In vitro antimicrobial assessment of Cuban propolis extracts

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Propolis is a resinous mixture of different plant exudates collected by honeybees. Currently, propolis is widely used as a food supplement and in folk medicine. We have evaluated 20 Cuban propolis extracts of different chemical types, brown (BCP), red and yellow (YCP), with respect to their in vitro antibacterial, antifungal and antiprotozoal properties. The extracts inhibited the growth of Staphylococcus aureus *and* Trichophyton rubrum *at low µg/mL concentrations, whereas they were not active against* Escherichia coli *and* Candida albicans*. The major activity of the extracts was found against the protozoa* Leishmania, Trypanosoma *and* Plasmodium*, although cytotoxicity against MRC-5 cells was also observed. The BCP-3, YCP-39 and YCP-60 extracts showed the highest activity against* P. falciparum*, with 50% of microbial growth (IC_{50}) values of 0.2 µg/mL. A positive correlation between the biological activity and the chemical composition was observed for YCP extracts. The most promising antimicrobial activity corresponds to YCP subtype B, which contains acetyl triterpenes as the main constituents. The present in vitro study highlights the potential of propolis against protozoa, but further research is needed to increase selectivity towards the parasite. The observed chemical composition-activity relationship of propolis can contribute to the identification of the active principles and standardisation of this bee product.*

Key words: propolis - Cuban - antibacterial - antifungal - antiprotozoal

Propolis is a resinous mixture collected from plant buds and exudates by honeybees; this substance is used as a building insulation material in beehives (Greenaway et al. 1990). Because of its wide use in folk medicine, propolis has been the subject of intense pharmacological and chemical study for almost 30 years (Bankova 2005). It has recently gained popularity as a food supplement and is used extensively in foods and beverages in various parts of the world (Peterson & Barnes 1991). Numerous studies have shown its versatility of therapeutic activities, including antimicrobial, anti-inflammatory, antitumoural, antioxidant and hepatoprotective functions (Banskota et al. 2001, Lotfy 2006).

The chemical composition of propolis is very complex and depends on the local flora at the site of collection (Marcucci 1995). Propolis samples from tropical zones display a highly variable chemical composition, including terpenoids, prenylated organic acid derivatives, lignans and flavonoids (Cuesta-Rubio et al. 2007, Duran et al. 2008, Salomão et al. 2008). In Cuba, with its great botanical diversity and richness of flora, extensive chemical studies have identified three main types of propolis, based on their secondary metabolite classes. The three types of Cuban propolis are known as brown (BCP), red (RCP) and yellow (YCP) (Cuesta-Rubio et al. 2007). In addition, various medicinal properties of Cuban propolis, such as antipsoriatic, anti-inflammatory, analgesic (Ledón et al. 1996) and antitumoural activities (Popolo et al. 2009), have been explored. Diverse studies of propolis have been conducted against infectious diseases, such as acute cervicitis (Quintana et al. 1996), parasitic vaginal infections (Santana et al. 1995), dental plaque (Gispert et al. 2000), facial septic injury (Quintana et al. 1997) and giardiasis (Miyares et al. 1988). In this study, we investigated the effects of 20 propolis extracts (BCP, RCP and YCP type), which were from different regions of Cuba, against bacteria (*Escherichia coli* and *Staphylococcus aureus*), fungi (*Trichophyton rubrum* and *Candida albicans*) and protozoa (*Plasmodium falciparum, Trypanosoma brucei brucei, Trypanosoma cruzi* and *Leishmania infantum*).

MATERIALS AND METHODS

Propolis extracts - Twenty Cuban propolis samples were provided by La Estación Experimental Apícola, Havana City, between October 2003-December 2004, from nine provinces (Supplementary data) of the eastern, central and western regions (Fig. 1). The propolis samples were extracted by maceration with 100% methanol (10 mL, 3 times) for 1 h at room temperature (RT), with occasional stirring. The extracts were filtered using paper filters and the solvent was evaporated at 40ºC under reduced pressure to obtain dry extracts. The extracts were dissolved in dimethyl sulphoxide (DMSO) (BDH, Poole, England) at 20 mg/mL and stored at 4ºC.

Each sample was characterised and classified using a combination of monodimensional nuclear magnetic resonance (NMR), high-performance liquid chromatography with photodiode array detector (HPLC-PDA) and HPLC with electrospray ionisation mass spectrometer (HPLC-ESI/MS) techniques, as previously described (Cuesta-Rubio et al. 2007). Briefly, to perform the NMR

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Received 1 February 2012
Accepted 18 July 2012

analysis, the propolis extracts were dissolved in $CDCl_3$ and a Bruker DRX-600 spectrometer was operated at 599.19 MHz for ^1H and 150.858 for ^{13}C. The UXNMR (Bruker, Karlsrühe, Germany) software package was used. The HPLC-PDA analysis was conducted on an Agilent 1100 series system, consisting of a G-1312 binary pump, a G-1328A Rheodyne injector, a G-1322A degasser and a G-1315A PDA, equipped with a µ-Bondapack C-18 column (250 x 4.6 mm i.d., particle size 10). The elution solvents used were water and methanol. The flow rate was 1 mL/min and PDA data were recorded with a 200-600 nm range, with three preferential channels as the detection wavelengths (320, 280 and 254 nm). The Liquid chromatography-mass spectrometry analysis was performed using a Surveyor LC pump and a Surveyor Autosampler, coupled with an LCQ Advantage ion trap mass spectrometer (Thermo Finnigan, San Jose, CA), equipped with Xcalibur 3.1 software. HPLC separations were accomplished using a binary gradient composed of 10% water and 90% acetonitrile v/v. The data were acquired in the full scan and the MS/MS scanning modes and the maximum injection time was 50 ms. The composition of each extract is shown in Tables I-III; this information was used to classify the propolis samples into BCP, RCP and YCP types, according to their chemical constituents (Supplementary data).

Microorganisms and cells - The following microorganisms were used in this study: *E. coli* ATCC8739, *S. aureus* ATCC6538, *T. rubrum* B68183, *C. albicans* B59630, chloroquine-susceptible *P. falciparum* Ghana, suramin-sensitive *T. b. brucei* Squib-427, nifurtimox-sensitive *T. cruzi* Tulahuen CL2 and *L. infantum* MHOM/MA(BE)/67. Cytotoxicity was tested with human simian virus 40-immortalised lung fibroblasts (MRC-5 SV_2 cells, European Collection of Cell Cultures, United Kingdom).

Reference drugs - Erythromycin, chloramphenicol and tamoxifen were purchased from Sigma-Aldrich (Bornem, Belgium) and miconazole and flucytosine were kindly supplied by Janssen Pharmaceuticals (Beerse, Belgium). Benznidazol, chloroquine, miltefosine and suramine were generously provided by World Health Organization-Special Programme for Research and Training in Tropical Diseases (WHO-TDR).

Test plate production - The experiments were performed in 96-well plates (Greiner, Germany) at fourfold dilutions in a dose-titration range from 64-0.25 µg/mL. The dilutions were performed by a programmable precision robotic station (BIOMEK 2000, Beckman, USA). Each plate also contained medium controls (blanks: 0% growth), infected untreated controls (negative control: 100% growth) and reference controls (positive control). The tests were performed in duplicate.

Biological screening tests - The integrated panel of microbial screens for the present study and the standard screening methodologies were adopted, as described by Cos et al. (2006).

Antibacterial activity - *S. aureus* and *E. coli* were cultured at 37ºC in Mueller-Hinton broth medium. Assays were performed by adding 5 x 10^3 colony-forming unit (CFU)/well. After 17 h incubation, the bacterial viability was assessed fluorimetrically by adding resazurin (Räz et al. 1997) for 30 min at 37ºC. The fluorescence was measured using a GENios Tecan fluorimeter (excitation 530 nm, emission 590 nm).

Antifungal activity - *T. rubrum* and *C. albicans* were cultured in RPMI medium at 27ºC and 37ºC, respectively. Assays were performed by adding 5 x 10^3 CFU/well. After seven days (for *T. rubrum*) or 24 h (for *C. albicans*) of incubation, the fungal viability was assessed fluorimetrically by adding resazurin (Räz et al. 1997) for 24 h at 27ºC for *T. rubrum* and for 4 h at 37ºC for *C. albicans*.

Antiplasmodial activity - Parasites were cultured in human A^+ erythrocytes at 37ºC under a low-oxygen atmosphere (3% O_2, 4% CO_2 and 93% N_2) in a modular incubation chamber (Trager & Jensen 1976). The culture medium was RPMI-1640 supplemented with 0.5% (g/v) Albumax™. Two hundred microlitres of infected human red blood cell suspension (1% parasitaemia, 2% haematocrit) was added to each well of the plates with test compounds and the plates were incubated for 72 h. After the incubation, the test plates were frozen at -20ºC. Parasite multiplication was measured by the Malstat method (Makler et al. 1993). One hundred microlitres of the Malstat™ reagent was transferred into a new plate and mixed with 20 µL of the haemolysed parasite suspension for 15 min at RT. After the addition of 20 µL of a nitro-blue tetrazolium (2 mg/mL)/phenazine ethosulphate (0.1 mg/mL) solution and a 2 h incubation at RT in dark conditions, the optical density (OD) was read at 655 nm (Biorad 3550-UV microplate reader). The percentage of growth inhibition was calculated in comparison to the blanks.

Antitrypanosomal activity - Trypomastigotes of *T. brucei* were cultured at 37ºC and 5% CO_2 in Hirumi-9 medium (Hirumi & Hirumi 1989) supplemented with 10% inactivated foetal calf serum (FCSi). Assays were performed by adding 1.5 x 10^4 trypomastigotes/well. After 72 h of incubation, parasite growth was assessed fluorimetrically by adding resazurin (Räz et al. 1997) for 24 h at 37ºC.

T. cruzi amastigotes were maintained on MRC-5 cells in minimal essential medium (MEM) supplemented with 20 mM L-glutamine, 16.5 mM sodium hydrogen carbonate and FCSi (5%) at 37ºC and 5% CO_2. To determine the in vitro anti-trypanosomal activity, 4 x 10^3 MRC-5 cells and 4 x 10^4 parasites were added to each well of a test plate with the compound. After incubation at 37ºC for seven days, the parasite growth was assessed by adding the β-galactosidase substrate chlorophenol red β-D-

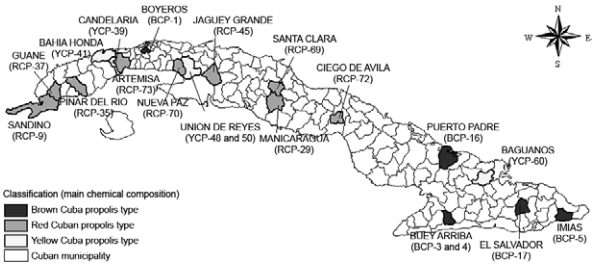

Fig. 1: geographic distribution of Cuban propolis samples studied.

galactopyranoside (Buckner et al. 1996) for 4 h at 37ºC. The colour reaction was read at 540 nm and the OD values were expressed as a percentage of the blank controls.

Antileishmanial activity - *Leishmania infantum* amastigotes were collected from an infected donor hamster and used to infect primary peritoneal mouse macrophages. To determine the in vitro antileishmanial activity, 3 x 10^4 macrophages were seeded in each well of a 96-well plate. After 48 h incubation at 37ºC and 5% CO_2, the cells were washed and infected with *L. infantum* amastigotes at a multiplicity of 15 parasites per macrophage. Two hours after infection, pre-diluted compounds were added and the plates were further incubated for 120 h at 37ºC and 5% CO_2. Then, the cells were fixed with methanol and stained with 10% Giemsa stain for microscopic reading. The total parasite burden, defined as the average number of amastigotes per cell, in the treated wells was compared with that of the control wells.

Cytotoxicity assay - MRC-5 cells were cultivated in MEM, supplemented with L-glutamine (20 mM), 16.5 mM sodium hydrogen carbonate and 5% FCSi at 37ºC and 5% CO_2. For the assay, 10^4 MRC-5 cells/well were seeded onto the test plates containing the pre-diluted compounds and incubated at 37ºC and 5% CO_2 for 72 h. Cell viability was assessed fluorimetrically 72 h after the addition of resazurin.

Statistical analysis - In each experiment, the 50% of microbial growth (IC_{50}) and human cell growth (CC_{50}) inhibition values were determined using Probit analysis (StatView®). The results were expressed as the means ± standard deviation of two independent experiments.

Numerical cluster analyses - Five samples of the YCP type were treated as operational taxonomic units. Based on previous data obtained using the NMR, HPLC-PDA and HPLC-ESI/MS techniques (Table III), the chemical

TABLE I

Proportion of compounds in brown Cuban propolis (BCP) samples

	Proportion of compounds identified in brown Cuban propolis (µg/100 µg of propolis extracts)[a]					
Compounds	BCP-1	BCP-3	BCP-4	BCP-5	BCP-16	BCP-17
Nemorosone	13.1	12.2	28.5	22.3	9	35.2
Propolone A	+	+	+	+	+	+
Propolone B	+	+	+	+	+	+
Propolone C	+	+	+	+	+	+
Propolone D	+	-	-	-	-	-
Garcinielliptone I	-	-	+	+	-	+
Hyperibone B	+	+	-	-	-	+

a: mean value of three replications, standard deviations below 10%; +: detected as minor compound; -: not detected.

TABLE II

Proportion of compounds in red Cuban propolis (RCP) samples

	Proportion of compounds identified in red Cuban propolis (µg/100 µg of propolis extracts)[a]								
Compounds	RCP-9	RCP-29	RCP-35	RCP-37	RCP-45	RCP-69	RCP-70	RCP-72	RCP-73
Isoliquiritigenin	7.1	7.2	6.3	8.1	6.6	1.7	2.1	1.4	1.4
Liquiritigenin	3.9	3.7	2.3	4.1	5.8	1.9	1.8	1.4	2.1
Biochanin A	-	-	-	-	-	-	-	-	-
Formononetin	12.8	18.4	1.6	11.3	+	3.2	3.5	2.1	2.9
Vestitol	29.7	25.0	34.7	27.2	49.5	15.3	19.9	19.1	24.1
Neovestitol	12.6	10.6	15.3	6.9	12.9	6.2	8.8	11.7	7.9
Isosativan[b]	25.3	21.3	10.3	31.5	20.7	3.0	2.9	2.8	2.5
Medicarpin[b]			18.4			14.8	13.8	16.3	14.9
Homopterocarpin	+	+	+	+	+	+	0.2	+	+
Vesticarpan[c]	1.9	3.2	0.5	3.4	+	1.1	+	+	+
3,8-dihydroxy-9-methoxy pterocarpan[c]									
3-hydroxy-8,9-dimethoxy pterocarpan	1.3	2.5	1.4	1.7	4.4	2.5	1.6	2.1	1.4
3,4-dihydroxy-9-methoxy pterocarpan	2.6	3.4	3.8	4.7	+	2.1	2.5	3.9	2.2

a: mean value of three replications, standard deviations below 10%; *b, c*: value given reflects the sum of both compounds; +: detected as trace; -: not detected.

constituents of each sample were scored as present (1) or absent (0). Similarly, the activities of each sample against the eight microorganisms were classified as present (1) for a score ≥ 4 (IC_{50} < 2 μg/mL, score 4; IC_{50} < 1 μg/mL, score 5; IC_{50} < 0.5 μg/mL, score 6) or absent (0) for a score ≤ 3 in each sample (IC_{50} > 2 μg/mL). A binary table was used to find associations between the chemical composition and antimicrobial activities, using Jaccard's coefficient (Sneath 1957). The unweighted pair-group method with arithmetic averages was used to construct a dendrogram. Bootstrap analysis was performed on 2,000 replicates and the FreeTree software version 0.9.1.50 was used to conduct all numerical analysis (Pavlicek et al. 1999). The statistical testing of the trees concordance was performed using the program Treept (Flegr & Záboj 1997, Flegr et al. 1998).

RESULTS

The different propolis extracts showed activity against *S. aureus* and *T. rubrum* at low μg/mL concentrations, whereas at the highest test concentration of 64 μg/mL, no activity was observed against *E. coli* and *C. albicans* (Table IV). Most propolis samples showed antiparasitic activity (Table V). Nevertheless, cytotoxicity against MCR-5 cell lines was found with CC_{50} values ranging from 2-29 μg/mL, indicating a low selectivity of activity. Out of all of the propolis samples, BCP-3, YCP-39 and YCP-60 showed the highest activity against *P. falciparum* (IC_{50} ≤ 0.2 μg/mL), with selectivity indices (CC_{50}/IC_{50}) of 48, 73 and 10, respectively.

In a cluster analysis study (Fig. 2), the most interesting results were obtained for YCP. The YCP-39 and YCP-60 samples were grouped in a cluster showing high activity against *Plasmodium*, trypanosomatids and *T. rubrum*. The YCP-48 and YCP-50 samples were less active and non-specific and therefore were grouped in another cluster. The YCP-41 samples were displayed as a single group, with activity against the trypanosomatids. A significant statistical association (p = 0.029) was demonstrated between the trees obtained for chemical composition and antimicrobial activity of YCP extracts.

DISCUSSION

Infectious diseases are the second leading cause of death, despite the introduction of many antimicrobial agents in the 20th century (Taylor & Wright 2008). Most of the current antimicrobials were discovered from natural sources (Newmann & Cragg 2007).

In this study, we have evaluated the antimicrobial activity of Cuban propolis extracts. Our interest in this natural product was raised by previous activities reported for propolis from Turkey (Ugur & Arslan 2004), Mexico (Quintero-Mora et al. 2008) and Brazil (Ayres et al. 2007). These studies investigated propolis activity against bacteria (*Shigella sonnei*, *Salmonella typhi* and *Pseudomonas aeruginosa*), *C. albicans* and *Leishmania amazonensis*. The chemical characterisation of Cuban propolis has revealed the presence of potential antimicrobial compounds, such as benzophenones, flavonoids and terpenoids (Cuesta-Rubio et al. 2007).

The propolis samples used in this study inhibited *S. aureus*, whereas no activity was observed against *E. coli*. These results suggest that Cuban propolis may possess a specific activity against Gram-positive bacteria, which was also observed for propolis from other geographical origins (Lofty 2006). In the literature, activity against *C. albicans* has also been reported for propolis samples from Brazil (Dota et al. 2011), Thailand (Umthong et al. 2009), Mexico (Quintero-Mora et al. 2008) and Iran (Ghasem et al. 2007). However, in our study, propolis samples did not show any activity against *C. albicans*, although there was an observed inhibitory effect against *T. rubrum*.

The major activity of the extracts was found against protozoa, although a high level of cytotoxicity was also observed. Several studies have been published on the antiprotozoal activity of propolis, including activity against

TABLE III

Proportion of compounds in yellow Cuban propolis (YCP) samples

Compounds	Proportion of compounds identified in yellow Cuban propolis (μg/100 μg of propolis extracts)[a]				
	YCP-39	YCP-41	YCP-48	YCP-50	YCP-60
24-methylene-9,19-ciclolanostan-3β-ol	0.2	1	0.8	0.8	0.4
α-amyrin	1	1.4	-	2.2	0.2
α-amyrone	0.2	0.2	0.2	0.4	0.2
β-amyrin	1.2	1.4	1	2.2	0.8
β-amyrin acetate	0.2	1	0.8	0.8	0.4
β-amyrone	-	-	-	0.2	-
Cycloartenol	1.6	6.6	0.2	1.8	0.8
Germanicol	-	-	0.6	0.4	-
Germanicol acetate	0.2	-	0.6	0.4	-
Lanosterol	-	-	-	-	0.6
Lanosterol acetate	-	-	0.2	0.2	-
Lupeol	1.6	1.2	3.4	2.4	0.6
Lupeol acetate	2.8	-	0.8	2.2	2.4

a: mean value of three replications, standard deviations below 10%; -: not detected.

L. amazonensis (Ayres et al. 2007), *T. cruzi* (Dantas et al. 2006) and *Acanthamoeba castellanii* (Topalkara et al. 2007). Cytotoxicity has also been reported on different normal and cancer cell lines (Li et al. 2010, Umthong et al. 2011).

A significant number of papers addressing propolis chemistry have been published to date (Cuesta-Rubio et al. 2007, Duran et al. 2008, 2011). On this basis, researchers have begun to understand that the chemical composition of propolis is highly variable and, as a result, they have decided to compare the biological activity of propolis from different geographic regions of the world. However, few studies correlate the chemical composition with the biological activity of propolis samples collected from the same country (Bonvehi et al. 1994, Salomão et al. 2008). Our study is the first assessment to compare different types of Cuban propolis extracts for their antibacterial, antifungal and antiprotozoal activities in conjunction with their chemical composition. In general, most of the samples possess antimicrobial properties, but with high variability in bioactivity within samples of the same propolis type. This finding suggests that the propolis samples contain several different bioactive compounds. Studies on the activity of nemorosone (BCP type) against *P. falciparum* (Monzote et al. 2011), formononetin (RCP type) against *L. major* (Takahashi et al. 2006), liquiritigenin (RCP type) against *P. falciparum* (Khaomek et al. 2008), biochanin A (RCP type) against *L. chagasi* and *T. cruzi* (Sartorelli et al. 2009) and lupeol (YCP type) against *P. falciparum* (Ziegler et al. 2002) have already been reported.

It is well known that propolis samples present a wide variety of chemical compositions, including both the type and the proportion of compounds present in each sample. The association of the chemical compositions of propolis samples from different geographic regions with the biological activities of those samples has led to the identification of active principles, which is a fundamental tool to achieve standardisation of this bee product (Salomão et al. 2008). A positive correlation between the biological activity and the chemical composition was observed for the YCP samples. Recently, the YCP type has been subdivided into two subtypes, YCP-A and YCP-B (Marquéz et al. 2010). Notably, the most promising antimicrobial activity corresponds to YCP-B (including the YCP-39 and YCP-60 samples). The YCP-B subtype contains acetyl triterpenes as the main constituents, which could be responsible for the antimicrobial activity (Marquéz et al. 2010). Previous studies on the correlation between chemical composition and antimicrobial activity for *S. aureus* and *T. cruzi* have been reported, in which higher levels of 4-hydroxy cinnamic acid and derivatives were associated with a stronger biological activity of propolis samples from different regions of Brazil (Salomão et al. 2008). In our study, the association between antimicrobial activity and chemical composition was

TABLE IV

Activity of Cuban propolis extracts against bacteria and fungi

	IC_{50} ± SD (µg/mL)			
Products	*Staphylococcus aureus*	*Escherichia coli*	*Trichophyton rubrum*	*Candida albicans*
BCP-1	9.3 ± 1.8	> 64.0	7.05 ± 2.0	> 64.0
BCP-3	7.1 ± 2.1	> 64.0	6.7 ± 2.1	> 64.0
BCP-4	8.0 ± 3.3	> 64.0	16.7 ± 2.5	> 64.0
BCP-5	6.0 ± 2.2	> 64.0	11.9 ± 4.8	61.6 ± 3.4
BCP-16	13.7 ± 0.8	> 64.0	10.9 ± 3.3	> 64.0
BCP-17	4.7 ± 0.5	> 64.0	21.1 ± 20.9	> 64.0
RCP-9	7.7 ± 3.1	> 64.0	14.9 ± 6.5	> 64.0
RCP-29	15.2 ± 4.8	> 64.0	33.8 ± 11.1	> 64.0
RCP-35	6.6 ± 2.0	> 64.0	33.3 ± 9.9	> 64.0
RCP-37	6.2 ± 1.5	> 64.0	33.0 ± 11.3	> 64.0
RCP-45	4.4 ± 1.3	> 64.0	32.9 ± 7.4	> 64.0
RCP-69	21.0 ± 6.5	> 64.0	34.5 ± 11.1	> 64.0
RCP-70	25.9 ± 5.7	> 64.0	33.1 ± 12.5	> 64.0
RCP-72	6.0 ± 0.03	> 64.0	32.4 ± 9.6	> 64.0
RCP-73	10.0 ± 0.03	> 64.0	39.4 ± 0.03	> 64.0
YCP-39	13.6 ± 4.1	> 64.0	3.6 ± 7.1	> 64.0
YCP-41	28.7 ± 6.5	> 64.0	38.1 ± 10.2	> 64.0
YCP-48	49.0 ± 13.4	> 64.0	> 64.0	> 64.0
YCP-50	58.2 ± 8.1	> 64.0	29.5 ± 7.4	> 64.0
YCP-60	13.2 ± 5.3	> 64.0	3.8 ± 1.0	> 64.0
Erythromycine	6.6 ± 4.3	-	-	-
Chloramphenicol	-	6.9 ± 3.4	-	-
Miconazole	-	-	0.28 ± 0.18	-
Flucytosine	-	-	-	0.65 ± 0.14

Cuban propolis are known as brown (BCP), red (RCP) and yellow (YCP). IC_{50}: concentration of product that caused 50% of inhibition growth; SD: standard deviation.

TABLE V

Toxicity and activity against protozoa of Cuban propolis extracts

Products	CC_{50} ± SD (µg/mL)	IC_{50} ± SD (µg/mL)			
		Plasmodium falciparum	*Trypanosoma cruzi*	*Trypanosoma brucei*	*Leishmania infantum*
BCP-1	16.7 ± 3.9	11.6 ± 5.4	6.9 ± 0.8	10.2 ± 3.2	8.4 ± 0.4
BCP-3	9.6 ± 5.2	0.2 ± 0.1	5.8 ± 0.7	8.8 ± 2.0	8.8 ± 1.0
BCP-4	24.4 ± 1.6	10.8 ± 5.2	6.7 ± 0.3	16.3 ± 4.5	10.7 ± 2.9
BCP-5	16.3 ± 2.7	12.5 ± 6.9	4.5 ± 0.6	11.2 ± 5.0	8.4 ± 0.4
BCP-16	24.4 ± 5.4	9.7 ± 3.6	8.0 ± 0.1	8.2 ± 0.5	22.2 ± 2.6
BCP-17	10.7 ± 4.2	6.0 ± 3.9	4.1 ± 1.6	6.8 ± 2.7	7.5 ± 0.9
RCP-9	7.4 ± 0.6	1.8 ± 1.1	2.6 ± 1.0	2.0 ± 0.03	3.3 ± 1.0
RCP-29	20.9 ± 7.3	5.1 ± 1.2	9.0 ± 1.5	8.2 ± 0.1	7.5 ± 0.9
RCP-35	9.4 ± 0.5	1.5 ± 0.1	7.8 ± 0.04	1.8 ± 0.3	6.4 ± 0.6
RCP-37	7.0 ± 1.7	1.2 ± 1.2	4.7 ± 1.0	1.2 ± 1.0	5.2 ± 1.3
RCP-45	8.5 ± 0.2	2.3 ± 1.3	2.5 ± 0.8	2.1 ± 0.2	4.0 ± 2.8
RCP-69	29.0 ± 4.6	3.6 ± 2.8	7.3 ± 0.03	8.1 ± 0.2	16.1 ± 4.7
RCP-70	16.7 ± 6.2	5.6 ± 1.2	7.2 ± 0.7	8.1 ± 0.1	7.5 ± 0.9
RCP-72	8.5 ± 0.2	2.5 ± 1.3	3.1 ± 1.4	1.8 ± 0.4	6.0 ± 0
RCP-73	23.7 ± 1.9	6.4 ± 3.4	8.1 ± 0.8	8.3 ± 0.1	14.9 ± 4.6
YCP-39	14.7 ± 3.7	0.2 ± 0.1	4.1 ± 1.6	6.7 ± 1.7	6.0 ± 0
YCP-41	5.7 ± 3.4	6.4 ± 0.6	1.6 ± 0.2	1.5 ± 0.3	2.0 ± 0
YCP-48	19.2 ± 4.5	1.7 ± 0.6	7.0 ± 2.0	2.0 ± 0	6.8 ± 1.1
YCP-50	6.8 ± 1.1	1.1 ± 1.5	2.0 ± 0.1	2.8 ± 1.3	5.5 ± 0.7
YCP-60	2.1 ± 0.3	0.2 ± 0	2.1 ± 0.02	1.0 ± 0.3	3.2 ± 0
Tamoxifen	11.31± 0.9	-	-	-	-
Chloroquine	-	0.3 ± 0.1	-	-	-
Benznidazol	-	-	2.2 ± 0.5	-	-
Suramine	-	-	-	0.05 ± 0.05	-
Miltefosine	-	-	-	-	7.7 ± 4.5

Cuban propolis are known as brown (BCP), red (RCP) and yellow (YCP). CC_{50}: concentration of product that caused 50% inhibition of human cell growth; IC_{50}: concentration of product that caused 50% inhibition of microbial growth; SD: standard deviation.

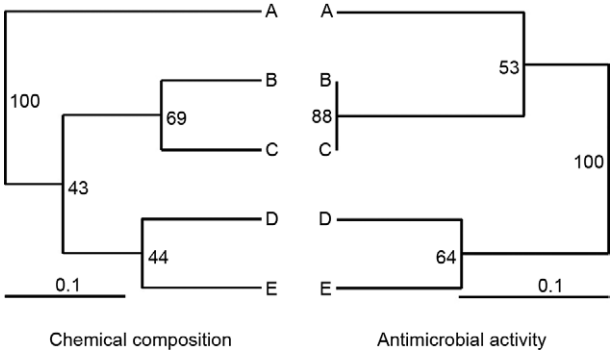

Fig. 2: cluster analysis of Cuban propolis samples type III according to their chemical composition and antimicrobial activity.

strong, demonstrating the relevance of intra-regional variation in propolis samples.

The present study corroborates the antimicrobial activities of propolis, particularly against protozoal parasites. The association of the chemical composition of propolis with its biological activities can lead to the identification of bioactive principles, which is a fundamental step in achieving standardisation of this bee product.

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