XVI Congreso Nacional de Biotecnología y Bioingeniería
21 al 26 de Junio de 2015 Guadalajara, Jalisco, México.
Guadalajara

PURIFICATION OF HEXANIC EXTRACT FROM *CITRUS SINENSIS* L. AND TOXICITY ASSESSMENT

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Key words: purification, Citrus sinensis, Artemia salina

Introduction. Purification of compounds of agro-alimentary importance such as *Citrus sinensis* L., require effective and accurate separation techniques whose foundation is based on the polarity of the compound and its affinity between the mobile phase and stationary (1). In addition to use as the biological assays *Artemia salina*, this allows selection of bioactive compounds, which were purified once in other models to evaluate to permit a more specific application as nutraceuticals (2). You also need a qualitative chemical characterization. The objective of this work was to purify the hexane extract of the epicarp of *Citrus sinensis* (HxECs), evaluate the toxicity by biological model of *A. salina* and determine its composition by qualitative chemical analysis.

Methodology. The HxECs was purified by vacuum liquid chromatography (VLC) using as eluent dichloromethane-acetone (100:0 → 0:100) acetone: methanol (0:100), with volumes of 200 mL at a flow of 15 cm^3/min, the fractions were coded as VLC_{1-12}. That were characterized by phytochemical qualitative screening, using specific tests for each compound. The cytotoxic effect of the fractions was determined on the biological system of *A. salina*, was verified by microdilution of the samples, (1000, 500, 250, 100, 50 ppm), upon ten nauplii *A. salina* in a microplate of 96 wells., the results were analyzed through simple linear regression by statistical program Graph pad prism 5 ®.

Results. In the chromatographic purification (VLC) were obtained 12 fractions from HxECs and were coded VLC_{1-12}, the highest percentage of recovery was found for the CLV_1 with 38 %, this depends on the group of metabolites that exists in each one of them, whose migration is caused due to the balance between the mobile phases, and the stationary (3).

The qualitative phytochemical screening of the fractions CLV_{1-12}, revealed the presence of steroids and triterpenes, coumarins, sesquiterpenlactones, saponins, flavonoids and alkaloids, which may contain unsaturation and the presence of phenolic compounds reported in other investigations oxhydrils (5), and the presence of carbohydrate was detected.

Briefly, the HxECs showed a median lethal concentration (LC_{50}) of 87.41 ± 2.01 ppm, the fractions F1 and F2 showed toxic activity on *A. saline* of ≥250 and ≥100 ppm values respectively. The F3 showed a toxicity value of LC_{50} of 24.29 ± 0.64 ppm, the F4 of 60.14 ± 0.50, F5, F6 and F7 a toxicity value of ≥ 500 ppm, while in fractions F8, F9, F10, F11 and F12 a toxicity value of ≥1000 ppm. The values mentioned above can be seen reflected in Table 1, as well as lethality criteria based on the criteria established by Gualdrón *et al*., 1997.

Table 1. Median lethal concentration (LC_{50}) of the fractions of the HxECs

Sample	Lc_{50} (ppm)	Lethality criteria
HxECs	87.41 ± 2.01	High
CLV_1	≥250	Moderate
CLV_2	≥100	High
CLV_3	24.29 ± 0.64	High
CLV_4	60.14 ± 0.50	High
CLV_{5-8}	≥ 500	Low
CLV_{9-12}	≥1000 ppm	Minimum

Conclusions. The purified compounds from EHxCs of the epicarp from *Citrus sinensis* (Orange) may be considered of importance in human health, because that could be used in the medium term through its consumption as a food or food supplement

Acknowledgement. The Consejo Nacional de Ciencia y Tecnología (CONACYT) for the funding of this research, for master studies.

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