Searching for flavonoids with tyrosinase inhibitory activity from extracts of *Dalea pazensis*

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INTRODUCTION

Melanin formation is one of the most important factors in determining the color of skin in mammals. Tyrosinase (Tyr), catalyzes two reactions in the biosynthesis of melanin, the hydroxylation of L-tyrosine (monophenolase activity) and L-Dopa's Oxidation to o-dopaquinone (diphenolase activity). Tyr presents two active sites, for these reason the inhibition kinetics of both reactions are different. Tyrosinase inhibitors have importance in treating diseases with pigmentation abnormalities and as whitening agents in cosmetics, but the toxicity for several of them, do important the research into new tyrosinase inhibitors.[1,2]

Our research group carried out the chemical and pharmacological studies of argentinian species from the *Dalea* genus reporting new flavonoids with important activity as Tyr inhibitors. Following with the study of the genus, recently we began studying *Dalea pazensis* a Bolivian species [3,4]

Thereon, we reported the monophenolase tyrosinase activity on different extracts (hexane, benzene, ethyl acetate and ethanol) obtained from roots, being the benzenic the most active. [5]

In this oportunity we inform the inhibition of diphenolase tyrosinase activity from the hexane, benzene, ethyl acetate and ethanol extracts.

In addition, we report the isolation, identification and diphenolase inhibitory activity of 2 ', 4'-dihydroxy-5' - (1 " ', 1' " - dimetilalil) -8-prenilpinocembrin (8PP), isolated from one of bioactive extracts and reported in other species of the genus.[4]

MATERIALS AND METHODS

Plant material: ***Dalea pazensis*** Rusby, was collected in Yotala, near of Sucre city. Plant material was identified by specialized staff of the Museum of the Real y Pontificia Universidad Mayor de San Francisco Xavier de Chuquisaca, Bolivia. A representative voucher specimen is on deposit as Portal E. & López C.D., 961A in the herbarium of the Museum.

Preparation of extracts: 35 g of dried roots were extracted with solvents of increasing polarity using a soxhlet extractor. Thus, different extracts hexane (0.41 g), benzene (0.51 g), ethyl acetate (0.53 g) and ethanol (1.06 g) were obtained[6].

Isolation and identification of 2 ', 4'-dihydroxy-5' - (1''', 1''' -dimethylallyl) -8-prenylpinocembrin (8PP): The benzene extract (0.51g) was subjected to column chromatography using silica gel chromatography as stationary phase and eluted with n-hexane/ethyl acetate (100:0 to 0:100) and giving ten fractions. Fraction 2 was purified using preparative TLC with chloroform/ethanol to yield 4mg of 8PP. 8PP was identifying by several spectroscopic techniques such as UV-Vis, IR, 1H and 13C NMR 1D and 2D were used.

Tyrosinase inhibitory activity: The assay was according with the methodology described by ***Rahman et al.***[7] using tyrosinase from mushroom (250 U / mL) and L-DOPA (0.25 mM) as substrate. The dopachrome formation was measured at 475nm. Kojic acid was used as reference inhibitor. The percent inhibition of tyrosinase activity was calculated as follows:

$$\% \text{ Inhibition} = \frac{A_{475nm}(\text{control}) - A_{475nm}(\text{w/inh})}{A_{475nm}(\text{control})}$$

Statistics: All assays were independently performed in triplicate, and results were expressed as mean ± SD of three separate experiments. The IC50 was estimated using the OriginPro Program 8 on a PC compatible computer.

RESULTS AND DISCUSSION

Figure 1: %Inhibition vs Extracts of *Dalea pazensis* at 1, 10 y 100µg/mL.

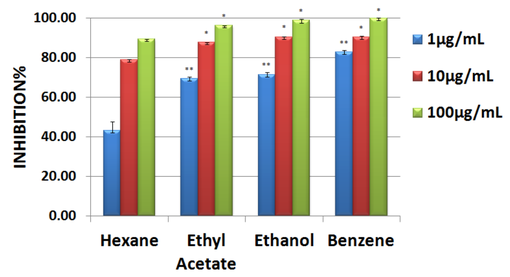
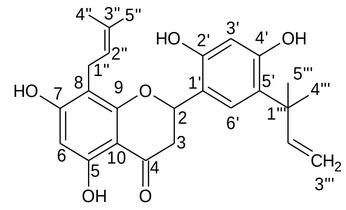


Table 1: % Inhibition of *Dalea pazensis* extracts at 1, 10, 100 µg/mL.

EXTRACTS	1µg/mL	10µg/mL	100µg/mL
Hexane	43.0 ± 4.4	78.8 ± 0.4	89.1 ± 0.1
Ethyl acetate	69.3 ± 1.0	87.6 ± 0.1	96.0 ± 0.2
Ethanol	71.4 ± 1.1	90.4 ±0.4	98.6 ± 0.8
Benzene	82.6 ± 1.0	90.2 ± 0.8	99.9 ± 0.2

The Benzene extract was the most active on the inhibitory diphenolase activity of tyrosinase. The decreasing order of inhibition is ethanol extract, ethyl acetate and hexane. These results are consistent with those reported on the inhibitory monophenolase activity.
Thus, we continued with the chemical study of this extract to determine the metabolite responsible for this activity.

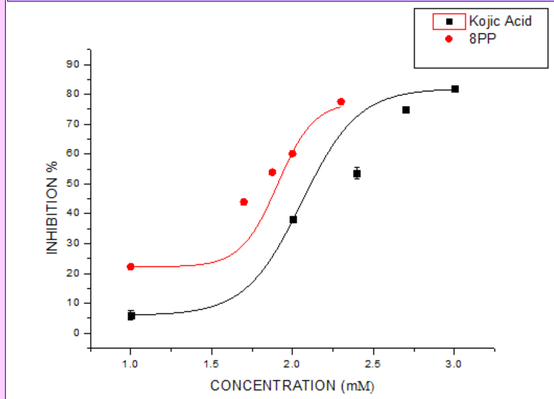
Figure 2: Chemical structure of 2 ', 4'-dihidroxy-5'-(1''', 1'''-dimethylalliyl)-8-prenylpinocembrin(**8PP**).



On the benzene extract was isolated and identified 2', 4'-dihydroxy-5' - (1 ''', 1''' - dimethylallyl) -8-prenylpinocembrin (8PP). Previously we reported that 8PP showed an important monophenolase inhibitory activity (IC50 = 2.305 ± 0.004 µM).Then we proceeded to assess its inhibitory diphenolase activity.

RESULTS AND DISCUSSION

Figure 3: Dose-dependent inhibition of mushroom tyrosinase activity by 8PP and reference inhibitor Kojic acid



8PP showed inhibitory activity of Tyr diphenolase with an IC_{50} of 80.7±0.4 µM being twice higher than Kojic Acid (129.6±0.3 µM).

CONCLUSION

This is the first report of the inhibition on diphenolase activity by 8PP, showing a higher activity than the reference inhibitor Kojic Acid.

We previously had reported an important inhibitory monophenolase activity by 8PP, being two times more active than the Kojic Acid. 8PP is more potent as an inhibitor on monophenolase activity, fact that could be explained for its structural similarity with the substrate of monophenolase reaction (L-tyrosine). [8,9]

According to these results, 8PP is presented as a possible candidate with potential applications in the pharmaceutical and cosmetics industry, being an important inhibitor of tyrosinase. This fact motivate us to search for other components that could contribute to the important inhibitory activity of Tyr observed in the benzene extract of *D. pazensis*.

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