RESEARCH ARTICLE

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

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Manuscript details:

Received: 09.10.2017
Accepted: 17.12.2017
Published : 31.12.2017

Editor:
Dr. Arvind Chavhan

Cite this article as:
Madhuri Vajha (2017) Investigation of inhibitory activity of *Sida cordifolia* on tyrosinase extracted from *Solanum tuberosum, International J. of Life Sciences*, 5 (4): 692-696.

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 monooxygenase, 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, blenorrhea, 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).

Fig : 1 *Sida cordifolia*

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

Table 1. pharmacological activities of different extracts of *Sida cordifolia*

Nature of extract	Activity studied	Reference
Aqueous extract of *S. cordifolia*	Inflammation of the oral mucosa, blenorrhea, asthmatic bronchitis and nasal congestion.	Franzotti *et al*. (2000).
The extracts of aerial and root parts	Central and peripheral analgesic activities	Ravi Kanth and Diwan (1999).
Methanol extract of root	Hypoglycaemic activity	
Hydroalcoholic extract	Acute toxicity and its action on the central nervous system (CNS)	Franco *et al*. (2005).
Methanol extract	Antifungal activity against F. verticillioides	Mahesh & Satish (2008).
Root extract	β-phenethylamines; carboxylated tryptamines; Quinazolinealkaloids; β-phenethylamine; ephedrine; ψ-ephedrine;*S*-(+)-N_b-methyltryptophan methyl ester; hypaphorine; vasicinone; vasicine; vasicinol; sympathomimetic amines; bronchodilator principle isolated	Ghosal *et al*. (1975).
Aqueous extract of *Sida cordifolia* leaves (AFSC)	The cardiovascular fraction	Medeiros *et al*., (2006).
(5′-Hydroxy methyl-1′-(1, 2, 3, 9-tetrahydro-pyrrolo [2, 1-b] quinazolin-1-yl)-heptan-1-one) (compound 1), isolated from *Sida cordifolia*	Analgesic and anti-inflammatory activities	Sutradharn *et al.* (2006).
Ethanol extracts of *Sida cordifolia*. leaf, stem, root, and whole plant	Anti–lipid peroxidation, free-radical scavenging, reducing power, nitric oxide scavenging, superoxide scavenging antioxidant assay, and further estimation of total phenolic content and HPTLC studies	Dhalwal *et al*. (2005).
Hydroalcoholic extract leaves	Antioxidant and biochemical profile	Kubavat & Asdaq (2009)
5,7-dihydroxy-3-isoprenyl flavone (**1**) and 5-hydroxy-3-isoprenyl flavone	Analgesic and anti-inflammatory activity	Sutradhar *et al.* (2008).
Ethanol extract of roots	Antistress, adaptogenic activity	Sumanth & Mustafa.(2009).
Aqueous fraction of the hydro alcoholic extract of the *Sida cordifolia* leaves	Vasorelaxantion	Santos *et al.* (2006).
Chloroform and methanol 80% ethanol extracts hexane, dichloro methane, ethyl acetate and butanol.	Analgesic and antiinflammatory activities of different extracts of S *ida cordifolia* Linn (SIC).	Sutradhar *et al.* (2006).
Methanolic extract	The anti-pyretic and anti-ulcerogenic properties	Philip *et al.* (2008).
Eighty percent concentrated ethanol extract of the roots	Analgesic activity Antibacterial activity	Momin *et al.* (2014).

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 4^0C. 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 4^0C. The enzyme in the supernatant was collected and saved for further use.

Tyrosinase enzyme Inhibitory Activity

Tyrosinase inhibitory assay was performed by Yoshimura *et a*l (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:

$$Inhibition\ (\%) = \frac{Abs\ (control) - (abs\ extract)}{Abs\ (control).} X\ 100$$

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 reseveratrol 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).

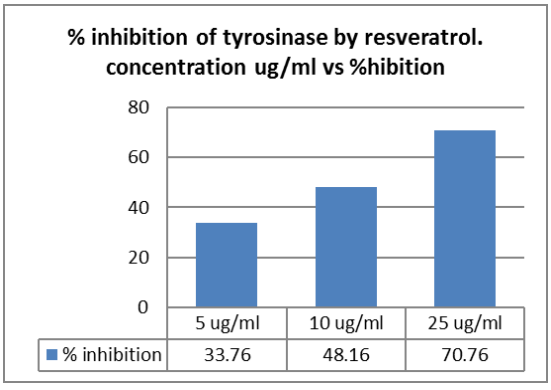

Fig. 2: Percentage inhibition of tyrosinase by different concentrations of resveratrol.
*values are mean of triplicate.
IC_{50} value of resveratrol is 12.83.

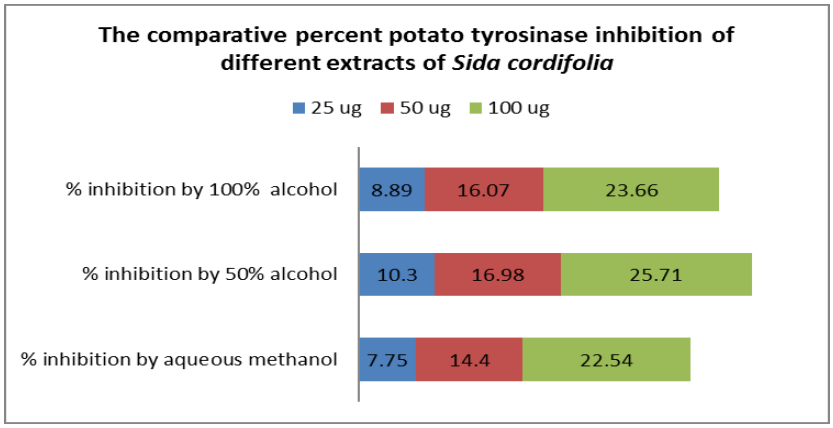

Figure 3: The percentage inhibition of Potato (Solanum tuberosum) tyrosinase by different types of extracts of *Sida cordifolia.*
*All test samples run in triplicates and one way ANOVA test was carried. % values are expressed as mean ± standard deviation (n = 3).

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 calytic site of enzymes (Sanchez-Ferrer *et al* 1995., Kim *et al*., 2007) Molecular docking studies revelaed the same that the binding orientations of the phenolic principles were in the tyrosinase binding packet and their orientations were laocated 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). IC_{50} 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.

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