ANTITYROSINASE AND ANTIOXIDANT ACTIVITIES OF VARIOUS PARTS OF MIMUSOPS ELENGI: A COMPARATIVE STUDY

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Abstract

The present study was undertaken to identify the new actives from plant origin for their antityrosinase and antioxidant activities to determine the anti-aging and skin whitening potential. The various parts (bark, fruit, flower and leaves) of Mimusops elengi were studied for their skin whitening and antioxidant potential in various solvents. The skin whitening ability of plant extracts was examined through tyrosinase inhibition assay. The antioxidant potential of the herb was investigated by 2, 2-diphenyl 1-picryl hydrazyl radical (DPPH) scavenging and ferric reducing power assay. Among the parts studied, the methanolic extract of M.elengi flowers showed the highest inhibition of tyrosinase with an IC\textsubscript{50} value of 401 µg which was followed by methanolic extract of leaves. The methanolic extract of flowers (96.57%) and fruits (97.15%) possessed the highest and almost similar inhibition of DPPH radical when compared to other parts studied. The ferric reducing ability of methanolic extract of M.elengi leaves was maximum when compared to all other parts. The flowers of M.elengi have superior skin whitening and antioxidant activities. Hence, it can be employed as a new active ingredient in skin whitening and anti-aging formulations.

Key words: Antityrosinase, antioxidant, anti-aging, Mimusops elengi, DPPH, IC\textsubscript{50} value, Ferric reducing power

INTRODUCTION

Skin is the important external defense organ of the body in living organisms. Hence, it is more prone to environmental factors which include UV light, drugs, pesticides, ozone, industrial waste, chemical solvents and pollutants. The exposure of skin to such environmental factors results in aging, hyperpigmentation, inflammation etc. Skin aging and hyperpigmentation pose an aesthetic problem in socioeconomic status. In skin aging, the degradation of collagen occurs in a rapid rate. As a result, the tensile strength of skin is impaired. The continuous exposure to various environmental factors leads to alterations in the connective tissue via the formation of lipid peroxides, enzymes and reactive oxygen species (Kaur et al., 2006).

Hyperpigmentation is caused by the key enzyme tyrosinase. It is a copper-containing monoxygenase that catalyses melanin synthesis in melanocytes. The accumulation of excessive epidermal pigmentation leads to various dermatological disorders such as freckling, age spots, and sites of actinic damage. So, tyrosinase inhibitors and antioxidant substances derived from herbs have become increasingly important in medication and cosmetics to prevent skin aging and hyperpigmentation (Briganti et al., 2003, Parvez et al., 2007).

Mimusops elengi Linn is a small to large evergreen ornamental tree which is highly used in traditional medicine for the treatment of various diseases. The main chemical constituents that contribute to the medicinal properties of this plant are quercetin, hydroquercetin, ursolic acid, betulnic acid, D-Mannitol, spinosterol, β-sitosterol glycoside, quercitol (Misra and Mitra, 1967), lupeol (Misra and Mitra, 1968), alkaloid isoretronecyl tiglate (Hart et al., 1968) and mixture of triterpenoid saponins (Varsheny and Badhwar, 1972).

The various medicinal properties of this plant have been studied and documented. The flowers are used for preparing lotion for wounds and ulcers, used as a brain tonic, expectorant, and for treating asthma. The saponins of fruit are
reported to have anti-inflammatory activity. Bark of *Mimusops elengi* possesses cardiotonic, alexipharmic (antidote), stomachic, antihelminthic, anti- HIV type I protease (Kusumoto et al., 1995), and astringent activities (Kirtikar and Basu, 1935). Bark extract is also given orally to cure diseases of gums and teeth (Singh et al., 1980). The pounded seeds pasted with oil are used for the treatment of obstinate constipation (Nusrat et al., 1995). The present study was an attempt to study the antioxidant and antityrosinase activities of various parts of *M.elengi*.

**MATERIALS AND METHODS**

**Collection of plant material**

The various parts (leaves, flowers, bark and fruits) of *M.elengi* were collected from the field in Coimbatore, Tamilnadu and authenticated. The materials were shade dried and ground into fine powder.

**Plant extract preparation**

1 gram of herbal powder was dissolved in 10 mL of water/methanol/petroleum ether. The mixture was heated in a boiling water bath at 60 °C for 60 minutes. It was cooled to room temperature and centrifuged at 6500 rpm for 10 minutes. The supernatant was filtered and the filtrate was used for analysis.

**Skin whitening assay**

**Antityrosinase assay (Lee et al., 2003)**

Tyrosinase (Phenoloxidase activity) 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 reactions, O-dopaquinone is rapidly transformed into melanin, which is measured at 492 nm in a spectrophotometer. Each plant extract was assayed for tyrosinase inhibition by measuring its effect on tyrosinase activity using a 96-well reader. The reaction was carried out in 50 mM potassium phosphate buffer (pH 6.8) containing 20 mM L-tyrosine and 312.5 U/mL mushroom tyrosinase at 30°C. The reaction mixture was pre-incubated for 10 min before adding the enzyme. The reaction mixture with the corresponding solvents (without plant material) served as control and without enzyme served as blank. The change in absorbance at 492 nm was measured. The percent inhibition of tyrosinase was calculated as follows:

\[
\% \text{ inhibition of tyrosinase} = \left( \frac{OD \text{ of Control} - OD \text{ of Test}}{OD \text{ of Control}} \right) \times 100
\]

**DPPH radical scavenging assay (Mensor et al., 2001)**

DPPH (2, 2-diphenyl 1-pirylic hydrazyl) is a commercially available, commonly used stable free radical, which is purple in colour. Antioxidant molecules when incubated, reacts with DPPH and converts it to diphenyl hydrazine, which is yellow in colour. The degree of discoloration of purple to yellow was measured at 520 nm, which is a measure of scavenging potential of plant extracts. 5 µl of plant extract was added to 195 µl of DPPH solution (0.1mM DPPH in methanol) in a microtitre plate. The reaction mixture was incubated at 25 °C for 10 minutes, after which the absorbance was measured at 520 nm. The DPPH with corresponding solvents (without plant material) served as control and with respective plant extracts served as blank. The DPPH radical scavenging activity of the plant extract was calculated as the percentage inhibition.

\[
\% \text{Inhibition of DPPH radical} = \left( \frac{OD \text{ of Control} - OD \text{ of Test}}{OD \text{ of Control}} \right) \times 100
\]

**Ferric reducing power assay**

The reducing antioxidant power of the plant extracts was determined by the method of Oyaizu (1986). Different concentrations of plant extracts in 1 ml of distilled water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [K₃Fe(CN)₆] (2.5 ml, 1% w/v). The mixture was incubated at 50°C for 20 min. Then, trichloroacetic acid (2.5 ml, 10% w/v) was added to mixture, which was then centrifuged for 10 min at 3000 rpm. The upper layer the of solution (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl₃ (0.5 ml, 0.1% w/v). The absorbance was measured at 700 nm against a blank using UV-Vis spectrophotometer after 30 mins. Increased absorbance of the reaction mixture indicates increase in reducing power. The reducing power of the plant material was expressed in terms mg of gallic acid equivalents/g of plant material.

**Determination of total phenolics (Mallick and Singh, 1980)**

Phenols react with phosphomolybdic acid in Folin-Ciocalteau reagent in alkaline medium and produce a blue colored complex (molybdenum blue) that can be estimated colorimetrically at 650 nm. Pipetted out different aliquots (0.2 to 2 ml) into test tubes. Made up the volume in each tube to 3.0 ml with distilled water. Added 0.5 ml of Folin-Ciocalteau reagent. After 3 minutes, added 2.0 ml of 20% (w/v) sodium carbonate solution to each tube. Mixed thoroughly, placed the tubes in a boiling water bath for exactly 1 minute, cooled and measured the absorbance at 650nm against reagent blank.

**Statistical analysis:**

Samples were analyzed in triplicate and the results were given as Mean ± S.D.

**RESULTS AND DISCUSSION**

**Tyrosinase inhibition assay**

The various parts (bark, flowers, fruits and leaves) of *M.elengi* in different solvents were studied for their antityrosinase activity (Fig 1). Among the parts tested, methanolic extracts of *M.elengi* flowers showed the maximum inhibition of tyrosinase (89.85%). This was followed by methanolic extract and aqueous extract of *M.elengi* leaves. The methanolic extract of all the parts...
exhibited the maximum inhibition of tyrosinase when compared to their corresponding aqueous and petroleum ether extracts. The petroleum ether extract of bark, flowers, fruits and leaves possessed the inhibition potential in the range of 23% - 33%. The aqueous extract of leaves exerted the percentage inhibition of 70.46% which was comparable to methanolic extract of the same. The least and almost the same inhibition potential of tyrosinase were seen in the aqueous extract of bark and flowers. The maximum inhibition potential of methanolic extract of this plant indicated that the active principle is highly soluble in methanol in all the parts.

The methanolic extract of M. elengi flowers exhibited highest antityrosinase activity. Hence it was further investigated for ascertaining the IC₅₀ value through dose dependent antityrosinase assay (Fig 2). The IC₅₀ value for the flowers was found to be 401 µg.

**DPPH scavenging assay**

Figure 3 shows the DPPH scavenging ability of various parts of *M. elengi* in different solvents. The methanolic extract of various parts showed the maximum inhibition of DPPH radical when compared to their corresponding aqueous and petroleum ether extract. The methanolic extract of fruits and flowers had the highest and almost the same inhibition of DPPH radical. The methanolic extract of *M. elengi* bark ranked second. This was followed by aqueous extract of fruits and methanolic extract of leaves. Petroleum ether extract of all the parts of this plant possessed the least inhibition of DPPH radical.

The methanolic extract of *M. elengi* flowers were showing good tyrosinase inhibition as well as DPPH inhibition. Hence, it was further studied for dose dependent
DPPH inhibition (Figure 4). The IC$_{50}$ value for methanolic extract of flowers for scavenging the DPPH radical was found to be 193.37 µg.

Ferric reducing power assay

The ferric reducing ability of various solvent extracts of *M. elengi* parts are given in the table 1. Among the parts studied for ferric reducing ability, methanolic extract of *M. elengi* leaves ranked first followed by aqueous extract. This was followed by methanolic extract of flowers. Petroleum ether extract of all the parts exhibited the least reducing power. The order of reducing power with respect to solvents is Methanol> Aqueous> Petroleum ether.

Table 1 represents the ferric reducing ability of various parts of *M. elengi*.

<table>
<thead>
<tr>
<th><em>M. elengi</em> parts</th>
<th>Solvents</th>
<th>Amount (mg of gallic acid equivalents/g of plant material)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bark</td>
<td>Aqueous</td>
<td>1.91</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>3.16</td>
</tr>
<tr>
<td></td>
<td>Petroleum ether</td>
<td>0.04</td>
</tr>
<tr>
<td>Fruits</td>
<td>Aqueous</td>
<td>0.445</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>1.21</td>
</tr>
<tr>
<td></td>
<td>Petroleum ether</td>
<td>0.07</td>
</tr>
<tr>
<td>Flowers</td>
<td>Aqueous</td>
<td>2.491</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>5.40</td>
</tr>
<tr>
<td></td>
<td>Petroleum ether</td>
<td>0.376</td>
</tr>
<tr>
<td>Leaves</td>
<td>Aqueous</td>
<td>9.80</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>11.81</td>
</tr>
<tr>
<td></td>
<td>Petroleum ether</td>
<td>0.076</td>
</tr>
</tbody>
</table>
Total phenol estimation

The amount of phenols present in the various solvent extracts of flowers and fruits were studied (Fig 5). The methanolic extract of *M. elengi* flowers possessed greater phenol content in comparison to the fruits. The order of phenol content with respect to solvents is: methanol > aqueous > Petroleum ether.

CONCLUSION

The current study implied that all parts of *M. elengi* showed good antityrosinase, DPPH scavenging and reducing power activities in methanol. This indicated that the compounds responsible for the above said activities were highly soluble in methanol. The flowers showed remarkable tyrosinase inhibitory activity in comparison to other parts. Bark, flowers and fruits exhibited comparable DPPH scavenging ability. However, their reducing power was less as compared to leaves. It was concluded from the study that flowers of *M. elengi* can be used as potent ingredients in depigmenting and antiaging formulations.

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