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PRELIMINARY SCREENING OF SOME SELECTED PLANTS FOR ANTI-TYROSINASE ACTIVITY

Maitreyi Zaveri*, Archita Patel

K.B. Institute of Pharmaceutical Education and Research, Gandhinagar-382023, Gujarat, India

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For Correspondence:

Maitreyi Zaveri

K.B. Institute of Pharmaceutical Education and Research, Gandhinagar-382023, Gujarat, India

E-mail:

khandharmaitreyi@gmail.com

ABSTRACT

Hyperpigmentation is a condition related with increased skin pigmentation through increased skin melanogenesis or deposition of Melanin in the tissues of the skin. Melasma, dark spots, solar lentigos, post inflammatory hyperpigmentation etc. are the hyperpigmentation condition. Nowadays people are more conscious about their look and want an even skin tone. Melanin is synthesized by the enzyme tyrosinase, by inhibiting this enzyme, the melanin production and its deposition in the skin can be decreased. So this concept can be used to treatmelanin related skin disorders. Some of the plants which are used traditionally to treat the dark spot skin condition. In the present study *Azadirachta indica, Rubia cordifoia, Grewia tiliifolia, Murraya koenigii, Solanum lycopersicum, Carica papaya, Ficus religiosa, Mangifera indica, Nerium oleander* were screened for their antityrosinase activity. Tomato showed the maximum inhibitory activity with an IC_{50} value of 4.08 µg/ml compared to that of Kojic acid having an IC_{50} value of 5.53 µg/ml which is a known tyrosinase inhibitor.

INTRODUCTION

Melanin is a key factor determining the color of the skin. The enzyme tyrosinase plays the most important role in melanin synthesis (melanogenesis). Several tyrosinase inhibitors have been proposed based on the view that melanogenesis can be controlled, and skin whitening products can be developed if tyrosinase activity can be suppressed[1]. Pigmentation disorders arise as a result of an increased production of melanin by melanocytes and/or elevated transfer of melanosomes from melanocytes to basal and suprabasal keratinocytes. Once produced, melanin is transferred to keratinocytes or into the dermis via any of the below. Damage to melanocytes in the basal layer allows for phagocytization by melanophages releasing melanin into the dermis[2]. Melanosomes are directly deposited, through their dendrites into the dermis. Macrophages migrate into the epidermis where the melanosomes are phagocytized, returning them to the dermis. The disorders of hyperpigmentation are melasma, solar lentigos, post inflammatory hyperpigmentation etc[3]. The type and amount of melanin synthesized by the melanocyte and its distribution pattern in the epidermis determines the actual color of the skin. Melanin forms through a series of oxidative reactions involving the amino acid tyrosine and the enzyme tyrosinase. The first step is the most critical because the remainder of the reaction sequence can proceed spontaneously at physiological pH. Here, tyrosinase converts tyrosine to dihydroxyphenylalanine (DOPA) and then to dopaquinone. Subsequently, dopaquinone is converted to dopachrome through auto-oxidation, and, finally, to dihydroxyindole or dihydroxyindole-2-carboxylic acid (DHICA) to form eumelanin (black-brown pigment). The latter reaction occurs in the presence of dopachrome tautomerase and DHICA oxidase. In the presence of cysteine or glutathione, dopaquinone is converted to cysteinyl DOPA or glutathione DOPA. Subsequently, pheomelanin, a yellow-red pigment, is formed[4].

MATERIALS AND METHODS

Chemicals:

Tyrosinase enzyme was purchased from Sigma Chemicals, USA and L-dopa and other chemicals were purchased from SRL chemicals, Mumbai, India and kojic acid from Mulbe laboratories, Mumbai, India and L-ascorbic acid from suvidhinath laboratories, Baroda, India.

Collection of plant material:

The selected plant parts were collected from Lallu Vrajlal Gandhi Udhyan, Ahmedabad, Gujarat, India and papaya and tomato were collected from local vegetable market, Gandhinagar and the drugs were authenticated by comparing morphological characters with those in the literature. and were authenticated by Dr. S.K.Patel, Head of The Botany Department, Government Science College, Gandhinagar. The voucher specimen PH/10/0019 was deposited in K.B.Institute of pharmaceutical Education and Research, Gandhinagar Fresh tomato and papaya were ground into fine paste and shade dried and the other plant materials were shade dried and powdered. Thereafter 10 g of plant material was extracted with 50 ml of methanol for 24 hours by reflux extraction process. The solvent was distilled to obtain dried plant extracts.

Preparation of extracts:

Each of the plant extracts were dissolved in DMSO and three concentrations of each plant were prepared, i.e 10, 100, 1000 µg/ml to assess the anti-tyrosinase activity.

Assessment of anti-tyrosinase activity:

Take 1 ml of the phosphate buffer, which was dispensed in a test tube. Then add (500 µl) 0.5 ml of mushroom tyrosinase enzyme solution, mixed it and incubated at 25^0C for 10 min. Then finally add 1 ml of the substrate solution. The absorbance was monitored continuously in a recording spectrophotometer at 475 nm for 20 min[5]. The % inhibition can be calculated as follows:

% inhibition= $[(A_{control} - A_{sample})/ A_{control}] \times 100$.

Statistical Analysis:

The IC_{50} values were calculated using a logarithmic non-linear regression curve derived from the plotted data using Graph Pad Prism software version 4.03 (Graph Pad Software, USA).

RESULTS

Assessment of antityrosinase activity:

Hyperpigmentation is a skin disorder resulting from melanogenesis and increased melanin deposition in the skin tissue resulting into melasma, solar lentigos, dark spots, post inflammatory hyperpigmentation. The antityrosinase activity model referred here is a model for preliminary screening. Some of the selected plants which are used traditionally viz. *Azadirachta indica, Rubia cordifoia, Grewia tiliifolia, Murraya koenigii, Solanum lycopersicum, Carica papaya, Ficus religiosa, Mangifera indicum, Nerium indicum* were screened for their antityrosinase activity. The activity was screened out at 25-30^0C, the tomato fruit extract showed the maximum % inhibition against tyrosinase with an IC_{50} value of 4.08 µg/ml, when compared with a known tyrosinase inhibitor, kojic acid showed an IC_{50} value of 5.53 µg/ml as shown in table 1. The significant differences between control and test samples were observed. ($p<0.05$) as shown in table 2. The present study revealed that majith root extract, papaya seed extract, dhamni leaf extract, peepal tree bark extract, showed poor tyrosinase inhibitory activity compared to the reference standard (i.e <50 %) while neem leaf, papaya fruit and mango bark showed 50 % inhibition. However, tomato fruit extract and nerium leaf extracts showed good IC50 values i.e 4.03 µg/ml and 54.2 µg/ml respectively, when compared with reference standard kojic acid and ascorbic acid having an IC50 value of 5.53 µg/ml and 6.4 µg/ml respectively as shown in table 3.

Table 1: Plants selected for Anti tyrosinase screening

Sr. No.	Botanical name of Plant	Common name of Plant	Part of plant used
1.	*Azadirachta indica*	Neem	Leaves
2.	*Rubia cordifolia*	Majith	Roots
3.	*Grewia tiliifolia*	Dhamni	Bark
4.	*Murraya koenigii*	Curry tree	Leaves

5.	*Solanum lycopersicum*	Tomato	Fruit
6.	*Carica papaya*	Papaya	Fruit
7.	*Carica papaya*	Papaya	Seed
8.	*Ficus religiosa*	Peepal tree	Bark
9.	*Mangifera indicum*	Mango tree	Bark
10.	*Nerium oleander*	Red oleander	Leaves

Table 2: Effect of methanolic extracts of the selected plants on tyrosinase inhibition at three different concentrations at 475 nm

Plant extracts / Std	Control Mean ± SEM (n=3)	10 µg/ml Mean ± SEM (n=3)	100 µg/ml Mean ± SEM (n=3)	1000 µg/ml Mean ± SEM (n=3)
kojic acid	0.872± 0.0019	0.357±0.0032	0.174±0.0019	0.078±0.0145
Ascorbic acid	0.87±0.03	0.429±0.0015	0.249±0.0003	0.18±0.0009
neem leaf extract	0.837±0.0032	0.778±0.0012	0.608±0.0012	0.410±0.0015
majith root extract	0.878±0.0035	0.811±0.0011	0.615±0.0003	0.241±0.0017
Dhaman bark extract	0.837±0.0007	0.545±0.0009	0.625±0.0009	0.812±0.0003
Curry tree leaf extract	0.82±0.0006	0.502±0.0003	0.328±0.0015	0.466±0.0006
Tomato fruit extract	0.825±0.0003	0.445±0.0015	0.239±0.0007	0.347±0.0010
Papaya fruit extract	0.85±0.0006	0.331±0.0006	0.425±0.0009	0.229±0.0006
Papaya seed extract	0.84±0.0003	0.814±0.0009	0.669±0.0003	0.501±0.0012
Peepal bark extract	0.84±0.0007	0.681±0.0003	0.703±0.0006	0.621±0.0003
Mango bark extract	0.844±0.0003	0.462±0.0010	0.422±0.0006	0.329±0.0006
Nerium leaf extract	0.834±0.0007	0.453±0.0012	0.399±0.0009	0.34±0.0006

Table 3: % tyrosinase inhibition and IC_{50} value of the methanolic extracts of the selected Plants

Plant extracts / Std	Concentration in µg/ml	% Inhibition	IC_{50} value
Kojic acid	10	59	
(std)	100	80	5.53 µg/ml
	1000	91	
Ascorbic acid	10	51	
(std)	100	71	6.38µg/ml
	1000	80	
Neem leaves	10	7	
	100	29	981µg/ml
	1000	51	
Majith roots	10	12	
	100	20	------
	1000	29	
Dhamni leaves	10	35	
	100	28	------
	1000	27	
Curry tree	10	38	
	100	60	82.6µg/ml
	1000	54	
Tomato	10	46	
	100	71	4.08µg/ml
	1000	58	
Papaya fruit	10	39	
	100	50	-------
	1000	27	
Papaya seed	10	4	
	100	25	-------
	1000	35	
Peepal tree bark	10	24	
	100	21	--------
	1000	34	
Mango bark	10	45	
	100	50	56.2µg/ml
	1000	61	
Nerium leaves	10	40	
	100	58	54.2µg/ml
	1000	60	

DISCUSSION

Hyperpigmentation disorders arise as a result of an increased production of melanin by melanocytes and/or elevated transfer of melanosomes from melanocytes to basal and suprabasal keratinocytes [1-2]. Tyrosinase is a copper-containing enzyme, which is widely distributed in microorganisms, animals and plants. Tyrosinase is a key enzyme for melanin biosynthesis and is involved in determining the color of mammalian skin and hair. Therefore, finding effective tyrosinase inhibitors, either from natural sources, is beneficial in the treatment of melanin-related disorders [5]. Various dermatological disorders, such as melasma, age spots and sites of actinic damage, arise from the accumulation of an excessive level of epidermal pigmentation. In addition, unfavorable enzymatic browning of plant-derived foods by tyrosinase causes a decrease in nutritional quality and economic loss of food products. The inadequacy of current conventional techniques to prevent tyrosinase action encourages to seek new potent tyrosinase inhibitors[6]. Tyrosinase enzyme which is the key enzyme for melanin production, firstly converts tyrosine to dopa, then dopa to dopaquinone, both reactions occurs through oxidation. Then dopaquinone gets subsequently converted to dopachrome, and then to melanin[3]. So, tyrosinase enzyme is viewed as the important target for the discovery and isolation of tyrosinase inhibitors for the treatment of hyperpigmentation disorders.

Here, the present study has been conducted for the preliminary screening of the plants which are used traditionally to improve the complexion or to treat the dark spots on the skin [7, 8] as they may have some action on the tyrosinase enzyme to treat the hyperpigmentation condition. Some of the plants which are used traditionally viz. *Azadirachta indica, Rubia cordifoia, Grewia tiliifolia, Murraya koenigii, Solanum lycopersicum, Carica papaya, Ficus religiosa, Mangifera indicum, Nerium indicum* were screened for their antityrosinase activity[9].

To our knowledge previous phytochemical investigations of these plants did not revealed the natural compounds responsible for tyrosinase inhibitory activity and tomato is often used in cosmetic formulation for skin whitening. These plants could represent potential source for tyrosinase inhibitors.

For the confirmation of this activity biological investigations must be done. The isolation of the active compounds will reveal new tyrosinase inhibitors for skin whitening and to treat hyperpigmentation which can be used as skin lighteners.

CONCLUSION

Based on our study, it can be concluded that the plant extracts such as tomato extract, nerium leaf extract, mango bark extract which have shown good results for tyrosinase inhibition, i.e % Inhibition. Knowledge of this can be used for the development of formulation, which can be useful for skin lightening and melanin related disorders.

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