

Review Form 1.6

Journal Name:	<u>European Journal of Medicinal Plants</u>
Manuscript Number:	Ms_EJMP_84813
Title of the Manuscript:	Chemical composition and pharmacological activity of a propolis extract from Ecuador
Type of the Article	Original Research Article

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Field Code Changed

General guideline for Peer Review process:

This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound. To know the complete guideline for Peer Review process, reviewers are requested to visit this link:

(<https://www.journalejmp.com/index.php/EJMP/editorial-policy>)

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PART 1: Review Comments

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
Compulsory REVISION comments	The article presents a very interesting and relevant topic. In general the article is well written and with important information on the subject. However, the authors need to explain some points below:	
Minor REVISION comments	<p>- Why was methanol used in the extraction process? The authors must justify the choice, mainly due to its toxicity;</p> <p>- Authors should better explain the derivatization process used and explain why they performed this step;</p> <p>- Authors need to make use of the retention index in order to improve the identification of compounds. Identification by mass spectrum alone in gas chromatography may be insufficient. At least the retention rate should be used to improve identification assertiveness. In this case, the programmed temperature retention index (LTPRI) of van den Dool and Kratz can be used. This is the main questioning point of the work, as the identification process may be compromised since the authors did not use standards for positive identification and did not use the retention index.</p>	<p>▲ Methanol is a common solvent used in the extraction process of propolis. It is better than ethanol for removing bees_wax because it is slightly more polar. In addition, methanol has a lower boiling temperature and therefore it can be easily removed after concentration to dryness under vacuum at 40° C. So, there is no traces of the solvent in the sample</p> <p>▲ The derivatization process is usual strategy in gas chromatography since it can increase the volatility of chemical compounds and favors their detection. More information was included in the manuscript</p> <p>Ten mg of the extract of propolis were dissolved in 1 mL of ethanol (90 %, v/v). One hundred µL of the solution was taken and evaporated to dryness under a stream of nitrogen. The residue was dissolved in 50 µL of piridin and 100 µL of N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) in a sealed glass tube for 30 min at 100 °C. The gas chromatograph (Agilent model GC 6890) coupled to a quadrupole mass spectrometer (HP 5973N) was used. The injector was heated to 310 °C and was on split mode with a split ratio of 1:50. Sample was separated on a 30 m × 0.25 mm i.d., 0.25 µm film thicknesses, HP-5MS column. The temperature of the chromatograph was programmed at 60 °C for 2 min, increasing to 310 °C with a ratio of 3 °C per min-1, followed by an isothermal process of 20 min. The final injection volume was 1 µL. The components of the sample were fractionated on the HP-5MS column (30m x 0.25 mm x 0.25 µm). The total run time was 100 min, operating by electronic ionization at 70 eV with a mass range of 35 - 700 uma. Helio was the carrier gas (Campo et al. 2008; Popova et al. 2010).</p> <p>A chemical structure was only assigned to those compounds very commonly found in propolis and whose mass spectra have been extensively analyzed (amyrin, sugars, fatty acids and so on). Even two very closely isomers as α- and β-amyrin can be easily differentiated by their mass spectra. For the other compounds, tentative structures were proposed on the basis of the well-known rDA fragmentation of flavonoids. It was not possible to define certain structures because they are minor compounds and due to the lack of standards. Chemical characterizations performed on the basis of mass fragmentations and comparisons with computer mass libraries are usually employed: Syed Salleh, S. N. A., Mohd Hanapiah, N. A., Ahmad, H., Wan Johari, W. L., Osman, N. H., & Mamat, M. R. (2021). Determination of total phenolics, flavonoids, and antioxidant activity and GC-MS analysis of Malaysian stingless bee propolis water extracts. Scientifica, 2021. Bozkuş, T. N., Değer, O., & Yaşar, A. (2021). Chemical characterization of water and ethanolic extracts of Turkish propolis by HPLC-DAD and GC-MS. Journal of Liquid Chromatography & Related Technologies, 44(1-2), 77-86. Marquez Hernandez, I., Cuesta-Rubio, O., Campo Fernandez, M., Rosado Perez, A., Montes de Oca Porto, R., Piccinelli, A. L., & Rastrelli, L. (2010). Studies on the constituents of yellow Cuban propolis: GC-MS determination of</p>

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		triterpenoids and flavonoids. Journal of Agricultural and Food Chemistry, 58(8), 4725-4730. Bankova, V., Bertelli, D., Borba, R., Conti, B. J., da Silva Cunha, I. B., Danert, C., ... & Zampini, C. (2019). Standard methods for Apis mellifera propolis research. Journal of Apicultural Research, 58(2), 1-49. Kartal, M., Kaya, S., & Kurucu, S. (2002). GC-MS analysis of propolis samples from two different regions of Turkey. Zeitschrift für Naturforschung C, 57(9-10), 905-909.
Optional/General comments	<div>- In the first paragraphs of the introduction, the authors do not cite the references used;</div> <div>- It would be interesting for the authors to include the geographic coordinates of the sample collection point;</div> <div>- In the experimental part, in the chromatographic part, the authors must specify other relevant information such as: injector temperature, ion source temperature, carrier gas;</div> <div>- The resolution and axes of figure 1 should be improved;</div> <div>- Authors must show the complete chromatogram;</div> <div>- References used in the work need to be updated.</div>	<div>As the reviewer suggested reference in the first paragraph was included in the manuscript</div> <div>As the reviewer suggested the geographic coordinates was included in the manuscript (Latitude: -3.562452, longitude: -80.056454)</div> <div>As the reviewer suggested this information was included in the manuscript</div> <div>As the reviewer suggested, the axes of figure 1 was improved</div> <div>No chromatographic signals were observed after 60 min. For that reason, the chromatogram is displayed up to 60 min. If we show the chromatogram up to 100 min, no relevant signals can be appreciated and the resolution of the figure will decrease appreciably. For that reason we added part of the chromatogramAs the reviewer suggested, updated references was included</div> <div>As the reviewer suggested the references was updated</div>

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PART 2:

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
Are there ethical issues in this manuscript?	(If yes, Kindly please write down the ethical issues here in details)	

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