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Preliminary Screening of Selected Plant Extracts for Anti Tyrosinase Activity

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Abstract

Tyrosinase inhibition is an important approach towards controlling hyper pigmentation. We aimed to screen alcoholic extracts of 11 plants extract for their tyrosinase inhibitory activity. These plants have been used traditionally in the treatment of skin ailments and for the improvement of skin complexion. The extracts were quantified for total phenols, alkaloids and tannins. *In vitro* tyrosinase inhibition was performed with kojic acid as the positive control. Cell viability was tested on B16 F0 melanoma cells. The extracts of *Rosa berberifolia*, *Punica granatum* and *Casiia angustifolia* showed more than 80% inhibition at 500 mg/ml concentration. Nine of the extracts were also shown to have a high phenolic content greater than 200 mg/g of the plant material. The tyrosinase inhibitory activity of the extracts of *Cassia angustifolia*, *Punica granatum* and *Rosa berberifolia* were comparable with that of the control, kojic acid. The three extracts also showed lesser than 50% cytotoxicity at the concentrations tested. From the screening assays, it is seen that three plants have appreciable tyrosinase inhibitory activity. Hence, these plants may be further evaluated for their use in cosmetics and hyper pigmentation.

Keywords: Cosmetics, Melanin, Plant Extracts, Phenolics, Skin Whitening, Tyrosinase Inhibition

1. Introduction

Melanin is a major contributor of skin pigmentation and protects skin against harmful ultraviolet radiation[1]. Melanogenesis is a complex process which includes melanin synthesis, transport and release of melanosome. Abnormal melanin deposition in the skin could be triggered by many patho-physiological and environmental factors. Melanin degradation has great cosmetic relevance and has prompted research and development in the area of natural and chemical agents interfering with melanin synthesis[2].

Tyrosinase is a copper-containing enzyme that is implicated in pigmentation[3]. This enzyme catalyzes the hydroxylation of l-tyrosine to 3,4-Dihydroxy-l-Phenylalanine (l-DOPA), and, the oxidation of l-DOPA to DOPA quinone. The latter is a substrate for the synthesis of pheomelanins or eumelanins, which are red-yellow or black-brown pigments[4]. Therefore, the regulation of tyrosinase has been useful for the treatment of pigmentation disorders and in the development of cosmetic whitening agents. Tyrosinase inhibitor is a target for reduction of melanogenesis[5].

Herbal medicines provide an interesting, largely unexplored source for development of potential new agents that can antagonize tyrosinase activity[6].

This paper will compare the biological evaluation of extract of 11 plants, in mushroom tyrosinase inhibition, against a well known positive control: Kojic acid. The cytotoxicity of the extracts was also tested in B16 F0 cells and the phytochemical properties of the extracts were quantified.

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2. Materials and Methods

2.1 Reagents

L-tyrosine, L-DOPA, mushroom tyrosinase and kojic acid, 3-(4,5-Dimethyl–Thiazol-2-yl)-2,5-Diphenyl Tetrazolium Bromide (MTT) and Dimethyl Sulfoxide (DMSO) were purchased from Sigma (St. Louis, MO, USA).

2.2 Preparation of Extract

Eleven plants (Table 1.) used in this study were collected from Tamil Nadu Agricultural University, Coimbatore. To prepare the alcoholic extract, 20 g of each powdered plant material was extracted with 300 ml of chloroform: methanol (2:1) and concentrated in a flash evaporator.

2.3 Estimation of Total Phenols

Total phenol content in the plants extract was estimated by Folin Ciocalteau method[7].

2.4 Estimation of Alkaloids

Alkaloids were estimated by gravimetry method[8].

2.5 Estimation of Tannins

Tannins were estimated using Folin-Denis reagent as mentioned by[9].

2.6 Mushroom Tyrosinase Inhibition Assay

Extract of the plants were dissolved in Dimethyl Sulfoxide (DMSO) to a final concentration of 20 mg/ml. This extract stock solution was then diluted to 500mg/ml in 50mM potassium phosphate buffer (pH 6.5). Kojic acid (1% concentration) was used as a positive control. 70ml of each sample solution of different concentrations (200-500 mg/ml) were combined with 30ml of tyrosinase (333 Units/ml in phosphate buffer, pH 6.5) in triplicate in a 96-well microtitre plates. After incubation at room temperature for 5 min, 110ml of substrate (2mM l-tyrosine) were added to each well. Microtitre plates were incubated for 30min at room temperature. Absorbance was measured at 475nm using an enzyme-linked immunosorbent assay reader.

Tyrosinase inhibitory activity was calculated with the following formula:

$$\text{tyrosinase inhibition (\%)} = [1-(\text{OD475 of sample}/\text{OD475 of control})]\times 100.$$

2.7 Cell Culture

Murine melanoma B16 F0 cells (National Centre for Cell Sciences, Pune) grown in RPMI 1640 medium (GIBCO, USA) supplemented with 10% heat-inactivated fetal bovine serum (GIBCO, USA), 1% penicillin/streptomycin (GIBCO, USA) were cultured at 37°C in a humidified atmosphere of 5% CO_2.

2.8 Assay of Cell Viability

Cell viability was determined using the MTT method. For experiments, cells were plated in 24-well plates at 1×105 cells/well. After 24 h, the test sample was added to each well and incubated for 24 h. Cell survival was determined in a colorimetric assay at 570 nm employing

Table 1: Selected plants used in this study and their identified uses on skin ailments

Sl. No.	Scientific Name	Part used	Uses	Reference
1	*Punica granatum*	rind	skin toner	[12]
2	*Ananus comosus*	fruit	firming skin	[13]
3	*Rosa berberifolia*	petals	skin care	[14]
4	*Brassica rapa*	tap root	cure skin cancer	[15]
5	*Mentha piperrita*	leaves	anti inflammatory	[16]
6	*Trigonella foenum- graecum*	seeds	skin whitener	[17]
7	*Nigella sativa*	seeds	treat skin infections	[18]
8	*Casiia angustifolia*	flower	skin moisturizer	[19]
9	*Acalypha indica*	leaves	scabies, skin diseases	[20]
10	*Solanum nigrum*	fruit	improve complexion	[14]
11	*Hedychium spicatum*	rhizhome	improve complexion	[21]

mitochondrial dehydrogenase activity in active mitochondria to form purple formazan. Cell viability was calculated as follows:

Cell viability (%) = (absorbance of the sample tested/ absorbance of the medium only) ×100.

3. Results and Discussion

Among the extracts tested, the extract of *Casia angustifolia*, *Rosa berberifolia* and *Punica granatum* showed more than 80% inhibition of tyrosinase activity at 500 mg/ml concentration. Kojic acid showed 80 % inhibition at 10mg/ml. All the samples significantly inhibited tyrosinase activity in comparison with the negative control ($P < 0.05$) (Table 2).

In the past few decades, a number of polyphenol tyrosinase inhibitors from both natural and synthetic sources, including flavonoids, stilbenes, and terpenoids, have been intensively investigated[10]. Estimation of total phenolics, alkaloids and tannic acid indicated that all the three extracts which showed higher tyrosinase inhibition also had higher concentration of alkaloids, tannins and phenolics (Table 2).

Phenolics have been found to be the most plentiful chemical component in plant kingdom, carrying out multiple biological effects[11]. The extracts which showed higher phenolic content also had much higher tyrosinase inhibitory activity in this study.

Cytotoxicity assay (Figure 1.) revealed that the plants extract were not cytotoxic to the melanoma cells

Table 2: Phytochemical analysis and tyrosinase inhibition activity of selected plant extracts

Sl. No.	Scientific Name	Total phenols (µg/g)	Alkaloids (µg/g)	Tannins (µg/g)	Tyrosinase inhibition (%) at 500 µg/ml
1	*Punica granatum*	340	101	2120	82.3±5
2	*Ananus comosus*	222	99	1160	55±2
3	*Rosa berberifolia*	258	80	1640	84±2
4	*Brassica rapa*	194	65	927	55.6±2.5
5	*Mentha piperrita*	250	41	221	37.6±3.05
6	*Trigonella foenum- graecum*	200	84	1140	52±3.6
7	*Nigella sativa*	232	98	780	49.6±2.08
8	*Casiia angustifolia*	258	45	1132	80.6±2.08
9	*Acalypha indica*	250	14	300	45.7±4.04
10	*Solanum nigrum*	219	22	666	8.8±1.25

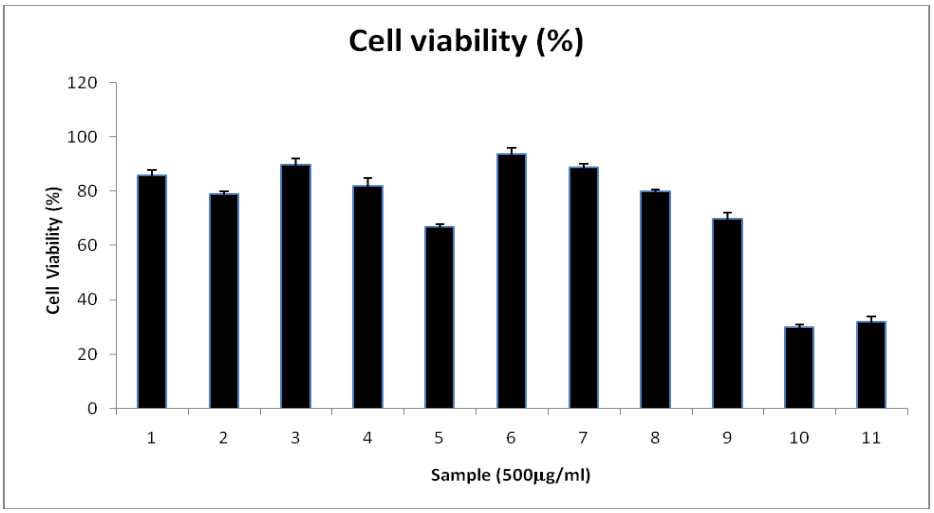

Fig. 1. Cell viability of mouse B16 F0 cells treated with plant extracts.
1- *Punica granatum*, 2- *Ananus comosus*, 3- *Rosa berberifolia*, 4- *Brassica rapa*, 5- *Mentha piperrita*, 6- *Trigonella foenum- graecum*, 7- *Nigella sativa*, 8- *Casiia angustifolia*, 9- *Acalypha indica*, 10- *Solanum nigrum*, 11- *Hedychium spicatum*

at the concentration at which they exhibited highest tyrosinase inhibitory activity. Except *Solanum nigrum* and *Hedychium spicatum,* others showed more than 50% cell survival at the concentration tested for tyrosinase inhibition.

4. Conclusion

From the screening assays, it is seen that the extract of *Casiia angustifolia*, *Punica granatum* and *Rosa berberifolia* have appreciable tyrosinase inhibitory activity at a concentration of 500 mg/ml. These extracts were not toxic to the cells at tested concentration. Hence, these plant species may be further evaluated for their use in cosmetic applications and hyper pigmentation properties.

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6. References

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