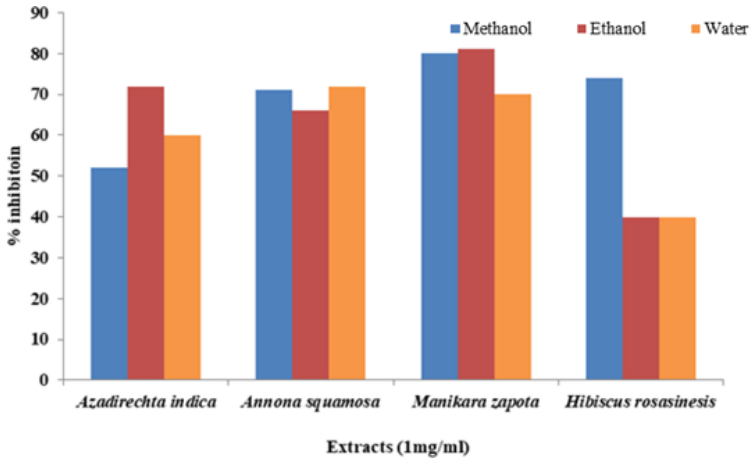

Figure 1. Tyrosinase enzyme inhibition percentage

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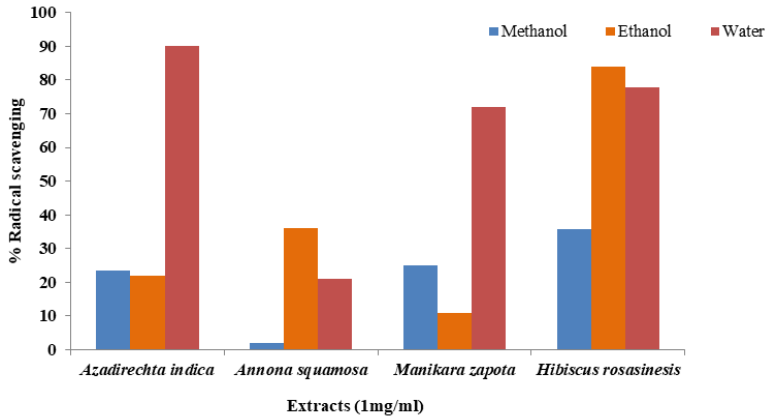

Figure 2. Antioxidant activity plant extracts

RESULT AND DISCUSSION

Phytochemical test

Qualitative tests of various solvent extractions reveal that the extracts are rich in flavonoids as almost all the tests are positive towards it except *Annona squamosa*, followed by phenolic compounds. Some of the extracts were also showing the presence of sugars, alkaloids, glycosides, fixed oil, and fats (Table 1).

Table 1. Phytochemical screening of four Medicinal Plants

Phytochemical Tests	*Azadirachta indica*			*Annona squamosa*			*Manikara zapota*			*Hibiscus rosa-sinesis*		
	Ethanol	Methanol	Distilled water	Ethanol	Methanol	Distilled water	Ethanol	Methanol	Distilled water	Ethanol	Methanol	Distilled water
Flavonoids	-	-	+	-	-	+	-	-	+	-	-	+
Flavonoids alkaline test	+	–	–	–	–	–	–	–		+	–	–
Phenolic compounds A. $FeCl_3$	+	-	-	-	-	-	–	–	–	+	–	–
B. Lead acetate	--	-	-	-	+	-	-	-	-	-	-	-
Alkaline test reagent (alkaloid)	+	-	-	-	-	+	-	-	+	-	-	-
Mayer's reagent	-	+	-	+	-	-	+	-	-	-	+	-
Glycosides	-	-	-	-	-	+	-	-	-	+	-	-
Saponin (Borntragers test)	–	–	–	–	–	–	–	–	–	–	–	–
Fixed oil & fat 1) spot test	+	-	-	-	+	-	-	-	+	-	+	-
2) Saponification	-	-	-	-	-	+	+	-	-	+	-	-
Sugars 1) Fehling solution	-	-	-	-	-	+	-	+	-	-	-	-
2) Barfoed test	+	-	-	-	-	-	-	-	-	+	-	-
3) Benedict test	-	+	-	-	+	-	-	-	-	+	-	-

Purification of enzyme

The tyrosinase enzyme was purified at 0.251 U/µg specific activities which represents the 4.04-fold purification after column chromatography (Figure 3). The eluent showing maximum activity was pooled together for further characterization.

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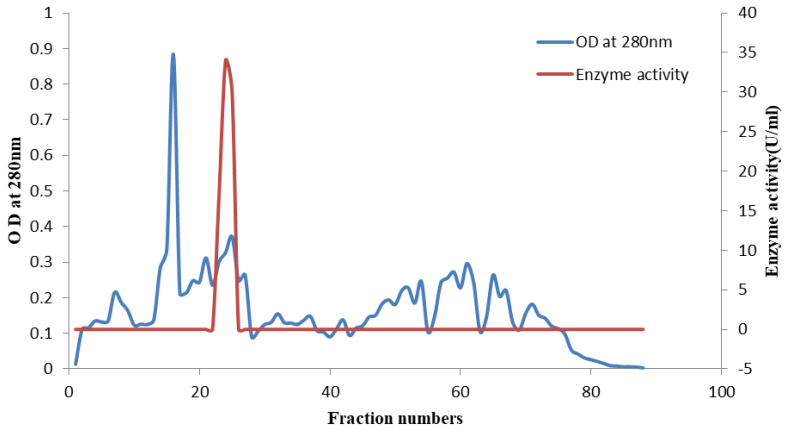

Figure 3. Fraction collection by column chromatography

The dialyzed enzyme sample was subjected to DEAE-Column chromatography. The enzyme was obtained in a 0.2 M NaCl fraction at fraction number 27. The specific activity of the enzyme after all purification steps was found to be 2 U/µg proteins with 17.09 fold purification. Ethanolic and methanolic extract of *Azadirachta indica* shows maximum inhibition as 81% and 80% respectively as compared to all the other extracts whereas water extract of *Azadirachta indica*, ethanolic, and water extracts of *Hibiscus rosa-sinesis* showing good antioxidant activity. These activities are contributed by the active phytochemical constituent present in the extracts as shown in the table.

CONCLUSION

The systematic study to explore the tyrosinase inhibitory activity of selected plants. The study included extraction, purification, and inhibition of the enzyme. Plant materials selected were *Azadirachta indica* (Neem), *Manikara zapota* (Chiku), *Annona squamosa* (Sitaphal), and *Hibiscus rosa-sinesis* (China rose) extracted serially from low to high polar solvent. The extracts were used for antioxidant and inhibition of purified enzymes from the mushroom. Enzyme purification was done by sequential ammonium sulphate precipitation, dialysis followed DEAE-Cellulose column chromatography. The enzyme precipitates on 85% saturation of ammonium sulphate, dialyzed and loaded on pre-equilibrated DEAE-cellulose column for further purification at 1ml/min flow rate. Purification shows the 26 fractions giving very good enzyme activity. The specific activity of the enzyme after all purification steps was found to be 2 U/µg proteins with 17.09 fold purification.

Conflicts of Interest

All contributing authors declare no conflicts of interest.

Source of Funding

None

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