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 Cassia 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 pheomelans or eumelans, 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:

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

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

| 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 piperita | leaves    | anti inflammatory | [16]       |
| 6      | Trigonella foenum-graecum | seeds | skin whitener | [17]        |
| 7      | Nigella sativa  | seeds     | treat skin infections | [18]       |
| 8      | Casia angustifolia | flower | skin moisturizer | [19]       |
| 9      | Acalypha indica | leaves    | scabies, skin diseases | [20]       |
| 10     | Solanum nigrum  | fruit     | improve complexion | [14]       |
| 11     | Hedychium spicatum | rhizome | improve complexion | [21]       |
mitochondrial dehydrogenase activity in active mitochondria to form purple formazan. Cell viability was calculated as follows:

\[
\text{Cell viability (\%) = \left( \frac{\text{absorbance of the sample tested}}{\text{absorbance of the medium only}} \right) \times 100.}
\]

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\textsuperscript{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\textsuperscript{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](image)

**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 piperita*       | 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 piperita*, 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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