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Potential uses of Hylocereus undatus (Haworth) Britton & Rose by-products: antimicrobial activity and flavonoid content from aerial parts extracts[1]

Usos Potenciales de los Subproductos de Hylocereus undatus (Haworth) Britton & Rose: Actividad Antimicrobiana y Contenido de Flavonoides de Extractos de Partes Aéreas

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Resumen

Para determinar los posibles usos de los subproductos de las partes aéreas de *Hylocereus undatus* como agentes antiinflamatorios y antimicrobianos, se obtuvieron los extractos orgánicos y se evaluaron utilizando el edema en oreja de ratón inducido por TPA y el método de macrodilución en tubo respectivamente. Se realizó la cuantificación colorimétrica de compuestos de tipo fenólico, la cuantificación de flavonoides totales, así como la identificación de algunos metabolitos no polares por CG-EM. En la evaluación de la actividad antiinflamatoria, el extracto metanólico mostró un 25,16% de inhibición del edema. En los ensayos antimicrobianos, todos los extractos mostraron inhibición contra todas las cepas, principalmente contra *C. albicans*. Por otro lado, el extracto de acetato de etilo mostró tanto la mayor cantidad de compuestos de tipo fenólicos, así como de flavonoides totales. Finalmente, el análisis CG-EM reveló la presencia de algunos compuestos bioactivos bien conocidos como el β-sitosterol, el ácido 4-metoxicinámico, entre otros. El estudio demostró que los subproductos de *H. undatus* pueden usarse como fuente de compuestos de tipo fenólicos bioactivos útiles en el desarrollo de agentes antifúngicos.

Palabras clave: *Hylocereus undatus*; subproductos; flavonoides; *C. albicans*; antimicrobiano.

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Abstract

To determine the possible uses of *Hylocereus undatus* aerial parts by-products as anti-inflammatory and antimicrobial agents, the organic extracts were obtained and evaluated using the TPA-induced mice ear edema, and the tube-macro-dilution method respectively. The colorimetric quantification of total phenolic and flavonoid compounds, as well as the identification of some non-polar metabolites by GC-MS, was performed. In the evaluation of anti-inflammatory activity, the methanolic extract showed 25.16 % of edema inhibition. In the anti-microbial assays, all extracts showed inhibition against all strains, mostly against *C. albicans*. On the other hand, the ethyl acetate extract showed the highest amount of total phenolics and flavonoids. Finally, the GC-MS analysis revealed the presence of some well-known bioactive compounds like β-sitosterol, 4-methoxycynnamic acid among other compounds. The study demonstrated that *H. undatus* by-products can be used as a source of bioactive phenolics useful in the development of antifungal agents.

Keywords: *Hylocereus undatus;* by-products; flavonoids; *C. albicans*; antimicrobial.

Introduction

Mexico is the country with the highest diversity of cactus in America. Taxonomical and ecological studies have confirmed that more than 600 species are found in the country, of which around 80% are endemic, grouped in about 100 genera. Many of the species in this genus have a relevant biological, cultural, economic and ethnobotanical importance (Godínez-Alvarez & Ortega-Baes, 2007). One of those genera is *Hylocereus*; the genus is endemic of North and Central America, but widely used all over the world as an important crop (Le Bellec, et al., 2006). The genus contains about 14 species of which fruits are traditionally used as food and medicine, whereas aerial parts are considered by-products. *Hylocereus* species around the world are mainly *H. undatus*, *H. monacanthus* and *H. megalanthus* (Ortiz-Hernández & Carrillo-Salazar, 2012). *Hylocereus* fruits (dragon fruit or pitahaya) and seeds had become an interesting subject of many studies to investigate their chemistry due to their economic importance, finding betalains as the major colorful compound in fruits and essential fatty acids (namely linoleic and linolenic) in seeds (Stintzing, et al., 2002); also, dragon fruit extracts have shown high antioxidant activity in DPPH model (VH, et al., 2012). In another study, *H. undatus* branches, rind, fruit pulp, and flowers have been reported as a promoter of wound healing in a diabetic rat model (Ghosh & Gaba, 2013; Woo, et al., 2011). Despite the research on *Hylocereus* fruits have increased in the fields of medicine, agronomy and industry, the chemistry and biological activities of aerial parts are still unknown because they are considered by-products. In the search of new sources of bioactive compounds such as flavonoids among other phenolic compounds, our group has decided to explore some by-products. In this opportunity, we begin a research project with aerial parts of *H. undatus*. As far as we know, the chemical composition and the bioactivities of aerial parts of *H. undatus* haven't been studied before; therefore, the aim of the present short communication was to evaluate the anti-inflammatory and antimicrobial activities of different organic extracts of *H. undatus* as well as the first exploration of chemical composition through the quantification of total phenolic and flavonoid contents and the exploration of the non-polar compounds by GC-MS, to find possible uses of *H. undatus* by-products.

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1. Experimental

1.1. Reagents

12-*O*-tetradecanoylphorbol-13-acetate (TPA), gallic acid, catechin, and all solvents were obtained from Sigma-Aldrich. Folin-Ciocalteu reagent (FCR), Sodium Carbonate, Sodium Nitrate, Aluminum Chloride, and Silica-Gel were obtained from J.T. Baker.

1.2. Plant material

The branches of *H. undatus* were collected in Cuautla, Morelos state, at coordinates 18°48’15.48” N and 98°56’01.20” W, in June 2013, and identified by Teresa Terrazas at Instituto de Biología, UNAM assigned with voucher code T. Terrazas-1001. The fresh plant material (406 g) was cut into slides, dried and macerated at room temperature with hexane, ethyl acetate and methanol during 24h. After this, the macerates were filtered and concentrated under vacuum to obtain the hexanoic (Hy-HX), ethyl acetate (Hy-AO) and methanolic (Hy-MH) extracts, respectively.

1.3. Anti-inflammatory activity

The anti-inflammatory activity was determined using the TPA-induced edema test as previously described (Salazar, et al., 2011), with slights modifications (Soto-Cabrera, et al., 2015). Extract concentration applied in both sides of ears was 1 mg/ear; indomethacin was used as a reference. The experiment was conducted by threefold. The Standard Error of the Mean was calculated, and the results were expressed as the arithmetic main ± SEM. All data were analyzed by ANOVA, the values of * $p \leq 0.05$ are considered as statistically different.

1.4. Antimicrobial activity

The antimicrobial assay was performed under the tube-macro-dilution method (NCCLS) using Mueller-Hinton broth for bacteria and Sabureaud broth for fungi. Each extract was used at concentrations: 0.500 - 0.015 mg/ml. The strains used were *Escherichia coli* (ATCC 35218), *Staphylococcus aureus* (ATCC 33862), *Staphylococcus epidermidis* (ATCC 12228), *Staphylococcus enterica* (ATCC 13076), and *Klebsiella pneumoniae* (ATCC 10031) bacteria and *Candida albicans* (ATCC 33862) yeast and *Aspergillus niger* fungus; standardized at 0.5 of the

McFarland scale (1x10^8). Positives controls were used: ofloxacin (0.005 mg/mL) for bacteria and clotrimazole (0.1 mg/mL) for fungi. The Minimum Inhibitory Concentration (MIC) was determined as described before (Nurmahani, et al., 2012).

1.5. Total Phenolic Content and Flavonoids Quantification

The Folin-Ciocalteu method was used for the quantification of total phenolic content (TPC) as previously described (Torres, et al., 2014). The calibration curve was prepared with gallic acid. TPC was calculated by interpolation, r^2=0.9834. The SD was calculated. The results are expressed in milligrams equivalents of gallic acid (mgeqGA)/gram of dry plant material ± sd. (Chew, et al., 2011). On the other hand, the spectrophotometric aluminum chloride method was used for total flavonoids content as previously described (Torres, et al., 2014). The ethanolic solutions of each extract were prepared at 100mg/ml and 10 µl of each were placed in the wells of the microplate. The SD was calculated, and the total flavonoid content was determined by interpolation, r^2=0.9972. The results are expressed in milligrams equivalents of catechin per dry g of plant material ± sd. Both quantifications were performed by fivefold. All data were analyzed by ANOVA, the values of * $p \leq 0.05$ are considered as statistically different.

1.6. GC-MS analysis

The GC-MS analysis was developed in a 6890N (Agilent Technologies) gas chromatograph equipped with a 5973nN (Agilent Technologies) mass spectrometer fitted with 60m x 0.25mm DB-1 Agilent 122-1061 capillary column at carrier gas flow (Helium) of 1.5 ml/min with splitless. The conditions and general procedures were done as previously described (Soto-Cabrera et al., 2015). The mass spectrometer was at 70 eV with a source and a quadrupole of 240 and 180 eV respectively, with a mass range (m/z) of 40-500 Da. The samples were compared with the NIST library.

2. Results and discussion

Tropical fruits by-products are an important source of bioactive compounds such as antioxidants, antimicrobials among others (Nguyen et al., 2019). In the production of dragon fruit, the branches and aerial parts are considered waste material. In this preliminary work, we are searching for

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bioactivity of the organic extracts of by-products of *H. undatus*, mainly anti-inflammatory, and antimicrobial activities, as well as exploring for the first time, the chemical composition of the bioactive extracts through the search of polyphenols and flavonoids, together with the first exploration of non-polar components of organic extracts in gas chromatography.

The branches of *H. undatus* (T. Terrazas-1001) were extracted by maceration with different organic solvents. The plant material was removed by filtration and the solvent was removed under vacuum.

The extracts were dissolved in acetone and evaluated in the anti-inflammatory assay. All extracts showed weak inhibition of edema induced by TPA at a single dose of 1 mg/ear. Hy-MH showed the highest edema inhibition percentage with 25.16 ± 2.22*, followed by Hy-AO: 19.75 ± 3.26 and Hy-HX: 9.74 ± 1.30. The inhibition of edema is not relevant to us, thus we decided to do not continue with the exploration of the anti-inflammatory assays.

On the other hand, in the antimicrobial assay, fungus and yeast showed higher sensibility than bacteria when organic extracts were incorporated into the broth. The MIC values observed against *C. albicans* were 0.062 mg/mL for all extracts. The MIC values for all extracts are shown in Table 1. Compared with the antimicrobial standards used, ofloxacin for bacteria; clotrimazole for fungi, organic extracts of *H. undatus* showed minor effectiveness against bacterial strains, but as well as one order in magnitude more effectiveness than clotrimazole against *C. albicans*.

The total phenolic content of organic extracts was determined. Hy-AO extract has the higher content with 570.2 mgeqGA/g ± 0.217*, followed by the Hy-MH extract with 137.3 ± 0.038, while in Hy-HX phenolic compounds were not detected with the methodology used. The high TPC of Hy-AO extract is relevant to us because the aerial part of *H. undatus* could be considered as a novel source of bioactive phenolics. Additional experiments are necessary to know the identity of phenolics.

Table 1.
Antimicrobial assay. Minimum Inhibitory Concentration (MIC, mg/mL) of H. undatus extracts against selected microorganisms.

Strain|Extract	Hy-AO	Hy-HX	Hy-MH	Control*
E. coli	0.060	0.125	0.125	0.005
S. aureus	0.250	0.500	0.125	0.005

S. enterica	0.125	0.125	0.125	0.005
S. epidermidis	0.250	0.250	0.125	0.005
K. pneumoniae	0.125	0.250	0.125	0.005
C. albicans	0.062	0.062	0.062	0.100
A. niger	0.125	0.125	0.125	0.100

Hy-AO = ethyl acetate extract; Hy-HX = hexanoic extract; Hy-MH = methanolic extract. *Control: ofloxacin for bacteria; clotrimazole for fungi.

In flavonoid quantification assay, Hy-AO extract showed also the higher amount with 489.99 mg ± 0.023*, followed by the Hy-MH: 272.40 mg ± 0.002* and the Hy-HX 143.80 mg ± 0.017*. The content of flavonoids in Hy-AO is consistent with TPC described above. The results suggest that the main kind of phenolic-like compounds in Hy-AO are flavonoids. Some additional experiments must be done to determine the nature of the major flavonoid components of *H. undatus*. Finally, in gas chromatography, some non-polar compounds from each extract were separated and identified by comparison of their retention times and mass spectrometry data. This exploratory analysis was carried out for the first time in the organic extracts of branches of *H. undatus* with the intention to identify some bioactive compounds previously described in Cactaceae. The most interesting extract we found in the species was Hy-AO. This extract was subjected to a separation in GC-MS. In the preliminary examination of GC-MS data of Hy-AO were found some free fatty acid, cinnamic acid derivatives like 4-methoxycinnamic acid, some medium and long-chain fatty acid esters, together with β-sitosterol (Figure 1).

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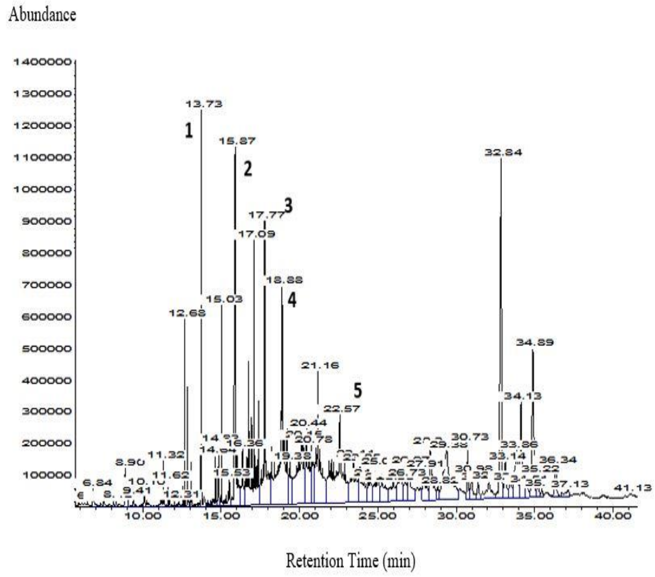

Figure 1. GC-MS chromatogram of ethyl acetate extract. Identified compounds: 4-methoxycinnamic acid (2); hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester (3); 9,12-octadecadienoic acid (4); β-sitosterol (5).

The overall results obtained indicate that organic extracts of *H. undatus* branches failed to decrease edema formation induced by TPA. Although all extracts showed the presence of flavonoids, which many of them are well-known anti-inflammatory agents (Ginwala et al., 2019), the topical application of extracts to mice ears could explain the absence of activity, since it is recognized that some flavonoids fail to be absorbed through mice skin (Paleco, et al., 2014).

In contrast, Hy-AO was able to decrease the viability of all strains of microorganism, mainly against *C. albicans* a well-known opportunist pathogen whit relevant importance in nosocomial infections (Cortegiani, Misseri & Chowdhary, 2019). These results contribute to the knowledge of aerial parts of *H. undatus* since only the antimicrobial activities of fruit extracts have been previously described (Tenore, et al., 2012). The high amounts of total phenolic compounds, together with the presence of some flavonoids, both determined in colorimetric quantifications, can explain the bioactivity of Hy-AO. In addition of the above, the identification in GC-MS of common antifungal (especially against *C. albicans*) natural products as β-sitosterol (Choi et al., 2017; Mainen et al., 2004), and 4-methoxycynnamic acid (Sova, 2012), could contribute to explain the diminution of *C. albicans* viability. Although all extracts showed the inhibition of the growth of all bacteria strains, the magnitude of the MIC values is not relevant to our group.

4. Conclusions

The results provide new information about the chemical composition of aerial parts of *H. undatus*. With the results obtained, it is possible to propose the use of byproducts from the species as raw material to further research and development of new phenolic/sterol-based antifungal agents against *C. albicans* and related species.

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