Investigation of inhibitory activity of *Sida cordifolia* on tyrosinase extracted from *Solanum tuberosum*

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**ABSTRACT**

The study was carried out to estimate antimelanogenesis activities of alcoholic extracts of *Sida cordifolia*, taking resveratrol as standard. The different concentrations of alcoholic extracts were subjected to preliminary phytochemical screening for the identification of different phytochemicals and was found to have ephedrine, pseudoephedrine, sterculic, malvalic and coronaric acid, saponins, betaphenethylamine, hypaphorine and ecdysterone and the results were compared with that standard. Tyrosinase or phenol oxidase is a copper containing monoxygenase, a principal enzyme for melanin synthesis. Tyrosine inhibitors are the substances which reduce or block melanin synthesis leading to skin whitening. A number of potent tyrosinase inhibitors are from synthetic, semi-synthetic and natural origins. For the purpose of the development of a skin-whitening agent, the present study intends to analyse the inhibitory activity of alcoholic extract on Potato tyrosinase at different concentrations.

**Key words:** Phytochemicals, *Sida cordifolia*, antioxidant, antimelanogenesis and anti-inflammatory activity.

**INTRODUCTION**

*Sida cordifolia* L. (Malvaceae) known as Bala in Ayurveda (Indian system of medicine) for its antirheumatic and antipyretic activities (Muzaffer et al., 1991). It is also used in folk medicine for the treatment of inflammation of the oral mucosa, blenorrea, asthmatic bronchitis, aching joints, cough, wheezing, edema and nasal congestion. The plant contains mainly alkaloids, fatty oils, steroids, resin, resin acids, mucin and potassium nitrate. The seeds show demulcent and laxative effects as well as the root infusion was found to possess astringent, diuretic and tonic properties and help in treating hemiplegia, facial paralysis and in urinary disorders (The Wealth of India, 1972; Rastogi and Malhotra, 2001). There are scanty reports on the analgesic, anti-inflammatory and hypoglycaemic activities of the plant. The present study focuses on the evaluation of these activities from aerial and root extracts of *Sida cordifolia* (Figure 1).
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Oil preparation is also useful to relieve from pain, swelling disorder. The extracts of different parts of the plant are reported to be used in Ayurvedic system of medicine for a variety of purposes (Table 1). These activities affirm the presence of biologically active compounds in the plant. Further investigations are in progress for the isolation of bioactive molecules and the establishment of the mechanism of actions to produce potential bioactive molecules from this plant.

Skin pigmentation is mainly displayed through melanin content which is synthesized in body through process of melanogenesis. This melanogenesis

![Fig 1 Sida cordifolia](image)

### Table 1. Pharmacological activities of different extracts of *Sida cordifolia*

<table>
<thead>
<tr>
<th>Nature of extract</th>
<th>Activity studied</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous extract of <em>S. cordifolia</em></td>
<td>Inflammation of the oral mucosa, blenorrhea, asthmatic bronchitis and nasal congestion.</td>
<td>Franzotti et al. (2000).</td>
</tr>
<tr>
<td>The extracts of aerial and root parts</td>
<td>Central and peripheral analgesic activities</td>
<td>Ravi Kanth and Diwan (1999).</td>
</tr>
<tr>
<td>Methanol extract of root</td>
<td>Hypoglycaemic activity</td>
<td>Franco et al. (2005).</td>
</tr>
<tr>
<td>Methanol extract</td>
<td>Antifungal activity against F. verticilloides</td>
<td>Ghosal et al. (1975).</td>
</tr>
<tr>
<td>Root extract</td>
<td>β-phenethylamines; carboxylated tryptamines; Quinazolinealkaloids; β-phenethylamine; ephedrine; ψ-ephedrine;S-(+)-Np-methyltryptophan methyl ester; hypaphorine; vasicinone; vasicine; vasicinol; sympathomimetic amines; bronchodilator principle isolated</td>
<td>Medeiros et al., (2006).</td>
</tr>
<tr>
<td>Ethanol extracts of <em>Sida cordifolia</em></td>
<td>Analgesic and anti-inflammatory activities</td>
<td>Sutradharn et al. (2006).</td>
</tr>
<tr>
<td>Ethanol extract of roots</td>
<td>Antistress, adaptogenic activity</td>
<td>Sumanth &amp; Mustafa (2009).</td>
</tr>
<tr>
<td>Aqueous fraction of the hydro alcoholic extract of the <em>Sida cordifolia</em> leaves</td>
<td>Vasorelaxant</td>
<td>Santos et al. (2006).</td>
</tr>
<tr>
<td>Chloroform and methanol 80% ethanol extracts hexane, dichloro methane, ethyl acetate and butanol.</td>
<td>Analgesic and anti-inflammatory activities of different extracts of <em>Sida cordifolia</em> Linn (SIC).</td>
<td>Sutradhar et al. (2006).</td>
</tr>
<tr>
<td>Methanolic extract</td>
<td>The anti-pyretic and anti-ulcerogenic properties</td>
<td>Philip et al. (2008).</td>
</tr>
<tr>
<td>Eighty percent concentrated ethanol extract of the roots</td>
<td>Analgesic activity Antibacterial activity</td>
<td>Momin et al. (2014).</td>
</tr>
</tbody>
</table>
is regulated by ‘tyrosinase’ a rate limiting enzyme which is widely distributed in nature. As a result, several plants have been screened for their tyrosinase inhibitory activity (Lee et al., 1997). It is also reported the correlation between higher percent of total polyphenol content, antioxidant activity and tyrosinase inhibition. Some skin lightening cosmeceutical products were recently developed from rhizomes of ginger (Rozanida et al., 2006). The rhizomes and leaves of some plants of zingiberaceae family showed antioxidant and tyrosinase inhibitory properties along with high content of polyphenols (Chan et al., 2008). Sida cordifolia, with its ephedrine and pseudoephedrine has gained a lot of interest and is now sold by many of these companies (Ghosal et al., 1975).

MATERIALS AND METHODS

Plant material

The whole plant Sida cordifolia was collected from surrounding of Vijaywada and identified at Taxonomy division, Krishna University, Machilipatnam. A voucher specimen was deposited at the same division.

Extraction and bio-assay fractionation:

The plant parts were shade dried and pulverized to fine powder. The powdered leaf (500 g) was extracted with four volumes of alcohol four times. Total extract was combined and fine filtered and one sixth of part was directly concentrated to dry powder in rotary evaporator and made powder for phytochemical and bioactivity studies. The balance extract was concentrated to aqueous stage in rotary evaporator and was extracted.

Extraction of tyrosinase from potato

Potato was cut into pieces and homogenised in a blender with 100 ml of sodium fluoride. For about one minute at high speed. The homogenate was filtered through several layers of cheese cloth. Equal volume of saturated ammonium sulphate was added to homogenate. So that soluble potato proteins become insoluble and get precipitated. The soluble tyrosinase is one of these proteins. The homogenate is subjected to centrifugation at 1500 x g for 5 min at 4°C. Pellet was collected after discarding supernatant. To the pellet, 60ml of citrate buffer (pH 4.8) was added. Stirring was continued for 2 min in cooling conditions. Recentrifuged at 300 x g for 5 min at 4°C. The enzyme in the supernatant was collected and saved for further use.

Tyrosinase enzyme Inhibitory Activity

Tyrosinase inhibitory assay was performed by Yoshimura et al (2005). The assay mixture contained Test reaction mixtures were prepared by adding 250 µl of potato enzyme, 50 µl of different concentrations of drug and 1250 µl of L-dopa (8Mm). The reaction was started by the addition of substrate (L-dopa). Then the reaction mixture was incubated for 1min at 37°C and absorption of the sample was measured at 475 nm against a blank and IC_{50} values were calculated using linear regression analysis. All the assays were performed in triplicate. The percent inhibition of tyrosinase activity was calculated as follows:

\[
\text{Inhibition (\%) = } \frac{\text{Abs (control) - } (\text{abs extract})}{\text{Abs (control)}} \times 100
\]

RESULTS AND DISCUSSION

Tyrosinase inhibitory activity of leaves of Sida cordifolia

Tyrosinase inhibitory activity of plant extract including aqueous extracts, 50% alcoholic extracts and 100% alcoholic extracts were studied along with control standard-resveratrol 5ug/ml, 10ug/ml and 25ug/ml of reserveratrol showed 33.76%, 48.16% 70.76% (Figure 2). and 25 µg, 50 µg and 100µg aqueous extracts of Sida cordifolia showed 7.75%, 14.4% and 22.54% respectively and 50% alcoholic extracts showed 10.3%, 16.98% and 25.71% respectively and 100% alcoholic extracts 8.89%, 16.07% and 23.66% of inhibition respectively (Fig 3).

![Fig. 2: Percentage inhibition of tyrosinase by different concentrations of resveratrol. *values are mean of triplicate. IC_{50} value of resveratrol is 12.83.](image-url)
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**Figure 3:** The percentage inhibition of Potato (Solanum tuberosum) tyrosinase by different types of extracts of Sida cordifolia.

*All test samples run in triplicates and one way ANOVA test was carried. % values are expressed as mean ± standard deviation (n = 3).

Tyrosinase is known to be key enzyme for melanin biosynthesis through the hydroxylation of tyrosinase and the oxidation of L-Dopa. Tyrosinase inhibition by some phenolic compounds may be due to interaction with metal ions in the catalytic site of enzymes (Sanchez-Ferrer et al. 1995, Kim et al. 2007) Molecular docking studies revealed the same that the binding orientations of the phenolic principles were in the tyrosinase binding pocket and their orientations were located in the hydrophobic binding pocket surrounding the binuclear copper active site (Kubo et al., 1999).

The results of ANOVA analysis show significant differences (p<0.05) in the means of inhibition (%) of tyrosinase (µg/ml). IC₅₀ values are more than 100.

**CONCLUSION**

The purpose of the study was to identify natural tyrosinase inhibitor from Sida. Tyrosinase enzyme causes undesirable browning especially in cut vegetables and fruits and leads decrease in nutritional and then marketing values. The research study revealed that the extracts of Sida cordifolia can be used to the ultimate goal of developing new antityrosinase inhibitor. However, more research is needed in isolating active principle from Sida cordifolia so that it can be practically used and are compatible with the safety food additive.

**Conflicts of interest:** The authors stated that no conflicts of interest.

**REFERENCES**


