ARTICLE / INVESTIGACIÓN

In vitro inhibition of xanthine oxidase by hydroalcoholic extracts of *Corynaea crassa* Hook. F

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Abstract: *Corynaea crassa* is a hemiparasitic plant native to America, used for the treatment of erectile dysfunction and has been shown to have antimicrobial properties. However, it lacks other biological studies to justify its use in traditional medicine. The objective was to examine the *in vitro* inhibitory activity of xanthine oxidase in hydroalcoholic extracts of the species from Ecuador and Peru. The extracts obtained by maceration and the Allopurinol used as the reference drug, at concentrations of 10, 30, 40, 50 and 60 µg/mL, were tested to measure the degree of *in vitro* inhibition of xanthine oxidase employing spectrophotometric determination at 295nm, which is associated with the formation of uric acid. The two extracts showed significant inhibitory activity on xanthine oxidase in a concentration-dependent manner, with the highest percentages being observed at the highest concentrations, being higher for the extract from the Ecuadorian species with enzyme inhibition percentages comparable to Allopurinol. Median inhibitory concentration (IC_{50}) values of 15.35µg/mL and 17.42µg/mL were observed for the extracts from Ecuador and Peru, respectively, although the activity was more notable for the reference drug, which was shown to be an IC_{50} of 12.21 µg/mL. The results concluded the basis for the potential use of *C. crassa* in the treatment of hyperuricemia.

Key words: xanthine oxidase, hydroalcoholic extracts, Allopurinol.

Introduction

The species *Corynaea crassa* Hook. F (Balanophoraceae) is a hemiparasitic plant native to America, found in various countries, including Bolivia, Colombia, Costa Rica, Ecuador, Mexico, Panama, Peru and Venezuela. In Ecuador, it is located in the provinces of Azuay, Carchi, Chimborazo, Cotopaxi, Imbabura, Loja, Morona Santiago, Napo, Pichincha, Sucumbios, Tungurahua and Zamora Chinchipe[1].

In previous studies, the antioxidant activity of hydroalcoholic extracts of the species was evaluated, with similar or superior results to the tested reference substances (vitamin C and Trolox)[2]. Other studies demonstrated the anti-inflammatory effect of the aqueous and hydroalcoholic extracts of the plant from Ecuador and Peru[3] and the erectogenic effect on induced sexual dysfunction in rodents[4].

Phytochemical studies revealed by liquid chromatography-mass spectrometry the presence of catechin, quercetin, and a flavanone glycoside)[2]. Other investigations by gas chromatography-mass spectrometry allowed the detection of terpenoids and fatty acids as significant compounds[3]. On the other hand, Malca *et al.*[5], reported sterols, triterpenoids, flavonoids, tannins and anthocyanins.

In Ecuador, *C. crassa* is little used due to the population's ignorance. Because it develops in the Andean forests and lacks sufficient scientific studies to support said ancestral knowledge, the present investigation was carried out to examine the *in vitro* inhibitory activity of xanthine oxidase in hydroalcoholic extracts of the species from Ecuador and Peru.

Materials and methods

The species *Corynae crassa* was collected in August 2018, coming from Ecuador (Yanachoca reserve in the north of Pichincha Province (00° 05′S, 78° 33′E, 3700 m elevation)) and Peru (province of La Libertad, department of Santiago de Chuco, Agasmarca (08°07′53″S, 78°03′23″E, 2900 m elevation). One specimen from each collection was identified in the herbarium of the Faculty of Natural Sciences of the University of Guayaquil. It was deposited under the coupon specimen of 13,115 and 13,116, respectively. The whole plant was worked with. The hydroalcoholic extracts were obtained by maceration from 20 g of the dry drug in 100 mL of the hydroalcoholic mixture at 80%. Subsequently, they were concentrated to dryness in a rotary evaporator (Buchi REF 120) at a temperature of 50 °C and redissolved in dimethylsulfoxide (Sigma-Aldrich) for *in vitro* analysis.

Xanthine oxidase inhibition assay

The inhibitory activity of xanthine oxidase (XO) was assayed spectrophotometrically under aerobic conditions, according to the procedure described by Nguyen *et al.*[6]. The assay mixture consisted of 50 mL of test solution, 35 mL of 70 mM phosphate buffer (Sigma, Aldrich) (pH=7.5), and 30 mL of xanthine oxidase enzyme solution (Sigma, Aldrich) (0.01 units / mL in 70 mM phosphate buffer, pH=7.5), was prepared immediately before use. After preincubation at 25 °C for 15 min, the reaction was started by adding 60 mL of substrate solution (150 mM xanthine (Sigma, Aldrich) in the same buffer). The assay mix was incubated at 25°C for 30 min. The reaction was stopped by adding 25 mL of 1N HCL,

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Figure 1. *Corynaea* is a monotypic genus of parasitic plants, belonging to the family Balanophoraceae. Its only species: *Corynaea crassa* Hook.f., is native to America.

and the absorbance at 290 nm was measured in a spectrophotometer (RAY LEIGH UV-1601 of Chinese origin). The enzyme solution was added to the assay mix after adding 1N HCL, and a blank was prepared in the same way. One XO unit is defined as the enzyme needed to produce 1 mol of uric acid/min at 25 °C.

XO inhibitory activity was expressed as the percentage inhibition of XO in the above assay system, calculated as (1 – B/A) x 100, where A and B are the activities of the enzyme without and with test material, respectively. The extracts (from samples from Ecuador and Peru) and the Allopurinol (Sigma) used as a positive control were prepared at concentrations of 10, 30, 40, 50 and 60 µg/mL.

Statistical analysis

The results were expressed as mean/standard deviation (SD), and the experiments were carried out in triplicates. A one-way analysis of variance (ANOVA) was used to determine the differences between groups, followed by the Duncan test with $p \leq 0.05$. The mean inhibitory concentration (IC_{50}) of the reference drug and the extracts was calculated from the mean values of the data of three determinations. The Statgraphics statistical program Plus version 5.1 was used to process the experimental data.

Results

The inhibitory activity of xanthine oxidase (XO) was demonstrated by the hydroalcoholic extracts of C. crassa, with enzyme inhibition percentages more significant than 50% from a concentration of 30 µg/mL. The results of this study demonstrated that the active extracts inhibited XO in a dose-dependent manner. The results of the in vitro XO inhibitory activity of test samples and control are shown in table 1.

Discussion

Xanthine oxidase (XO) inhibitors are generally used for the treatment of gout and hyperuricemia since they can hinder the enzymatic reactions involved in the synthesis of uric acid, reduce the formation of uric acid and relieve the symptoms of said disease, a very painful medical condition caused by high levels of uric acid. Scientists have recently attempted to find new safe XO inhibitors from various plants[7,8].

In the Balanophoraceae family, especially in the *Balanophora* genus, many phytochemicals have been found with a hyperuricemic effect[7,9]. However, in the genus *Corynaea* ofthe same family, the inhibitory activity of the enzyme xanthine oxidase has not been studied, which allowed this research to be carried out on *C. crassa*.

Five concentrations of hydroalcoholic extracts of species from Ecuador and Peru were tested, and Allopurinol as a reference drug, which prevents the formation of uric acid and is the mainstay of prophylactic treatment for hyperuricemia in patients undergoing chemotherapy[10,11].

XO is an important enzyme that converts xanthine and hypoxanthine to uric acid; thus, an increased rate of XO activity leads to excessive uric acid production[11]. In the present investigation, the reference drug and the two tested extracts (Ecuador and Peru) were able to reduce the activity of the said enzyme with mean inhibitory concentration values of 12.21, 15.35 and 17.42 µg/mL, respectively. The extract from the Ecuadorian sample showed enzyme inhibition

Concentration (µg/mL)	XO inhibitory activity (%)		
	Allopurinol	Ecuador extract	Peru extract
10	43.01/2.11[a]	40.01/1.91[ab]	38.77/0.92[b]
30	71.35/0.70[a]	70.12/0.63[ab]	68.27/1.75[b]
40	79.93/1.39[a]	75.21/1.20[b]	71.44/0.68[c]
50	81.87/1.63[a]	78.66/1.01[a]	73.20/2.19[b]
60	88.31/0.96[a]	86.25/1.04[a]	83.36/2.04[b]
IC_{50}	12,21	15,35	17,42

Values represent Mean / Standard deviation (n =3)

Different letters in a row show significant differences ($p < 0.05$) according to Duncan test

Table 1. *In vitro* xanthine oxidase inhibitory activity of *C. crassa* extract.

percentages comparable to Allopurinol at all concentrations tested (10, 30, 40, 50 and 60 µg/mL). An analysis of the results concerning the literature reports[9] demonstrated the excellent capacity of the extracts to inhibit xanthine oxidase, compared to the species *Balanophora laxiflora* (Balanophoraceae), where at a concentration of 10 µg/mL of the crude extract of the plant, an inhibition of 31.7% was obtained, lower than the experience carried out where the evaluated extracts registered values of 40.01% (Ecuador) and 38.77% (Peru). In addition, the IC_{50} values(the concentration required to inhibit uric acid formation by 50%) of crude extract was 28.2 µg/mL, while for the extracts under study, the IC50 was 15.35 and 17.42 µg/mL.

Comparison with other investigations of the species *Balanophora subcupularis* P.C. Tam (IC50= 48.41 µg/mL), also confirms the high property of *C. crassa* extracts as inhibitors of the enzyme xanthine oxidase. In contrast, *Balanophora tobiracola Makino* (Balanophoraceae) showed higher activity (IC_{50}=11.87 µg/mL)[7].

Natural products have been taken as an ideal source of bioactive compounds with specific pharmacological activities. Inhibitors of xanthine oxidase have been identified and isolated in many plant extracts[12,13]. Several bioactive compounds, including polyphenols, saponins, terpenoids, phenylethane glycosides, and alkaloids, among other compounds, are effective inhibitors of XO[13,14].

Previous studies with hydroalcoholic extracts of *C. crassa* demonstrated the presence of a variety of metabolites, including catechin flavonoids, quercetin glycoside, and a flavanone glycoside[2], terpenoids (triterpenoids and sesquiterpenoids), saponins, tannins, among others compounds[3] that could contribute to the inhibition of xanthine oxidase.

Conclusions

The results of this research provide scientific evidence for the first time on the inhibitory capacity of *C. crassa* xanthine, and the species may be considered a potential resource in the treatment of hyperuricemia and gout.

Author Contributions

AJLB proposed the concept of this study and analyzed the results and wrote the initial draft; YIGG. Investigation, writing-review and editing.

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Conflicts of Interest

The authors declare no conflict of interest.

Bibliographic references

1. Tropics. Flora Mesoamericans. Corynaea crassa Hook. f. [Online]. 2012. (Cited 2020 Dec 10. Available from: http://www.tropicos.org/name/03000026?projectid=3
2. Lopez, A.J.; Gutiérrez, G.Y.I.; Miranda, M.M.; Choez, G.I.A.; Ruiz, R.S.G.; Scull, L.R. Pharmacognostic, Phytochemical, and Anti-Inflammatory Effects of Corynaea crassa: A Comparative Study of Plants from Ecuador and Peru. Pharmacognosy Research 2021, 12, 394-402. Doi: 10.4103/pr.pr_42_20
3. Lopez, A.J.; Gutiérrez, Y.I.; Miranda, M.; Choez, I.A., Ruíz, S.G., Scull, R. Pharmacognostic, phytochemical, and anti-inflammatory effects of Corynaea crassa: A comparative study of plants from Ecuador and Peru. Pharmacogn. Res. 2020, 12, 394-402. https://dx.doi.org/10.4103/pr.pr_42_20
4. Acaro, F.; Arroyo, J. Efecto del extracto de Corynaea crassa y selenio en la disfunción sexual inducida en Rattus norvegicus albinus. Rev. Peru. Med. Integr. 2019, 4(3), 83-89.
5. Malca, G.R.; Hennig, L.; Sieler, J.; Bussmann, R.W. Constituents of Corynaea crassa Peruvian Viagra. Rev. Brasileira de Farmacognosia 2015, 25, 92-7.
6. Nguyen, M.T.T; Awale, S.; Tezuka, Y.; Tran, Q.L.; Watanabe, H; Kadota, S. Xanthine oxidase inhibitory activity of Vietnamese medicinal plants. Biol. Pharm. Bull. 2004, 27, 1414-1421.
7. Tung, N.T.; Quan, N.V.; Anh, N.P.; Phuong, N.V.; Hung, N.Q. Preliminary Phytochemical Evaluation and In Vitro Xanthine Oxidase Inhibitory Activity of Balanophora subcupularis P.C. Tam and Balanophora tobiracola Makino (Balanophoraceae). Trop J Nat Prod Res. 2019, 3(1), 6-9. Doi.org/10.26538/tjnpr/v3i1.2

8. Rodriguez, S.A.; Murray, A.P.; Leiro, J.M. Xanthine Oxidase Inhibition by Aqueous Extract of Limonium brasiliense (Plumbaginaceae). Chem. Proc. 2021, 3, 123. https://doi.org/10.3390/ecsoc-24-08410
9. Ho, S.T.; Tung, Y.T.; Huang, C.C.; Kuo, C.L.; Lin, C.C.; Yang, S.C.; et al. The Hypouricemic Effect of Balanophora laxiflora extracts and derived phytochemicals in hyperuricemic mice. Hindawi. Evidence-Based Complementary and Alternative Medicine 2012, 2012. Doi:10.1155/2012/910152
10. Bernardes, A.C.; Brandão, C.G.; Araújo, M.C.; Guimarães, D.A. In vivo anti-hyperuricemic activity of sesquiterpene lactones from Lychnophora species. Revista Brasileira de Farmacognosia 2019, 29, 241-245.
11. Cicero, A.F.G.; Fogacci, F.; Cincione, R.I.; Tocci, G.; Borghi, C. Clinical effects of xanthine oxidase inhibitors in hyperuricemic patients. Med Princ Pract 2021,, 30, 122-130. DOI:10.1159/000512178
12. Liu, Y.; Hou, Y., Si, Y.; Wang, W.; Zhang, S.; Sun, S.; et al. Isolation, characterization, and xanthine oxidase inhibitory activities of flavonoids from the leaves of Perilla frutescens. Natural Product Research. 2019. https://doi.org/10.1080/14786419.2018.1544981
13. Liu, L.; Zhang, L.; Ren, L.; Xie, Y. Advances in structures required of polyphenols for xanthine oxidase inhibition. Food Frontiers 2020, 1, 152-167. DOI: 10.1002/fft2.27
14. Mehmood, A.; Zhao, L.; Wang, C.; Nadeem, M.; Raza, A.; Ali, N.; et al. Management of hyperuricemia through dietary polyphenols as a natural medicament: A comprehensive review. Critical Reviews in Food Science and Nutrition. 2019, 59(9), 1433-1455. https://doi.org/10.1080/10408398.2017.1412939