

Phytochemical constituents, antioxidant, cytotoxic and antimicrobial activity of the ethanolic extract of Mexican brown propolis.

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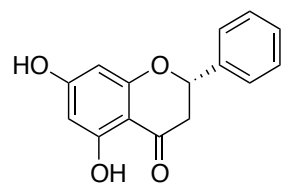
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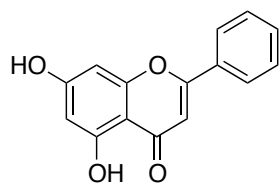
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Abstract: Propolis is a complex mixture of natural sticky and resinous components produced by honeybees from living plants. Extensive research has been dedicated to studying the biological properties and chemical composition of propolis from various geographical regions. However, the chemical data and biological properties of Mexican brown propolis are scant. The antioxidant activity of propolis (EEP) sample collected in Mexico and the isolated compounds is described. Cytotoxic activity was evaluated in human breast and cervical cancer cell lines. Cytotoxicity of EEP was evaluated in C6 cell line and cervical cancer (HeLa, SiHa, and CasKi) cell lines using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) assay. The antibacterial activity was tested using the minimum inhibitory concentration (MIC) assay. Twelve known compounds were isolated and identified by nuclear magnetic resonance spectroscopy (NMR). A total of 12 compounds were identified by means of headspace-solid phase microextraction with gas chromatography and mass spectrometry (HS-SPME/GC-MS-TOF). The main volatile compounds detected include nonanal (18.82%), α -pinene (12.45%), β -pinene (8.04%). EEP showed an anti-proliferative effect on glioma cells better than temozolomide, also decreased proliferation of cervical cancer cells, but its effectiveness was lower in compared to cisplatin.

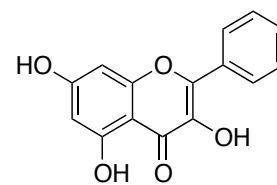
Keywords: Propolis, antioxidant activity, cytotoxic, antibacterial, Mexico, HS-SPME/GC-MS-TOF, NMR, volatile compounds, organic acids.



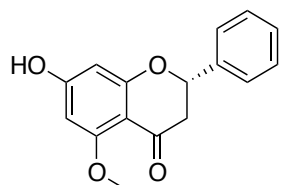
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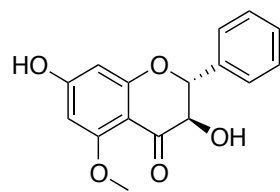
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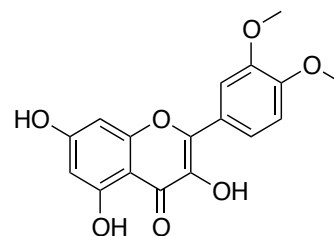
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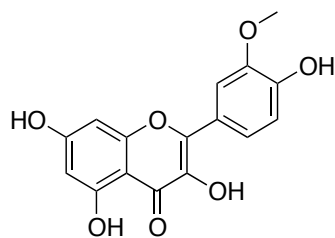
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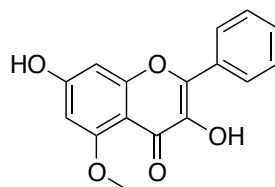
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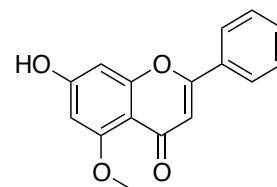
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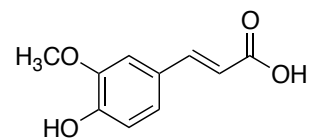
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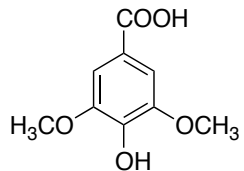
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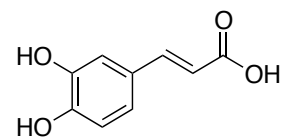
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Figure S1. Flavonoids isolated from EEP GUA-4

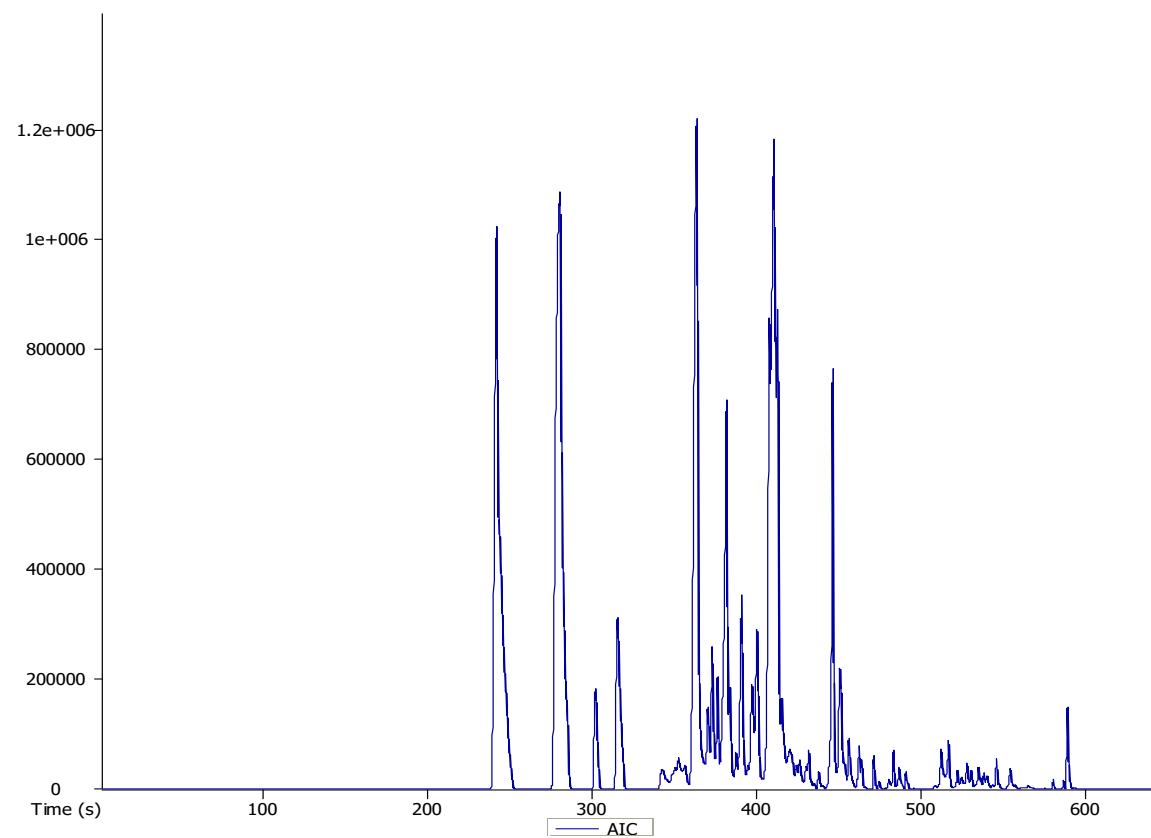


Figure S2. Analytical ion current chromatogram (AIC) of propolis GUA-4.



Figure 3. Major volatile compounds of EEP GUA-4.

Table S1. ¹H NMR (400 MHz) of the compounds isolated from propolis.

1H	pinocembrin (1) (MeOH- <i>d</i> ₄)	chrysin (2) (DMSO- <i>d</i> ₆)	alpinetin (4) (DMSO- <i>d</i> ₆)	5-O-metilpinobanksin ether (5) (DMSO- <i>d</i> ₆)
2	5.45 (1H, dd, <i>J</i> =16.0, 4.0)	-	5.46 (1H, dd, <i>J</i> = 16.0, 4.0 Hz)	4.32 (1H, d, <i>J</i> = 12.0 Hz)
3	3a 2.76 (1H, dd, <i>J</i> = 4.0, 16.0 Hz) 3b 3.09 (1H, dd, <i>J</i> = 16.0, 12.0 Hz)	6.98 (1H, s)	3a 2.60 (1H, dd, <i>J</i> = 4.0, 16.0 Hz) 3b 2.97 (1H, dd, <i>J</i> = 16.0, 12.0 Hz)	5.28 (1H, d, <i>J</i> = 12.0 Hz)
6	5.93 (1H, d, <i>J</i> = 4.0 Hz)	6.20 (1H, d, <i>J</i> = 2.0 Hz)	5.93 (1H, d, <i>J</i> = 2.0 Hz)	5.94 (1H, d, <i>J</i> = 2.0 Hz)
8	5.90 (1H, d, <i>J</i> = 4.0 Hz)	6.52 (1H, d, <i>J</i> = 2.0 Hz)	5.98 (1H, d, <i>J</i> = 2.0 Hz)	6.05 (1H, d, <i>J</i> = 2.0 Hz)
2', 6'	7.49 (2H, dd, <i>J</i> = 8.0, 4.0 Hz)	8.1 (2H, dd, <i>J</i> = 8.0, 4.0 Hz)	7.48 (2H, dd, <i>J</i> = 8.0, 4.0 Hz)	7.48 (2H, dd, <i>J</i> = 8.0, 4.0 Hz)
3'-5'	7.39 (3H, m)	7.55 (3H, m)	7.41 (3H, m)	7.41 (3H, m)
5-OH	-	12.63 (1H, s)	-	-
5-OCH ₃	-	-	3.72 (3H, s)	3.75 (3H, s)
3'-OCH ₃	-	-	-	-
4'-OH	-	-	-	-

Table S1. ¹H NMR (400 MHz) of the compounds isolated from propolis (cont).

1H	dillenetin (6) (DMSO- <i>d</i> ₆)	Isorhamnetin (7) (DMSO- <i>d</i> ₆)	5-O-metil-galangin (8) (DMSO- <i>d</i> ₆)	5-O-methyl chrysin (9) (DMSO- <i>d</i> ₆)
2	-	-	-	-
3	-	-	-	6.68 (1H, s)
6	6.18 (1H, d, <i>J</i> = 2.0 Hz)	6.17 (1H, d, <i>J</i> = 2.0 Hz)	6.34 (1H, d, <i>J</i> = 2.0 Hz)	6.38 (1H, d, <i>J</i> = 4.0 Hz)
8	6.10 (1H, d, <i>J</i> = 2.0 Hz)	6.45 (1H, d, <i>J</i> = 2.0 Hz)	6.47 (1H, d, <i>J</i> = 2.0 Hz)	6.55 (1H, d, <i>J</i> = 4.0 Hz)
2'	7.62 (1H, d, <i>J</i> = 2.0 Hz)	7.76 (1H, d, <i>J</i> = 2.0 Hz)	8.11 (2H, d, <i>J</i> = 8.0 Hz)	7.98 (2H, m)
6'	7.56 (1H, dd, <i>J</i> = 8.0, 2.0 Hz)	7.67 (1H, dd, <i>J</i> = 8.0, 2.0 Hz)	8.11 (2H, d, <i>J</i> = 8.0 Hz)	7.98 (2H, m)
3'	-	-	7.43-7.54 (3 H, m)	7.43-7.54 (3H, m)
4'	-	-	7.43-7.54 (3H, m)	7.43-7.54 (3H, m)
5'	6.94 (1H, d, <i>J</i> = 8.0 Hz)	6.92 (1H, d, <i>J</i> = 8.0 Hz)	7.43-7.54 (3H, m)	7.43-7.54 (3H, m)
5-OH	12.65 (1H, s)	12.44 (1H, s)	-	-
5-OCH ₃	-	-	3.81 (3H, s)	3.78 (3H, s)
3'-OCH ₃	3.84 (3H,s)	3.82 (3H,s)	-	-
4'-OCH ₃	3.78 (3H,s)	-	-	-