SHORT COMMUNICATION

Tyrosinase inhibition kinetic studies of standardized extract of Berberis aristata

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ABSTRACT
The stem bark and wood of Berberis aristata DC (Daruharidra) are one of the principal ingredients of traditional skin lighting and exfoliating scrub preparation in India. The standardised extract of B. aristata was screened to evaluate their in vitro antityrosinase activity and inhibition kinetics. Phytochemical and pharmacological studies were carried out with different solvent fractions of the methanol extract of B. aristata (MEBA). RP-HPLC analysis was used to determine the berberine content in extract and fractions of B. aristata. MEBA showed maximum berberine content. Extract and fractions of B. aristata contain the maximum amount of alkaloids than other constituents. In tyrosinase inhibition assay, MEBA was found to possess highest dose-dependent monophenolase and moderate diphenolase activity. The enzyme kinetic study revealed that MEBA possessed mixed type inhibition of monophenolase activity of tyrosinase. These bioactivities indicate that the MEBA has antihyperpigmentation potential in human skin.

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1. Introduction
Melanin, a mixture of heterogeneous biopolymers, is regulating the colour of the skin. It also plays a protective role by absorbing ultraviolet sunlight and removes reactive oxygen.
species from skin that may cause skin damage. Melanin biosynthesis is processed by tyrosinase, a copper-containing multifunctional enzyme in melanocytes. Two major actions of the enzyme are the hydroxylation of L-tyrosine and oxidation of tyrosine to L-dopa. Accumulation of an abnormal amount of melanin in the skin may result in hyperpigmentation. These phenomena have encouraged researchers to search for new potent tyrosinase inhibitors that can effectively use as the skin-whitening agents; such agents are being practised to protect hyperpigmentation or hypermelanosis (Chang 2009).

Berberis aristata DC (Family: Berberidaceae) also known as ‘Daruharidra’ is one of the traditionally used medicinal plants in Ayurvedic system of medicine. The wood of the plant is one of the principal ingredients of traditional skin lighting and exfoliating scrub preparation in India. Its paste is applied to the skin for a natural glow (Kumar et al. 2013). Rasont is another folk medicine in India, which is prepared from stem and root of B. aristata, often mixed with honey and used for sores and ulcerations of the skin. B. aristata has also been reported to be hepatoprotective, antidiarrhoeal, cardiotonic, anti-diabetic, antimicrobial, anticancer and anti-inflammatory. Berberine is a major isoquinoline alkaloid obtained from the plant and possesses a number of pharmacological activities. Protoberberine alkaloid’s karachine, aromoline, oxyberberine, oxyacanthine, berbamine and berberine have obtained from root bark of the plant (Potdar et al. 2012).

This work was purported to examine the antityrosinase potential of B. aristata extracts and standardised through the RP-HPLC techniques for quality evaluation of the methanol extracts of the plant.

2. Results and discussion

2.1. Quantitative phytochemical analysis

Percentage of yield of the methanol extract of B. aristata (MEBA) was 18% (w/w). The yield for its aqueous fraction of B. aristata (AFBA), ethyl acetate fraction of B. aristata (EFBA) and hexane fraction of B. aristata (HFBA) were found to be 55.71, 28.57 and 15.71% w/w, respectively, with respect to the MEBA. The amount of alkaloid in the MEBA extract was 51.97%. The alkaloid content of its different fractions was estimated to be 46.23% (AFBA), 30.65% (EFBA) and 26.92% (HFBA). The total phenol and flavonoid content of the plant extract was expressed as gallic acid equivalents (GAE) per gram of extract weight and quercetin equivalents (QrE) in per gram of extract weight. The extracts contain very low amount of flavonoids. The ethyl acetate and hexane fraction contain no flavonoids. The phenol content of the MEBA, AFBA, EFBA and HFBA extracts were 20.34 ± 0.129, 27.32 ± 1.364, 2.83 ± 1.171 and 2.33 ± 0.104 (mg GAE/g extract), respectively.

2.2. Quantification of berberine

From the HPLC chromatogram of standard of different samples, it was observed that for a separate, distinct peak of berberine, the retention time was 14.289. The standard calibration curve of berberine was found to be linear with $r^2 = 0.9956$ in five concentrations range 10–100 μg/mL. Highest concentration of berberine was found in MEBA, 1.06 ± 0.05% (w/w) followed by 0.23 ± 0.11%, 0.13 ± 0.01 and 0.12 ± 0.01 (w/w) in AFBA, EFBA and HFBA, respectively (Figure S1–S6). The HPLC method was validated in accordance with the International Conference on Harmonization guidelines (ICH). The limit of detection and limit of quantification was estimated according to the guideline. The recovery experiment was performed at three different concentrations of the standard and mean recoveries were found to be
97.18, 98.09 and 98.67%, respectively, which indicates good accuracy of the method. The %RSD value of intra- and inter-day precision was less than 2%. The berberine standardisation method is reproducible.

2.3. **Monophenolase and diphenolase activity of B. aristata**

L-tyrosine and L-dopa were used as substrates for the tyrosinase inhibition study of plant extracts. Berberine and four fractions were assayed at different concentrations, and their relative activities were expressed as IC$_{50}$ values (Table S1). Kojic acid showed a competitive mode of inhibition on monophenolase (L-tyrosine) activity and a mixed inhibitory effect on the diphenolase (L-dopa) activity of mushroom tyrosinase (Chang 2009). Results of monophenolase inhibitory activity by MEBA and AFBA showed significant ($p < 0.01$) inhibition of 97% and 78% at a concentration of 110 μg/mL correspondingly as compared to Kojic acid (Figure S7). The diphenolase activity of MEBA and AFBA was observed 50% inhibition at concentrations of 412.01 and 431.11 μg/mL, respectively.

2.4. **Determining inhibition kinetics via Lineweaver–Burk plot analysis**

MEBA showed the most potent monophenolase inhibition activity among the four different fractions of *B. aritata*. The kinetic behaviour of MEBA on the monophenolase activity of tyrosinase was studied. The plots of 1/V vs. 1/S gave a family of straight lines with different slopes that was calculated using double-reciprocal Lineweaver–Burk plots (LB plot). The results showed changes in both the apparent $V_{max}$ and the $K_m$, indicating that MEBA induced a mixed type of inhibition (Figure S8). The secondary replot of slope vs. MEBA concentration gives the $K_i$ value 8.21 ± 0.325 μg/mL ($n = 3$) (Figure S9a) and Y-intercept vs. MEBA concentration gives the $\alpha K_i$ value 45.33 ± 0.219 μg/mL ($n = 3$), respectively (Figure S9b).

Tyrosinase inhibitors are gaining popularity in the cosmetic industry due to their skin-whitening effects. A significant number of tyrosinase inhibitors obtained from both natural and synthetic origin, but their use is restricted due to the undesirable side effects; only some of them are used as skin-whitening agents (Wang et al. 2006). Thus, many fields have centred on natural additives in order to decrease the browning progression in skin and food. In the present study, MEBA was found to have potent tyrosinase inhibition activity, mainly monophenolase (L-tyrosine) inhibition with increasing dosage of the extract. From LB plot, it was found that MEBA showed mixed type inhibition of monophenolase activity of tyrosinase. The results showed that polar alkaloidal compounds may be accountable for tyrosinase inhibitory activity. Thus, this investigation led us to search for naturally occurring tyrosinase inhibitors from *B. aristata*, which can further be exploited for its possibly responsible phytoconstituents.

3. **Conclusion**

The results suggested that the MEBA and its aqueous fraction are a source for novel anti-tyrosinase compound, which showed moderate to potent tyrosinase inhibition potential. Based on these findings, we propose that *B. aristata* may be useful in preventing enzymatic browning reactions of food product, as well as hyperpigmentation of human skin.

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Disclosure statement
No potential conflict of interest was reported by the authors.

References
Chang TS. 2009. An updated review of tyrosinase inhibitors. Int J Mol Sci. 10:2440–2475.
Kumar S, Palbag S, Maurya SK, Kumar D. 2013. Skin care in ayurveda: a literary review. Int Res J Pharm. 4:1–3.
Potdar D, Hirwani RR, Dhulap S. 2012. Phyto-chemical and pharmacological applications of Berberis aristata. Fitoterapia. 83:817–830.
Wang KH, Lin RD, Hsu FL, Huang YH, Chang HC, Huang CY, Lee MH. 2006. Cosmetic applications of selected traditional Chinese herbal medicines. J Ethnopharmacol. 106:353–359.