

## IDENTIFICATION, ISOLATION AND CHARACTERIZATION OF SUBSTANCES WITH ANTIMICROBIAL ACTIVITY IN THE EXTRACT OF PROPOLIS FROM *Scaptotrigona aff. Postica*

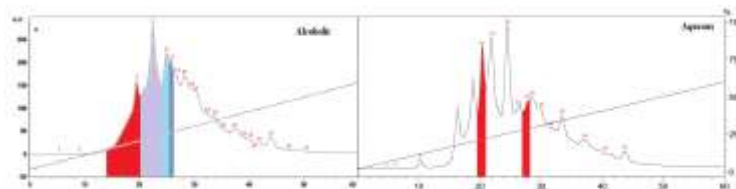
Mendonça RZ<sup>1</sup>, Cantero TM<sup>1</sup>, Nascimento, RM<sup>1</sup>, Negri G<sup>2</sup>, da Silva Junior PI<sup>3</sup>,

<sup>1</sup>Laboratory of Parasitology, Butantan Institute; <sup>2</sup>Laboratory of Phytochemist Department of Botany, Institute of Biosciences, University of São Paulo, <sup>3</sup>Laboratory of Applied Toxinology, Butantan Institute, Brasil <sup>1</sup>zucattelli@uol.com.br

**Keywords:** antimicrobial activity, propolis, *Scaptotrigona postica* bees

**Introduction.** Propolis is a resinous substance produced by bees. Its composition is diverse and varies according to the region they meet; more than three hundred bioactive compounds were identified in propolis from different species of bees, such as flavonoids and phenolic acids. Most studies on propolis are restricted to honeybees *Apis*, rare are the studies dealing with the propolis of stingless bees, among which we highlight those of the genus *Scaptotrigona aff. Postica*.

**Objective:** The objective of this work was to evaluate the antimicrobial potential of alcoholic and aqueous extractions of propolis of bee *S. postica*; **Material and Methods:** Propolis obtained from Barra do Corda, Brasil, was extracted into 70% methanol or ultrapure water (100 ml for every 10 grams). The supernatant was fractionated lyophilized in disposable C18 columns (SepPak). Chromatography of the sample was performed on an analytical reverse phase column C18, with a concentration gradient of 0% to 80% acetonitrile acidified for 60 minutes at a flow rate of 1.0 mL / min. The 40% ACN fractions, showing biological activity, were again purified. The samples obtained were analyzed for antimicrobial activity and cytotoxic assay by the MTT technique. The fractions with activity were submitted to mass spectrometry (CLAE-DAD-ESI-MS/MS) to identify the compounds.



**Figure 1.** Chromatogram of 40% ACN fraction. (A) Represents the 40% ACN eluted of the alcoholic propolis extraction on a preparative ODS Shim-pack in a 0% to 80% concentration gradient over 70 minutes at a flow rate of 8 ml / min. (B) Represents the eluted of the extract aqueous. Fractions with biological activity are highlighted in the graph above and the results of the test are shown in the table below.

Extraction Própolis	Fraction	Microorganismo		
		<i>Candida albicans</i>	<i>Micrococcus luteus</i>	<i>Escherichia coli</i>
Aqueous	3			+
Aqueous	9			+
Aqueous	13	+	+	
Aqueous	14	+	+	
Aqueous	15	+		
Aqueous	23	+		
Aqueous	25	+		
Aqueous	27		+	
Alcoholic	9		+	
Alcoholic	12		+	
Alcoholic	16		+	



**Results and Discussion:** Fractions were evaluated against four microorganisms *Micrococcus luteus* (A270), *Escherichia coli* (SBS 363), *Candida tropicalis* (IOC4560) and *Candida albicans* (MDM08). The alcoholic and aqueous extracts of the propolis after fractionated has resulted in three samples, each one de nominated 5, 40 and 80. They were lyophilized and resuspended in pure water or DMSO (5%). Then, they were tested against the strains of Gram-positive, Gram-negative and yeast. The tests were performed in liquid medium, as recommended by Bulet<sup>1</sup>. Initially the 5 and 40 samples, from both extracts demonstrate antibacterial activity, but only the 40% sample demonstrated capacity to inhibit Gram-positive bacteria. The samples were submitted to mass spectrometry. Some of the constituents obtained were: 7- (3-methoxy-2-methylbutyryl) -9-echimidinyl retronectine or methoxy echimidine derivative, methoxy-heptahydroxy-flavone glucuronide, catechin arabinoside, catechin rhamnoside and schaftoside (derived from apigenin).

Microorganism	Minimum Inhibitory Concentration (MIC)			
	alcoholic extract	aqueous	alcoholic extract semipurified	aqueous extract semipurified
<i>Staphylococcus aureus</i> ATCC29213	1000µg/mL	ND	1000µg/mL	1000µg/mL
<i>Bacillus megaterium</i> ATCC10778	62,5 µg/mL	500 µg/mL	62,5 µg/mL	62,5 µg/mL
<i>Micrococcus luteus</i> A270	125 µg/mL	250µg/mL	125 µg/mL	250µg/mL
<i>Escherichia coli</i> SBS 363	500 µg/mL	1000µg/mL	1000µg/mL	1000µg/mL
<i>Escherichia coli</i> D31	500 µg/mL	250µg/mL	1000µg/mL	250µg/mL
RESISTENT to streptomycin				

### Conclusion.

- Propolis has bioactive molecules against , *Escherichia coli*, *Micrococcus luteus*, *Bacillus megaterium* and *Staphylococcus aureus*;
- Total activity against resistant bacteria** was observed against *Escherichia coli* D31
- ACKNOWLEDGMENTS** – Instituto Butantan (IBu), CAPES, Fapesp,

