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In vitro inhibitory potential of methanolic extract of *Celosia argentea* var. *cristata* on tyrosinase, acetylcholinesterase and butyrylcholinesterase enzymes

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Abstract

In the current study, methanol extract of *Celosia argentea* var. *cristata* was tested for its inhibitory potential against tyrosinase, acetylcholinesterase and butyryl-cholinesterase enzymes at the concentration of 0.5 mM by ELISA microtiter plate assays. A significant tyrosinase inhibitory activity (63.6%), acetylcholi-nesterase inhibitory activity (80.3%) and butyrylcholinesterse inhibitory activity (68.2%) was shown by crude methanolic extract of *C. argentea* var. *cristata* with respective IC_{50} values of 268.5 ± 0.2 µg/mL, 73.6 ± 0.1 µg/mL and 132.8 ± 0.9 µg/mL. The result of this study reveals the use of *C. argentea* var. cristata in skin hyperpigmentation, Parkinson's disease and neurodegenerative disorders like Alzheimer's disease and dementia.

Introduction

Tyrosinase is an important enzyme involved in production of melanin which causes skin problems (Briganti et al., 2003). It also causes neurotoxicity associated with Parkinson's disease (Khan, 2007). While Alzheimer's disease is a neurodegenerative disorder associated with loss of memory and cognition (Blennow et al., 2006). It is due to the inefficiency of cholinergic function in the brain stated by cholinergic hypothesis (Perry, 1986) so cholinomimetic drugs can improve it. Discovery of novel tyrosinase, acetylcholinesterase and butyrylcholinesterase enzyme inhibitors from medicinal plants may open new road to the era of pharmacological research (Asanuma et al., 2003; Akhondzadeh et al., 2003; Wettstein, 2000).

Celosia argentea var. *cristata* (L.) O. Kuntze (Family: Amaranthaceae) commonly known as cockscomb, kulgha and sardabi (Kritikar and Basu, 1987) distributed worldwide including Pakistan (Prajapati and Pyrohit, 2003). Traditionally it is used to treat atherosclerosis (Wang et al., 2010), inflammation, diarrhea (Tova, 2004), dysentery (Shanmugam et al., 2011), abscess, cancer, pain and urinary tract infections. It is anti-tussive, expectorant, and hypotensive (Duke et al., 2002). It contains cochliophilin, isoflavone, cristatein, phenethyl-alcohol, kaempferol, quercetin, β-sitosteorol, octadecenoic acid, stigmasterol, saponins, celosin A-D (Xiang et al., 2010).

The plant possess hepatoprotective (Sun et al., 2011), anti-oxidant (Pyo et al., 2008) and antiviral activity (Begam et al., 2006). This study was conducted to evaluate the pharmacological potential of plant in the inhibition of three important enzymes i.e., tyrosinase, acetylcholine esterase and butrylcholine esterase to provide basis for its use in skin hyperpigmentation, Parkinson's disease, Alzheimer's disease and dementia.

Materials and Methods

Chemicals and drugs

Mushroom tyrosinase (EC 1.14.1.8.1, 30 U), Electric eel AchEstrase (Type-VI-S, EC 3.1.1.7), horse serum

BChEstrase (EC 3.1.1.8), L-dopamine, acetylthiocholine iodide, butyrylthiocholine chloride and DTNB (5,5'-dithiobis-(2-nitrobenzoic acid)) were purchased from Sigma chemicals Co. St Louis, MO, USA. All other chemicals were of the analytical grade available from Merck/Fluka/Sigma.

Collection and authentication of plant material

Whole plant of *C. argentea* var. *cristata* was collected from botanical garden of Bahauddin Zakariya University Multan and identified with the kind cooperation of an expert taxonomist (Prof. Altaf Dasti) from the Institute of Pure and Applied Biology at Bahauddin Zakariya University Multan, Punjab, Pakistan. A sample voucher (STW-231) was submitted to the herbarium of Institute of Pure and Applied Biology at Bahauddin Zakariya University Multan, Punjab, Pakistan.

Extraction of crude extract

The plant material was shade-dried and rendered free from soil and adulterated material and coarsely ground by electrical device. The powdered material was soaked into aqueous-ethanol (80%) for 72 hours with occasional shaking. The soaked material was rendered free from plant debris by passing through a muslin cloth and fluid portion was filtered through a fine filter paper (Williamson et al., 1998). The above mentioned extraction procedure was repeated twice on the plant debris and filtrate was subsequently combined before subjecting to evaporation under reduced pressure on a rotary evaporator to thick paste like mass of dark brown color, i.e., crude extract of *C. argentea* var. *cristata*, yielding approximately 11.1% (w/w).

Preliminary phytochemical analysis

Qualitative phytochemical analysis of crude extract was done for the pre-sence of alkaloids, anthraquinones, coumarins, saponins, flavonoids and tannins as reported elsewhere (Janbaz and Saqib, 2015)

Tyrosinase inhibition activity

Total volume of reaction mixture 100 µL containing 60 µL phosphate buffer (100 mM), pH 6.8, 10 µL mushroom tyrosinase enzyme (5 units) and 10 µL 0.5 mM test compound mixed in 96-well plate (Lee et al., 2009). Contents were pre incubated for 5 min at 37°C. After incubation, 20 µL of 10 mM L-dopamine was added as a substrate. Contents were mixed and incubated for further 30 min. Absorbance was taken at 490 nm using Synergy HT Biotek 96-well plate reader.

The enzyme inhibition (%) was calculated by the bellow formula.

%Inhibition = 100 - (absorbance of test sample/ absorbance of control × 100)

Acetylcholinesterase inhibition activity

The AChE inhibition activity was performed according to the method (Ellman et al., 1961) with slight modifications. Total volume of the reaction mixture was 100 µL. It contained 60 µL Na_2HPO_4 buffer with concentration of 50 mM and pH 7.7. Ten microliter test compound (0.5 mM/well) was added, followed by the addition of 10 µL (0.005 unit per well) enzyme. The contents were mixed and pre-read at 405 nm. Then contents were pre-incubated for 10 min at 37°C. The reaction was initiated by the addition of 10 µL of 0.5 mM/well substrate (acetylthiocholine iodide), followed by the addition of 10 µL DTNB, 0.5 mM/well. After 30 min of incubation at 37°C absorbance was measured at 405 nm using 96-well plate reader Synergy HT, biotek, USA. All experiments were carried out with their respective controls in triplicate. Eserine (0.5 mM/well) was used as a positive control. The percent inhibition was calculated by the help of following equation.

$$\text{Inhibition (\%)} = \frac{\text{Control} - \text{Test}}{\text{Control}} \text{ X } 100$$

Butyrylcholinesterase inhibition activity

The BChE inhibition activity was performed according to the method (Ellman et al., 1961) with slight modifications. Total volume of the reaction mixture was 100 µL containing 60 µL, $Na_2H\ PO_4$ buffer, 50 mM and pH 7.7. The 10 µL test compound 0.5 mM per well was added followed by the addition of 10 µL (0.5 unit per well) BChE (Sigma Inc.). The contents were mixed and pre-read at 405 nm and then pre-incubated for 10 min at 37°C. The reaction was initiated by the addition of 10 µL of 0.5 mM/well substrate (butyrylthiocholine chloride). Followed by the addition of 10 µL DTNB (5,5'-dithiobis-(2-nitrobenzoic acid)), 0.5 mM $well^{-1}$. After 30 min of incubation at 37°C, absorbance was measured at 405 nm using 96-well plate reader Synergy HT, Biotek, USA. All experiments were carried out with their respective controls in triplicate. Eserine (0.5 mM/well) was used as positive control. The percent inhibition was calculated by the help of following equation.

$$\text{Inhibition (\%)} = \frac{\text{Control} - \text{Test}}{\text{Control}} \text{ X } 100$$

Statistical analysis

In all of the enzyme inhibition activities, the experiments were performed three times and the results were expressed as mean ± standard error of mean (S.E.M) of three parallel measurements and the respective. IC_{50} values were calculated using EZ–Fit Enzyme kinetics software (Perrella Scientific Inc. Amherst, USA). Amherst USA software. The data was analyzed by an analysis of variance $p<0.05$. Results were processed by computer programs: Excel and Ezi Fit software.

Table I

Inhibitory effects of crude extract of *Celosia argentea* var. *cristata* on tyrosinase, acetylcholinesterase and butyrylcholinesterase

Sample	Conc. (mM)	%Tyrosinase inhibition	%AChE inhibition	%BChE inhibition	IC_{50} (µmol)		
					Tyrosinase	AChE	BChE
Crude extract	0.5 mM	63.6 ± 0.6	80.3 ± 0.8	68.2 ± 0.7	268.9 ± 0.2	73.6 ± 0.1	132.8 ± 0.9
Eserine	0.5 mM	-	91.3 ± 1.2	82.8 ± 1.1	-	0.0 ± 0.0	0.9 ± 0.0
Kojic acid	0.5 mM	93.5 ± 0.9	-	-	6.0 ± 0.1	-	-

All experiments are performed in triplicate and represented as mean± SEM; Standard drugs used are Kojic acid for tyrosinase inhibition and eserine for AChE and BChE inhibition

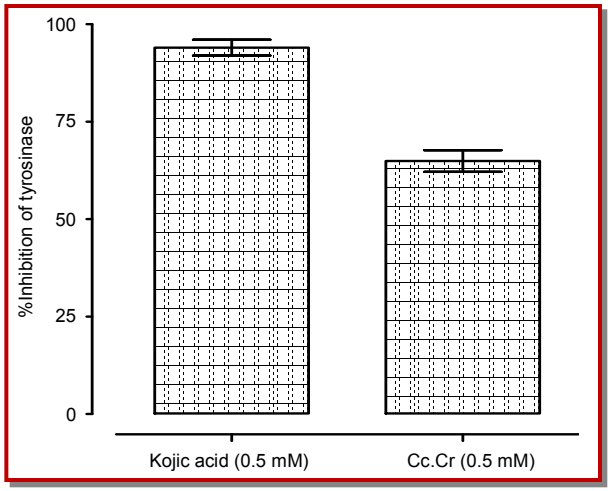

Figure 1: Showing percentage inhibition of tyrosinase by crude extract of *Celosia argentea* var. *cristata* (Cc.cr) and standard drug kojic acid

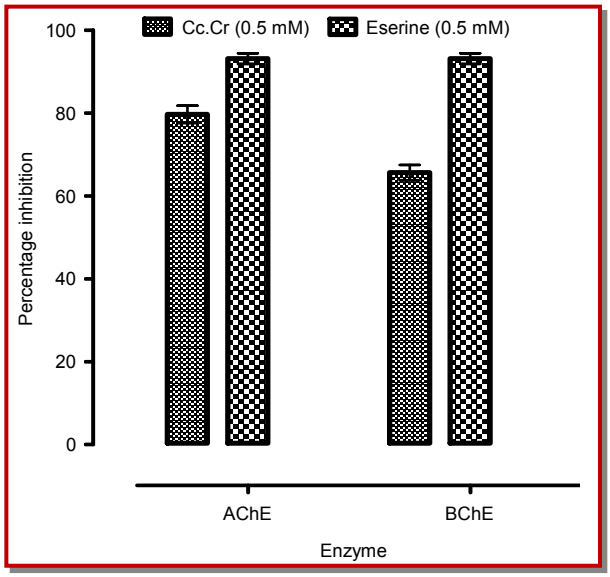

Figure 2: Showing percentage inhibition of acetyl cholinesterase inhibition (AChE) and butyryl cholinesterase (BChE) by crude extract of *Celosia argentea* var. *cristata* (Cc.Cr) and standard drug eserine

Results

The result of preliminary phytochemical analysis showed that extract of *C. argentea* var. *cristata* contains alkaloids, saponins, flavonoids, tannins and phenolic compounds.

Very moderate antityrosinase activity was seen with the methanolic extract of *Celosia argentea var. cristata* at the dose of 0.5 mg/mL which showed 63.6% inhibition of tyrosinase with 50% inhibition (IC_{50}) at a concentration of 268.9 ± 0.2 µg/mL ($p<0.05$) (Table I). The standard drug kojic acid, showed significant tyrosinase inhibition activity with the 0.5 mg/mL concentration showing a 93.5% inhibition of tyrosinase (IC_{50} 6.0 ± 0.1 µg/mL). The %tyrosinase inhibition activity of crude extract and kojic acid are shown in a Figure 1.

The 0.5 mg/mL of methanolic extract of *C. argentea var. cristata* showed 80.3% inhibition of acetylcholinesterase and 68.2% inhibition of butyrylcholinesterase with respective 50% inhibition of acetylcholinesterase (IC_{50}) at a concentration of 73.6 ± 0.1 µg/mL ($p<0.01$) and butyrylcholinesterase (IC_{50}) at 132.8 ± 0.9 µg/mL (Table I). The standard drug eserine, showed significant 91.3% acetylcholinestersae and 82.8% butyrylcholinesterase inhibition activity with the 0.5 mg/mL concentration. The IC_{50} values of the extract was found to be greater than the standard, eserine which showed an IC_{50} of acetylcholinesterase at concentration of 0.04 ± 0.0 µg/mL and IC_{50} of butyrylcholinesterase at 0.9 ± 0.0 µg/mL. The %acetylcholinesterase and butyrylcholines-terase inhibition activity of crude extract and standard, eserine are shown in a Figure 2.

Discussion

The skin is an important barrier that protects our body from damage due to its direct contact with the outside environment. Melanin is the important pigment in the skin. It protects our skin from UV damage by absorbing sunlight and removing reactive oxygen species. About

10% of skin cells in the innermost layer of epidermis produce melanin. Upon exposure to UV radiation, melanogenesis is initiated by the enzyme tyrosinase resulting in skin darkening (Vamos, 1981). As discussed earlier, tyrosinase is involved in the transformation of tyrosine to o-dopaquinone. The hyperpigmented skin is observed in various dermatological disorders namely melasoma, solar lentigines and ephelides (Maeda and Fukuda, 1991). The reactive oxygen species produced in the human body enhances the DNA damage, melanin biosynthesis and induces the proliferation of melanocytes (Yasui and Sakurai, 2003) which results in hyper pigmentation disorders. The production of melanin in the body can be reduced by several mechanisms including direct inhibition of tyrosinase enzyme, inhibiting the transport of melanosome to the stratum corneum, the supplementation of anti-oxidants (Pawelek and Kormer, 1982) as well.

The most common skin lightening and depigmentation agents available commercially are kojic acid, arbutin, catechins, hydroquinone (HQ) and azelaic acid (Maeda and Fukuda, 1996). Some adverse effects of these synthetic compounds are irreversible cutaneous damage, ochronosis etc. These adverse effects have led to the search for safer plant-based skin lightening ingredients. The aqueous methanolic extract of *C. argentea* var. *cristata* showed a moderate anti-tyrosinase activity with 63.6% inhibition. The preliminary chemical examination of the crude extract of *C. argentea* var. *cristata* has demonstrated the presence of phenolic and flavonoid compounds which may contribute to its tyrosinase inhibitory effect as reported earlier (Kubo et al., 2003; Nagendra et al., 2009). Anti-oxidant activity also affect tyrosinase activity (Kim et al., 2008). The potent skin whitening and anti-oxidant ability of *C. argentea var. cristata* make it a suitable candidate for the remedy of hyper pigmentation disorders of skin.

AChE (EC 3.1.1.7) comprise a family of enzymes which include serine hydrolases. They share about 55% of amino acid sequence identity and have similar catalytic properties. The different specificities for substrates and inhibitors are due to the difference in amino acid residues of the active sites of AChE and BChE (Cyglar et al., 1993). Acetylcholinesterase plays a prominent role as it participate in termination of acetylcholine based signal transmission through neurosynaptic cleft or neuromuscular junction (Ballard et al., 2005; Khaled et al., 2010).

Butyrylcholinesterase (BChE) is a non-specific ester hydrolyzing enzyme with no known endogenous physiological substrate (Quinn, 1987). It can hydrolyze various esters of choline and other compounds (Sun et al., 2001), e.g., cocaine, some organophosphorus compounds. BChE is produced in liver and enriched in blood circulation. In addition, it is also present in adipose tissues, intestine, smooth muscle cells, and white matter of the brain (Schwarz et al., 1995). H_1 and H_2 receptor antagonists have been shown to possess AChE inhibitory activities.

A deficit of ACh levels in CNS leads to conditions such as Alzheimer's disease (AD) which is the most common form of dementia, a progressive neurologic disease of the brain that leads to loss of mental ability severe enough to interfere with normal activities of daily living and decline in cognitive functions such as remembering, reasoning and planning. It affects parts of the brain that control thought, memory, and language. It is characterized by nerve-cell loss, abnormal tangles and plaques within nerve cells and deficiencies of several neurochemicals such as acetylcholine (ACh) and butyrylcholine (BCh), which are essential for the transmission of nerve messages. It was postulated that blocking the enzyme cholinesterase (ChE) induced hydrolysis of ACh and subsequent increase in Ach concentration in central synapses and enhancement of cholinergic functions provides the symptomatic improvement to AD patient (Schwarz et al., 1995; Pohanka et al., 2009).

ChE inhibitors were developed to improve the effectiveness of ACh by inhibiting its breakdown and increasing the levels in the brain or by strengthening the way nerve cells responds to it. Increased concentrations of ACh in the brain leads to increased communication between nerve cells and may temporarily improve or stabilize the symptoms of AD. These drugs appear to work best in the early and moderate stages of AD. It has been further suggested that dual inhibition of AChE and BChE enzymes should be one of the objectives in the treatment of cognitive dysfunction associated with AD (Pohanka et al., 2010).

The results obtained with the crude extract of *C. argentea var. cristata*, exhibits outstanding enzyme inhibitory activity against AChE (80.3%), and BChE (68.2%). The outstanding results obtained with the crude extracts of *C. argentea* var. *cristata*, indicate the need for further work on the isolation, purification and investigation of the active principles responsible for the extract inhibitory activity.

Conclusion

The crude extract of *Celosia argentea var. cristata* possesses inhibitory activity against tyrosinase, acetylcholine estrase and butrylcholineestrase enzymes which provide a base for its use in hyperpigmentation of skin, Parkinson's disease and other neurodegenerative disorders like Alzheimer's disease and dementia.

Competing Interest

The authors declare that they have no competing interests.

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