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Phytochemical Analysis and Tyrosinase Inhibitory Potential of *Cassia absus* (Seeds)

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ABSTRACT

Objective: To perform phytochemical analysis and investigation of the tyrosinase inhibitory activity of *Cassia absus* seeds.

Methods: Various extracts of *Cassia absus* seed were prepared such as ethanolic extract, *n*-hexane, chloroform, *n*-butanol and water fractions. Tyrosinase inhibitory activity was performed using Kim's methodology. The extracts were also subjected to phytochemical analysis.

Result: Phytochemical analysis showed presence of alkaloids, tannins, flavonoids, glycosides and saponins. Tyrosinase inhibition assay was performed for all the four fractions, *n*-butanol showed high activity of tyrosinase inhibition (86%) followed by crude ethanol extract (75%) and *n*. hexane (72%) and water (62%) as compared to kojic acid (97%).

Conclusion: From all the results, we found that that phytochemicals present in *C. absus* seed extract may be responsible for the tyrosinase inhibition activity. The data revealed *C. absus* seed extracts/fractions could be an addition to medicinal cosmetics.

Keywords: Anti-tyrosinase activity, *Cassia absus,* phytochemical analysis, tyrosinase, whitening.

INTRODUCTION

Skin color is due to melanin production and it is responsible for skin protection from UV light. Increase in melanin causes many skin ailments such as age spots, freckles, melasma, and actinic type hyperpigmentations [1]. So, in order to prevent hyper pigmentary disorders melanin inhibitors are used. Tyrosinase is a key enzyme involved in melanogenesis [2]. Tyrosinase inhibitors are being used to treat hyperpigmentation some of them are synthetic while others have natural origin. These agents include hydroquinones, arbutin, azelaic acid, ascorbic acid and kojic acid. Although, these chemicals are effective but they also have some serious side effects. Recently plant extracts have been gaining popularity to be used as skin-whitening agents in different cosmetic products, which have reported fewer side effects as compared to synthetic agents [3].

Cassia absus (chaksu) belongs to family leguminoseae [4]. It is widely spread across the world in Australia, Africa and Asia [5]. Various constituents are isolated from *Cassia absus* viz. chaksine, isochaksine, luteolin, alleloochemical, galactomannan, raffinose, linoleic acid, dodecanoic acid, octadecane, hexadecanoic acid, eicosanoic acid, β-sitosterol and ketooctadec-cis-15-enoic acid [6,7]. *C. absus* is widely used in traditional medicine [8]. Its seeds are used for infected skin treatment wound healing, astringent, anti-ulcer, anti-inflammatory, analgesic, cathartic, anti-cancer, diuretic and antihypertensive. It is also used as a remedy in cough, asthma, bronchitis, yaw, conjunctivitis, hemorrhoids, hepatic and renal diseases [5,9-19]. In literature review, there is no study reported for its anti-tyrosinase potential or its skin whitening effects. So, the current study evaluated the seeds of *C. absus* L. for its tyrosinase

inhibition activity along with its phytochemical analysis.

METHODOLOGY

Tyrosinase, L-DOPA, Kojic acid (Merck, Germany) were used. *Cassia absus* L. seeds were procured from the local herbalist. Seeds were authenticated and identified by a botanist Dr. Sarwar, Lecturer, Islamia University of Bahawalpur, Pakistan. A voucher specimen has been retained in the Faculty of Pharmacy, The Islamia University of Bahawalpur, Pakistan under the accession code 2201/L.S.

2kg dried seed powder of *Cassia absus* L. was macerated in ethanol. After 15 days the material was filtered through Whattman No.1 filter paper. Solvent was evaporated by rotary evaporator. Filtrate was reconstituted in distilled water and partitioned with different solvents on the base of polarity by using separating funnel and achieved *n*-hexane, chloroform, *n*-butanol and water fractions [20].

Tyrosinase Inhibition Assay

Kim's methodology [21] with slight modification was followed for tyrosinase inhibition assay. Tyrosinase (60 units), test compound (10µL) and 50mM potassium phosphate buffer of pH 6.8 (150µL) in each well were incubated at 30˚C for 15 minutes. After incubation, pre-read was taken at 480nm. 1mM L-DOPA as a substrate (10µL) per well was added and re-incubated at 30˚C for 30 minutes. After incubation, after read was taken at 480nm. Standard tyrosinase inhibitor used was Kojic acid. Following formula was applied for calculation of results.

$\%\text{inhibition} = 100 - (A_p \div A_c)\ 100$

While

A_p is the absorbance of plant extracts or fractions or standard

A_c is the absorbance of negative control

$IC_{50}$was calculated by making serial dilution of stock solution.

Statistical Analysis

Assay was done in triplicate and results denoted as Mean ± Standard error of mean. ANOVA followed by post hoc test was applied for comparison between different study groups. IBM SPSS version 20 was used for analysis of results.

RESULT AND DISCUSSION

Table **1** summarizes results of phytochemical analysis that showed presence of alkaloids, tannins, flavonoids, glycosides and saponins. The ferric chloride test performed for the tannins results in blue black color which indicated the presence of tannins. Other tests showed that hydrolysable tannins are present in *Cassia absus* seeds extract. A green fluorescence was appeared at the end of borax test performed for anthraquinone glycosides which confirmed the presence of anthraquinone glycosides. Yellow precipitate appeared in bromine test and reddish color produced by borntrager test also confirmed the presence of anthraquinone glycosides.

Test performed for flavonoids exhibited yellow color which revealed the presence of flavonoids. Persistence of frothing was observed for more than half hour when the test was performed for saponins which indicated the presence of high contents of saponins. Different tests are performed to identify the presence of cardiac glycosides. By Keller Killani method brown ring was not appeared at the interface, similarly kedde's and baljet test also gave negative results which confirmed the absence of cardiac glycoside but positive result with Lieberman-burchard test exhibited that cardiac glycosides are absent but phytosterols are present. Leibermanburchard test indicated positive result for all sterols double bond containing triterpenes, cholesterol and sitosterol [22].

Erdmmann's test performed for phenolics showed reddish color which confirmed the presence of phenolics. Color of sodium picrate paper remains same in guignard's test indicated the absence of cyanogenic glycosides. Tests performed for alkaloids produce precipitates of different colors which assured the presence of alkaloids.

Wagner test produced reddish brown precipitate. Mayer test presented cream color precipitate. Hager test gave yellow precipitate and Dragendorffs test gave orange precipitate. All these precipitates assured the presence of alkaloids.

Table 1. Results of phytochemical analysis.

S. No.	Chemical constituents	Standard	Tests Name	Result of standard	Result of sample
1	Saponin	*Glycyrrhiza glabra*	Froth test	+	+
			Emulsifying properties	+	+
2	Anthraquinone glycosides	*Aloe barbadensis*	Borax test	+	+
			Bromine water test	+	+
			Nitric acid test	+	+
			Borntrager test	+	+
3	Cyanogenic glycosides	*Prunus amygdalus*	Guignard test	+	-
4	Phenolic glycosides		Erdmann's test	+	+
5	Cardioactive glycosides	*Nerium oleander*	Keller killiani test	+	-
			Kedde's test	+	-
			Baljet test	+	-
			Liebermann- burchard test	+	+
6	Alkaloids	*Nicotiana tobaccum*	Mayer's reagent	+	+
			Wagner's reagent	+	+
			Hager's reagent	+	+
			Dragendorff's reagent	+	+
7	Tannins	Cinnamon	Ferric chloride test	+	+
			Bromine water	+	-
			Formalin test	+	-
			Sodium nitrite test	+	-
8	Flavonoids	Orange peel	Sodium hydroxide test	+	+

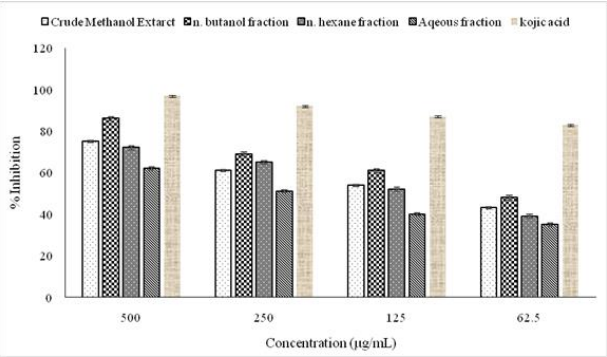

Figure 1. Shows the inhibitory effects of *C. absus* seed extract/ fractions against tyrosinase. In tyrosinase inhibition assay, crude ethanol extract and n-butanol fraction displayed significantly higher ($p \leq 0.05$) inhibition of tyrosinase.

Figure **1** demonstrates the percentages of tyrosinase inhibition of different fractions or extracts of *C. absus* seed at 500, 250, 125 and 62.5µg/mL. Kojic acid inhibited 97% in this study and was standard inhibitor of tyrosinase. Aqueous fraction showed 62% inhibition of tyrosinase that is far less than *n*. hexane fraction (72%) and crude ethanol extract (75%). The highest anti-tyrosinase activity among tested fractions was exhibited by *n*-butanol fraction (86%). Evaluation of serial dilutions showed gradual decrease in percent inhibition of tyrosinase with decrease in concentration of extract/fractions.

The inhibitory activity of crude ethanol extract (75%) and *n*-butanol fraction (86%) exhibited significantly higher ($p \leq 0.05$) inhibition of tyrosinase. Results were compared with Kojic acid, a potent inhibitor which inhibited tyrosinase by 97%.

C. absus seed extracts was studied for the first time for tyrosinase inhibition. In Pakistan, prevalence of hyperpigmentation and melisma is high due to severe sun exposure and harsh environments. The available treatment options are costly and have side effects. *Cassia absus*, therefore, can offer a possible and economic solution to the problem.

Our results indicated that the extracts of *C. absus* seeds effectively inhibit tyrosinase. This result was similar to that of a previous study, which showed specie of Cassia genus, *C. fistula* pods have

exhibited skin whitening effects [23]. So, *C. absus* seed extract may also reduce hyperpigmentation in human skin. Furthermore, many previous studies on individual chemical constituents responsible for antityrosinase activity also support the evidence of skin lightning effects of *C. absus*.

The present study showed the presence of flavonoids, tannins, alkaloids, saponins and phenolic glycosides in *C. absus* seed powder. The results matched to previous study showing presence of alkaloids, anthraquinones, flavone and flavonoids, carbohydrates, fatty acids, phenols and phenolic acids, proteins, steroids and triterpenesin *C. absus* seed extract [19]. Previous studies indicated that plants containing flavonoids, alkaloids, unsaturated fatty acids and minerals possess tyrosinase inhibitory potential [24].

Phenolics are most commonly used in skin lightening formulations. Skin formulations contain many herbal extracts that contain active ingredients acting synergistically with desirable results [25]. Fatty acids are reported to have promising effects for the treatment of hyperpigmentary disorders. Unsaturated fatty acids have more promising effects in reducing melanin production and tyrosinase inhibition as compared to saturated fatty acids [26]. *C. absus* seeds are rich in unsaturated fatty acids oleic acid, linoleic acid and linolenic acid [19]. Flavonoids also inhibit tyrosinase due to their copper chelating ability in the active site. Flavonoids i.e., quercetin and rutin showed anti-tyrosinase activity, *C. absus* seeds are also rich in flavonoids including quercetin and rutin[19]. So presence of essential fatty acids, phenols and flavonoids may be responsible for tyrosinase inhibition potential of different fractions of *C. absus* seeds.

CONCLUSION

This is the first study reporting tyrosinase inhibiting properties of *C. absus* seeds. The results revealed the potential of *n*-butanol (86%), crude ethanol extract (75%) and *n*. hexane (72%) from the seeds of *C. absus* in tyrosinase inhibition. Phytochemical analysis showed presence of alkaloids, glycosides, flavonoids, tannins and saponins are present in this plant may responsible for tyrosinase inhibition activities. Future studies should be performed for evaluation of exact mechanism of the tyrosinase inhibition of *n*-butanol, crude ethanol and hexane fractions from the seed of *C. absus*.

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