

Comparative antiproliferation of human prostate cancer cells by ethanolic extracts of two groups of Brazilian propolis

Yong K. Park, Cleber S. Moraes, Andreas Daugsch

State University of Campinas (UNICAMP), College of Food Engineering,
Department of Food Science, Campinas, SP., Brazil

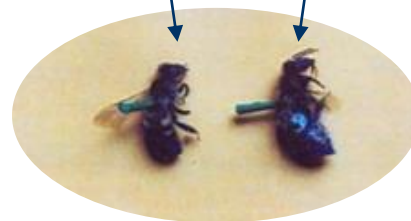
Two varieties of bees



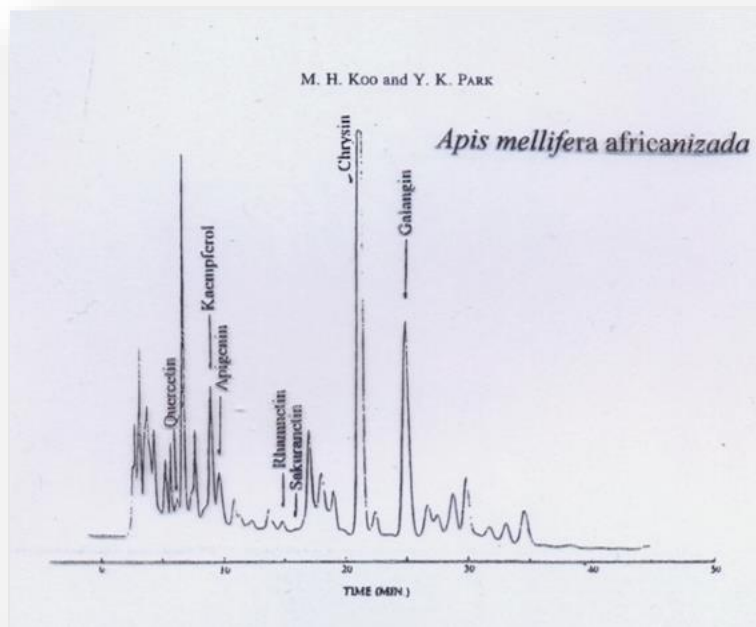
Africanized Apis mellifera



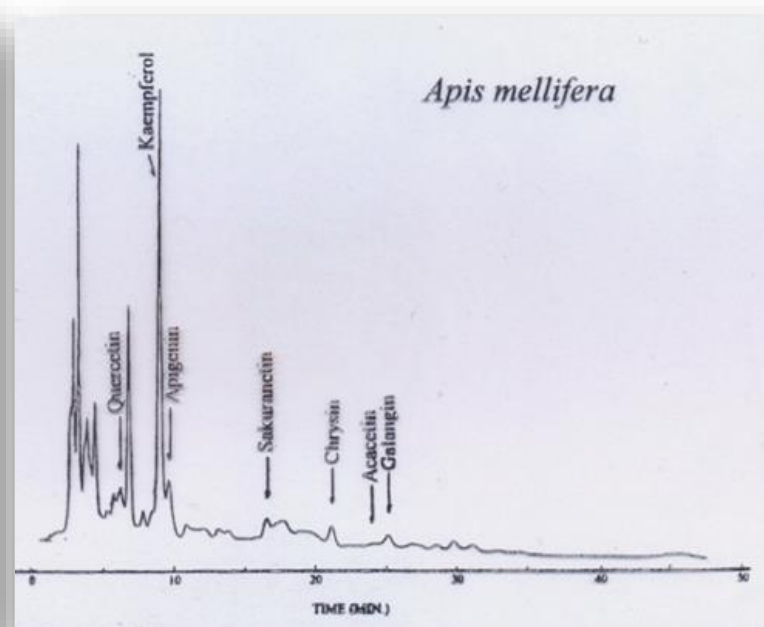
Apis mellifera



Reversed-phase HPLC Chromatograms of Ethanolic Extracts of Propolis (EEP) from two varieties of bees

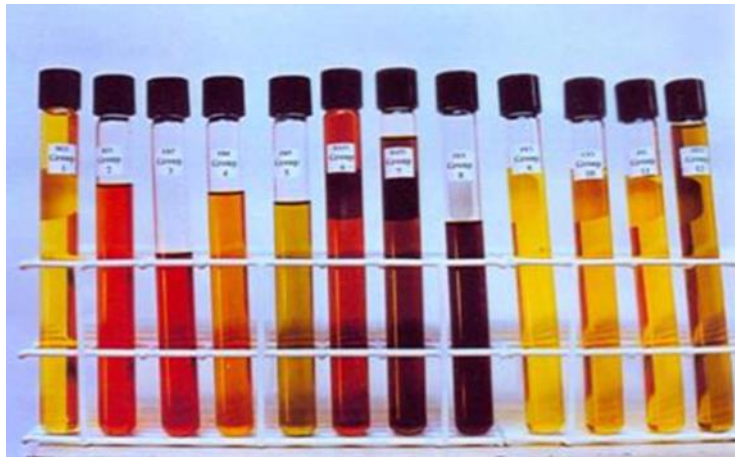


Apis mellifera africanizada

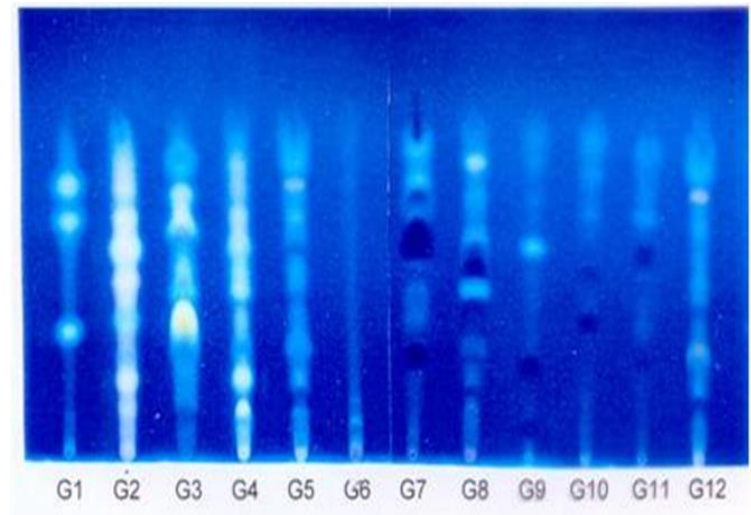


Apis mellifera

Appearance of Ethanolic Extracts of Brazilian Propolis



Appearance of Ethanolic Extracts of
12 Groups of Brazilian Propolis



RP-HPTLC of Ethanolic Extracts of
12 Groups of Brazilian Propolis

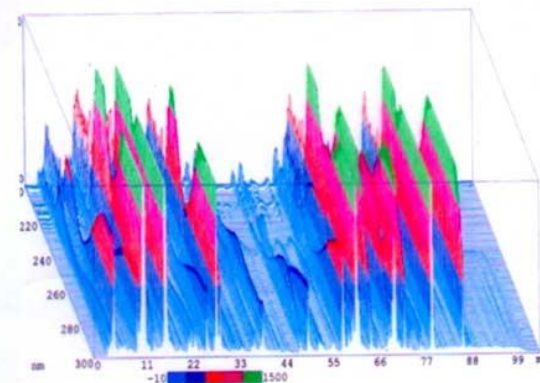
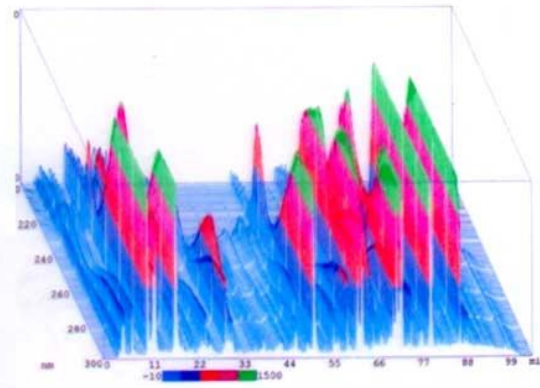
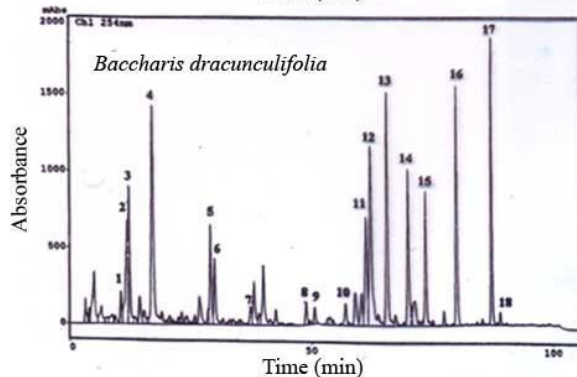
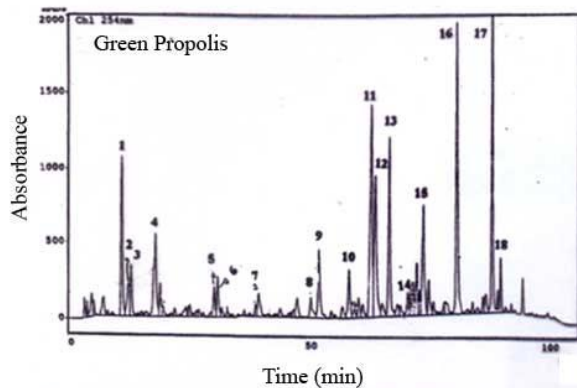
Baccharis dracunculifolia - Collection of propolis



Flower of *Baccharis dracunculifolia* - Collection of honey



RP-HPLC Chromatograms of methanolic extracts of Brazilian Green Propolis and bud exudates

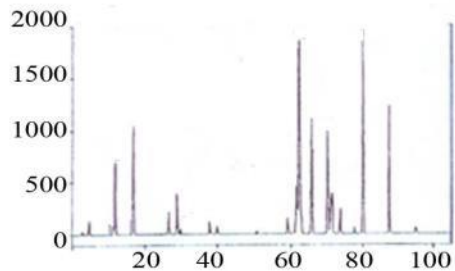


Number represent chemical compounds:

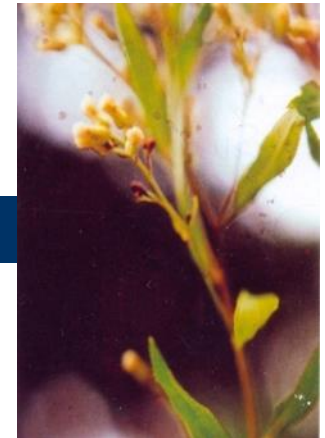
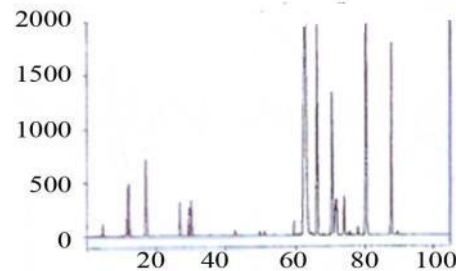
1 = Coumaric acid; 2 = Ferrulic acid; 7 = Kaempferol; 8 = Isosakuranetin; 11 = Kaempferide



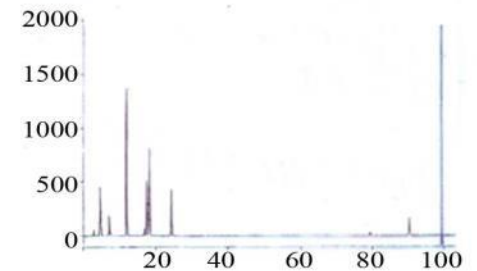
***Baccharis dracunculifolia*
(male)**



***Baccharis dracunculifolia*
(female)**



***Baccharis trinervis*
(female)**

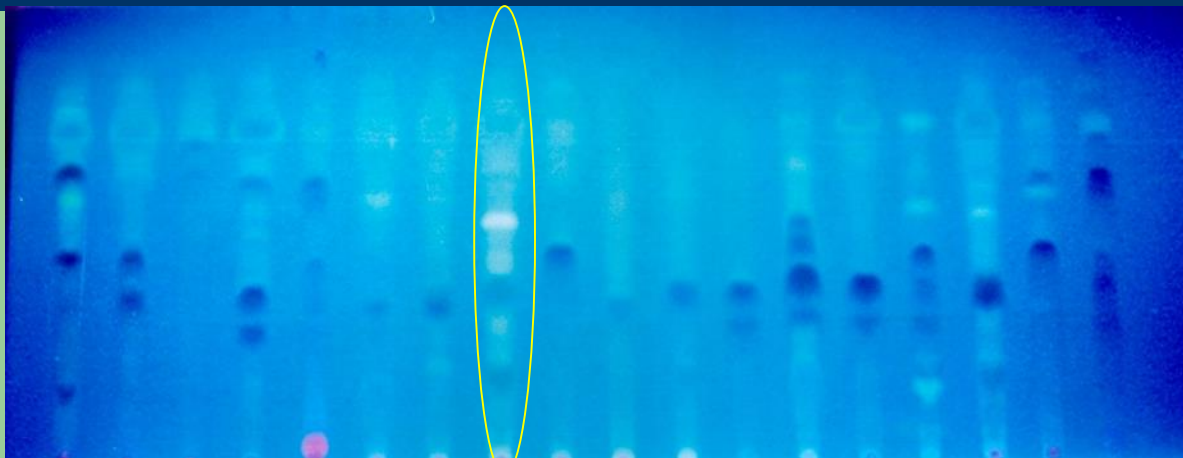


Baccharis dracunculifolia

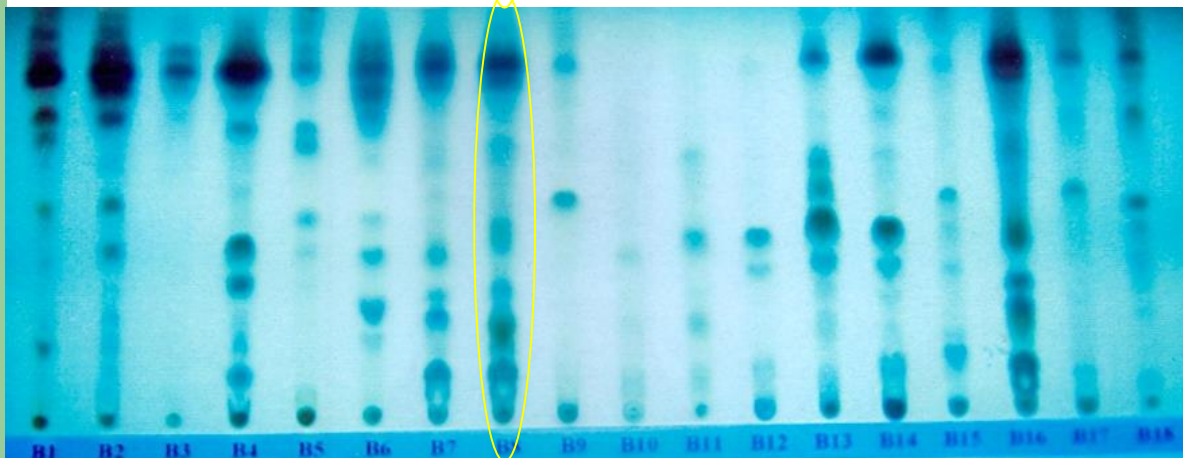


RPHPTLC of the ethanolic extracts of 18 *Baccharis* sp.

B8 is *Baccharis dracunculifolia*

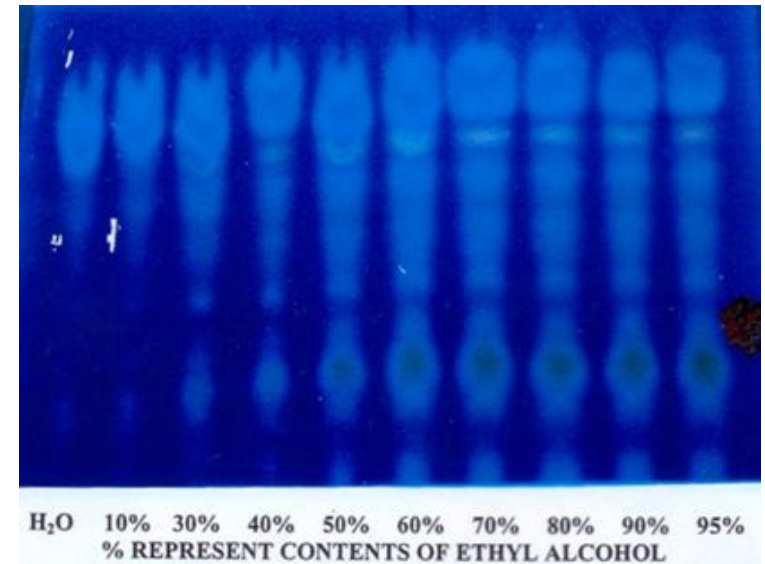
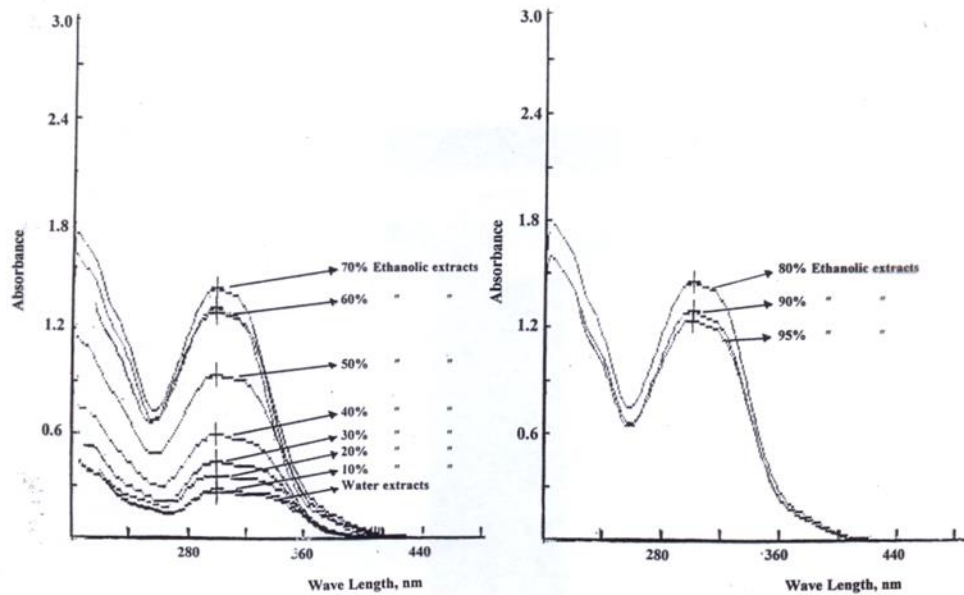


UV visualization 366 nm

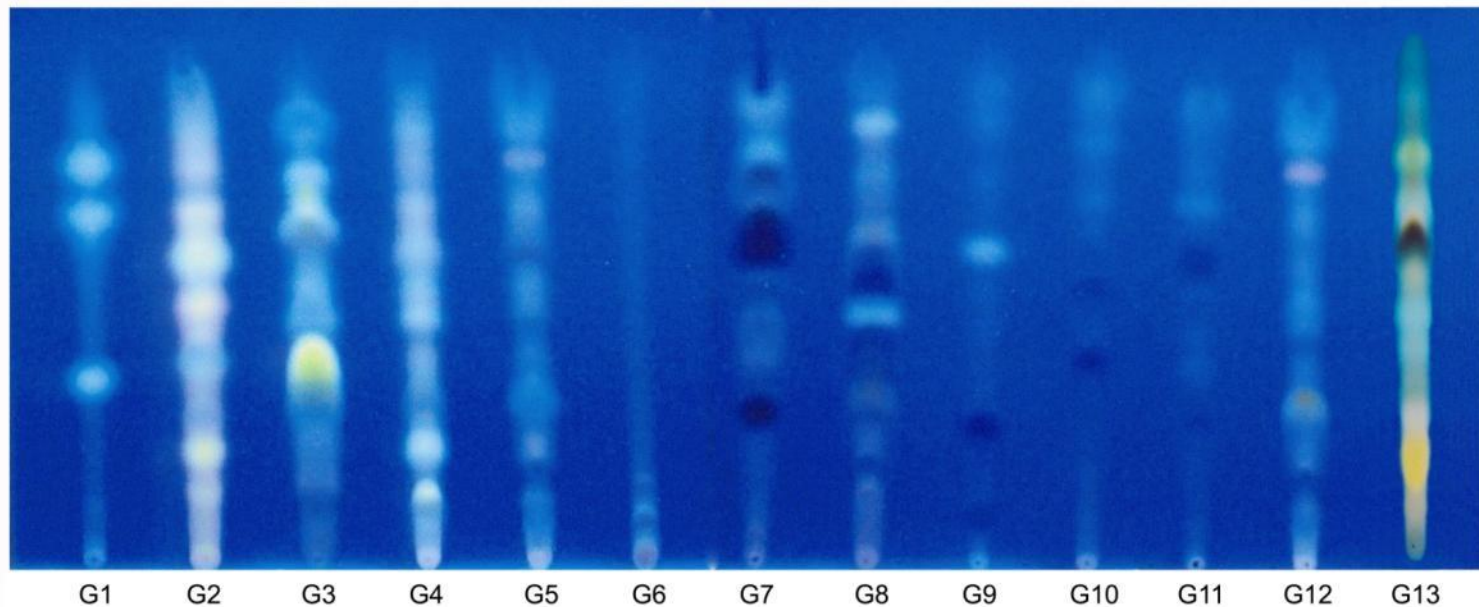


UV visualization 255 nm

Absorption spectra of water and different concentrations of ethanolic extracts of propolis



RPHPTLC of ethanolic extracts of Brazilian propolis



The Northeastern red propolis was classified as G13

Collection of propolis from red resinous exudates of *D. ecastophyllum* by Africanized *Apis mellifera*

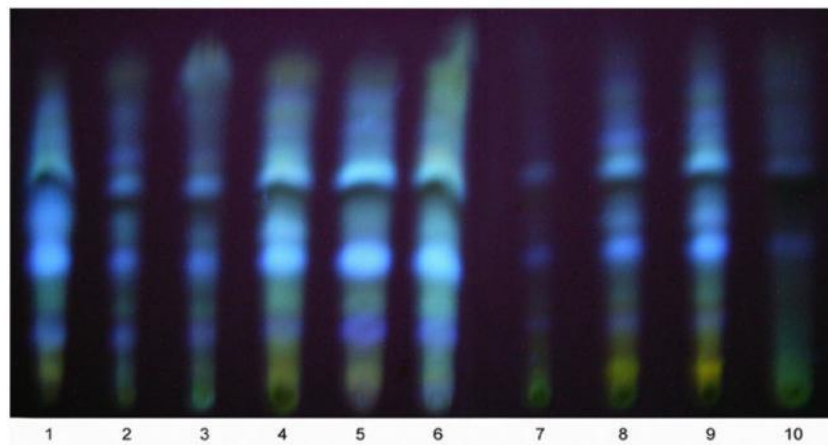
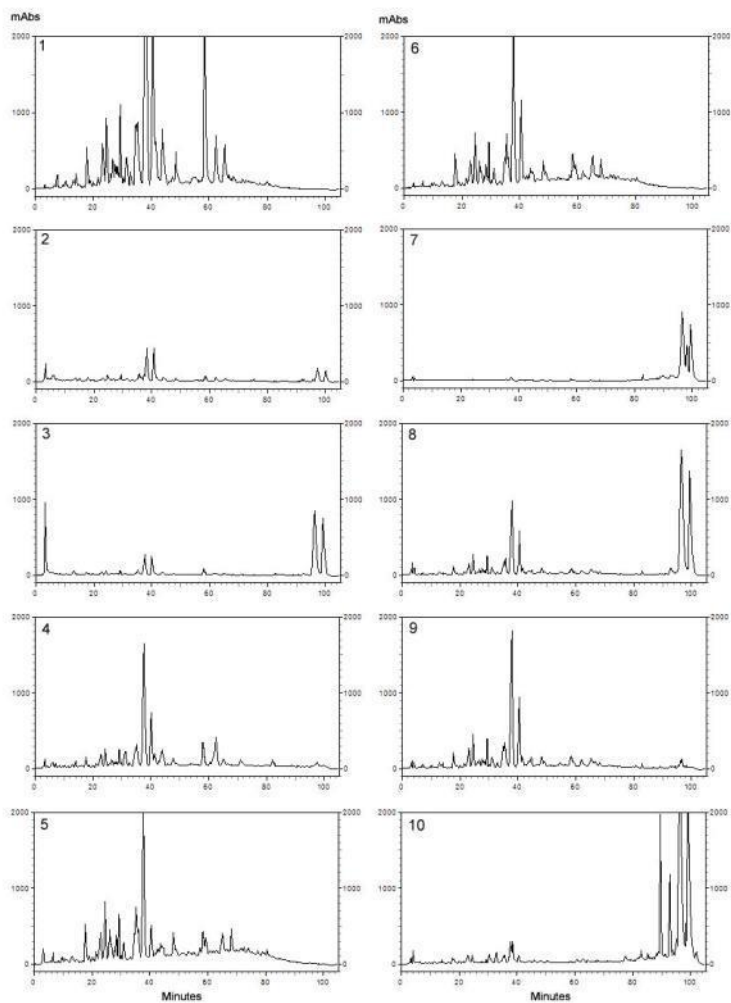


A - Secrete reddish exudates from a hole of branch of the tree

B - Bee is collecting the reddish exudates

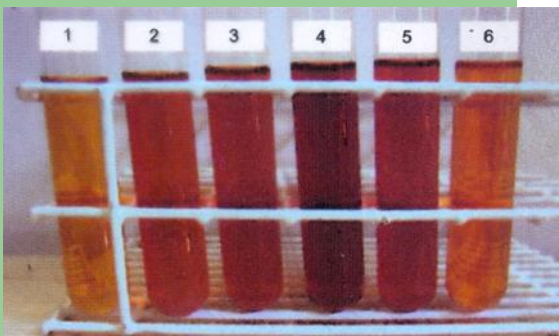
C - The collected exudates passed to the hind leg to make propolis

RPHPLC of ethanolic extracts of red propolis G13



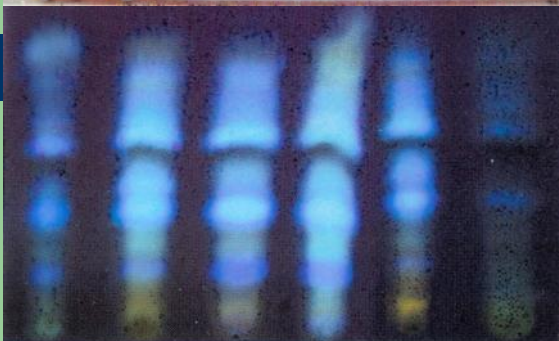
RPHPTLC of ethanolic extracts of red propolis G13

A

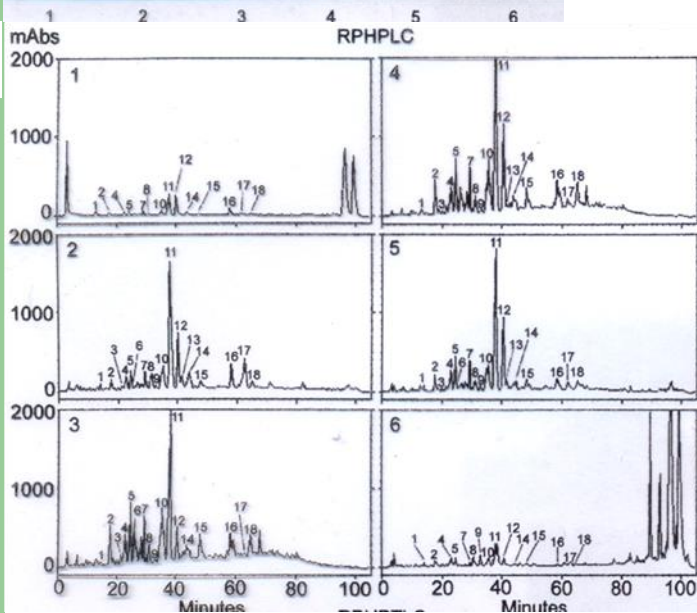


- Ethanolic extracts of 6 samples of red propolis in Maceió
- RPHPTLC of same samples
- RPHPLC of same samples
- Antimicrobial activity of same samples

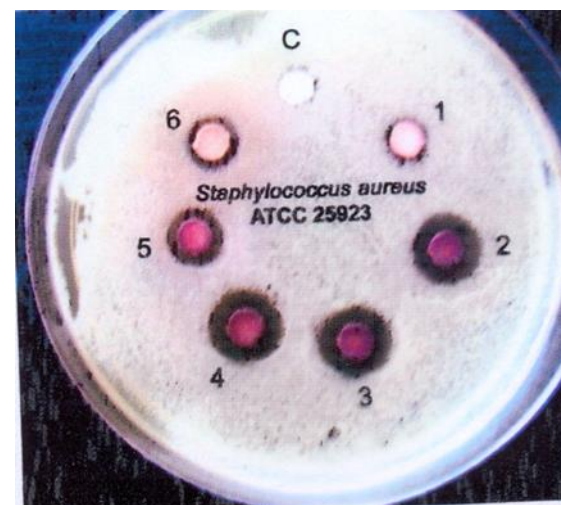
B

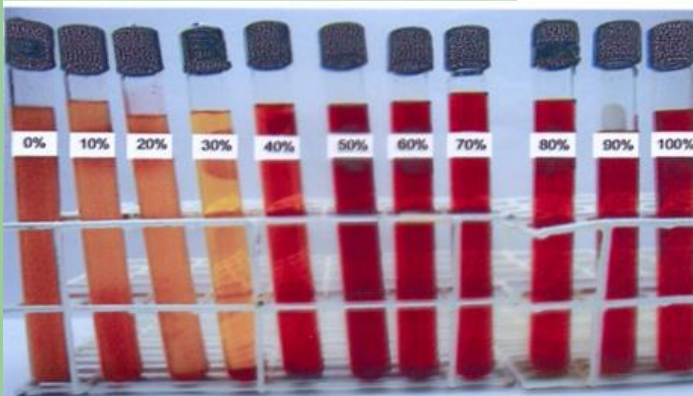


C

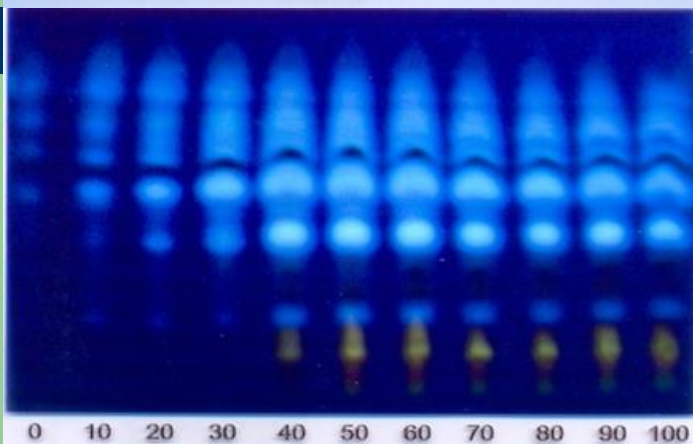


D

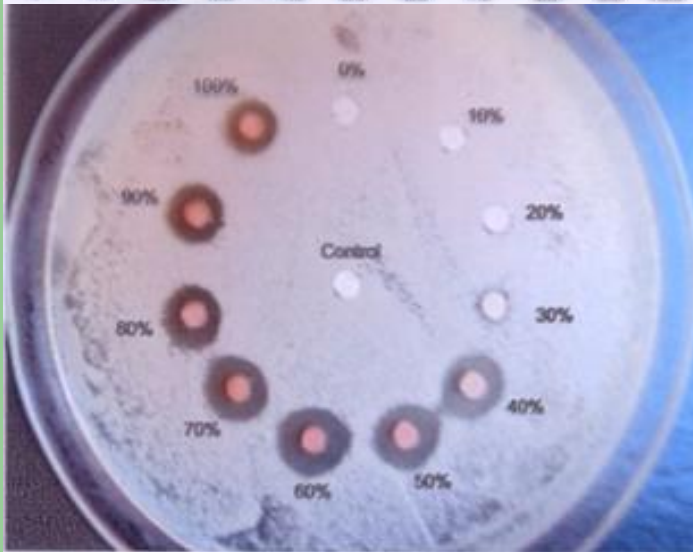




Extraction of red propolis in João Pessoa by different concentrations of ethanol



RPHPTLC of same samples



Antimicrobial activity (*Staphylococcus aureus*) of same samples

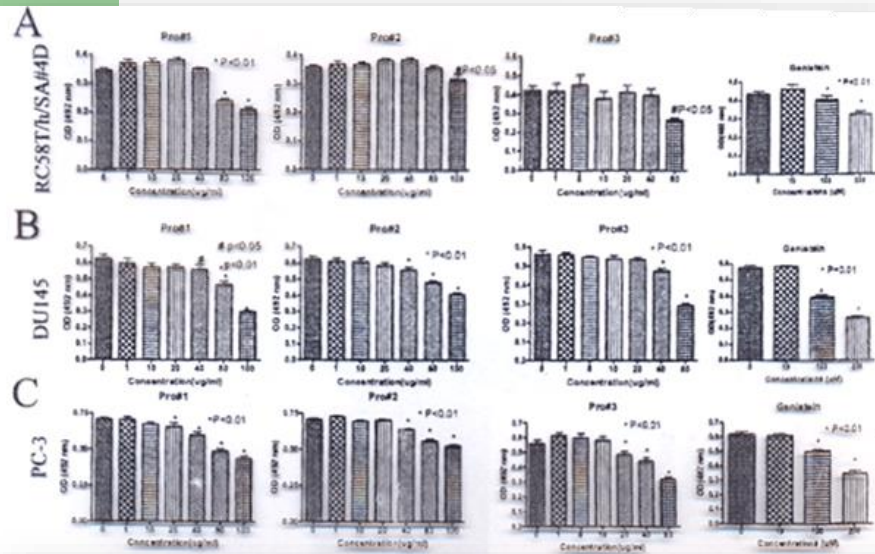
Recently, numerous biological properties of the propolis have been reported, including cytotoxic, antiherpes, antitumor, free radical scavenging, antimicrobial, anti-HIV activities, and suppressive effects of dioxin toxicity.

Brazilian propolis classified 13 groups based on physicochemical characteristics, and some of the ethanolic extracts of propolis inhibited the growth of solid tumor cell lines. Thus, **Propolis groups 1,2,6, and 7 are highly antagonistic to the growth of Nasopharyngeal carcinoma (KB)**

**Propolis groups 6 and 7 are also highly inhibitive against the growth of Ileocecal adenocarcinoma (HCT-8),
Renal carcinoma (CAKE-1),
Breast carcinoma (MCT-7)**

Previously, we reported that main botanical origin of propolis group 12, which is now increasingly used as a health food supplement and pharmaceutical purposes, were resins of *Baccharis dracunculifolia*. **Therefore, in the present study we have evaluated the effects of ethanolic extracts of the propolis group 12 and bud resins of botanical origin of propolis group 12, including propolis group 3 which was widely produced in Southern Brazil. Genistein was also used as positive control.**

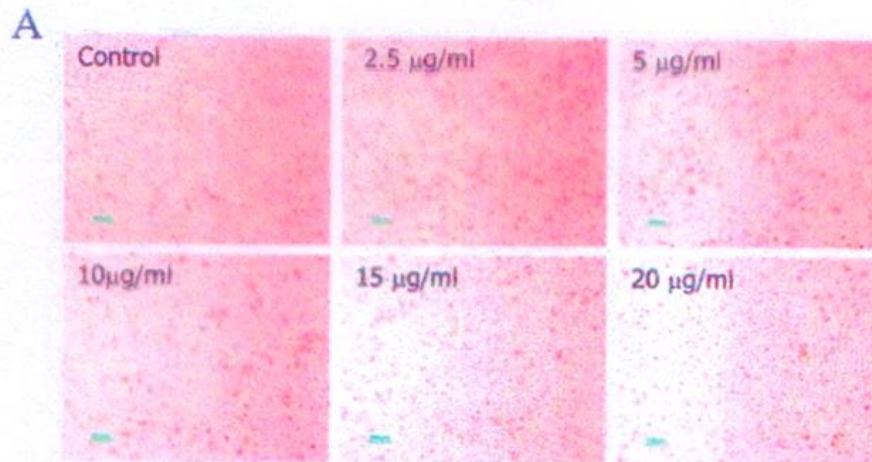
Inhibition of metastasis derived DU145 & PC-3 and primary tumor derived (RC-58T/h/SA#4) human prostate cancer cells by propolis



Propolis #1 - Botanical origin of propolis G12
 Propolis #2 - Propolis group 12
 Propolis #3 - Propolis group 3

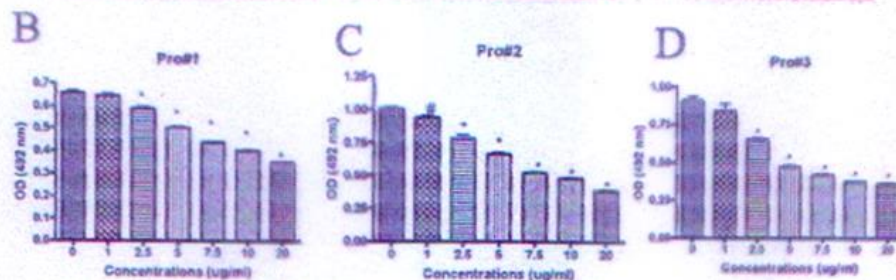
Cells		Propolis #1		Propolis #2		Propolis #3	
		80µg/ml	100µg/ml	80µg/ml	100µg/ml	40µg/ml	80µg/ml
DU145	2% serum:	80.2	93.0	76.9	79.9	65.1	88.29
	10% serum	31.0	64.4	28.3	43.1	20.8	63.9
PC3	2% serum	93.6	98.6	92.2	95.5	62.1	92.1
	10% serum	39.0	48.0	26.6	32.8	24.5	57.2
RC58T /h/SA#4	2%	88.2	95.3	83.0	90.8	42.0	84.7
	10% serum	51.4	65.8	0.02	19.18	9.6	56.2

Propolis inhibited cell proliferation in primary malignant tumor-derived human prostate cancer cell line (RC-58/h/AS#4)



A - Growth and morphology of RC-58/h/AS#4 cells exposed of various Doses of Propolis #1

B - The cells (RC-58/h/AS#4) were cultured In the KGM and on the following day, they were treated with propolis #1. After 48 hr treatment, the cells were washed with KGM medium and viable cells were counted



C - Same cells as described in **B**, were treated with propolis #2 (Propolis group 12)

D - Same cells as described in **B**, were treated with propolis #3 (Propolis group 3)

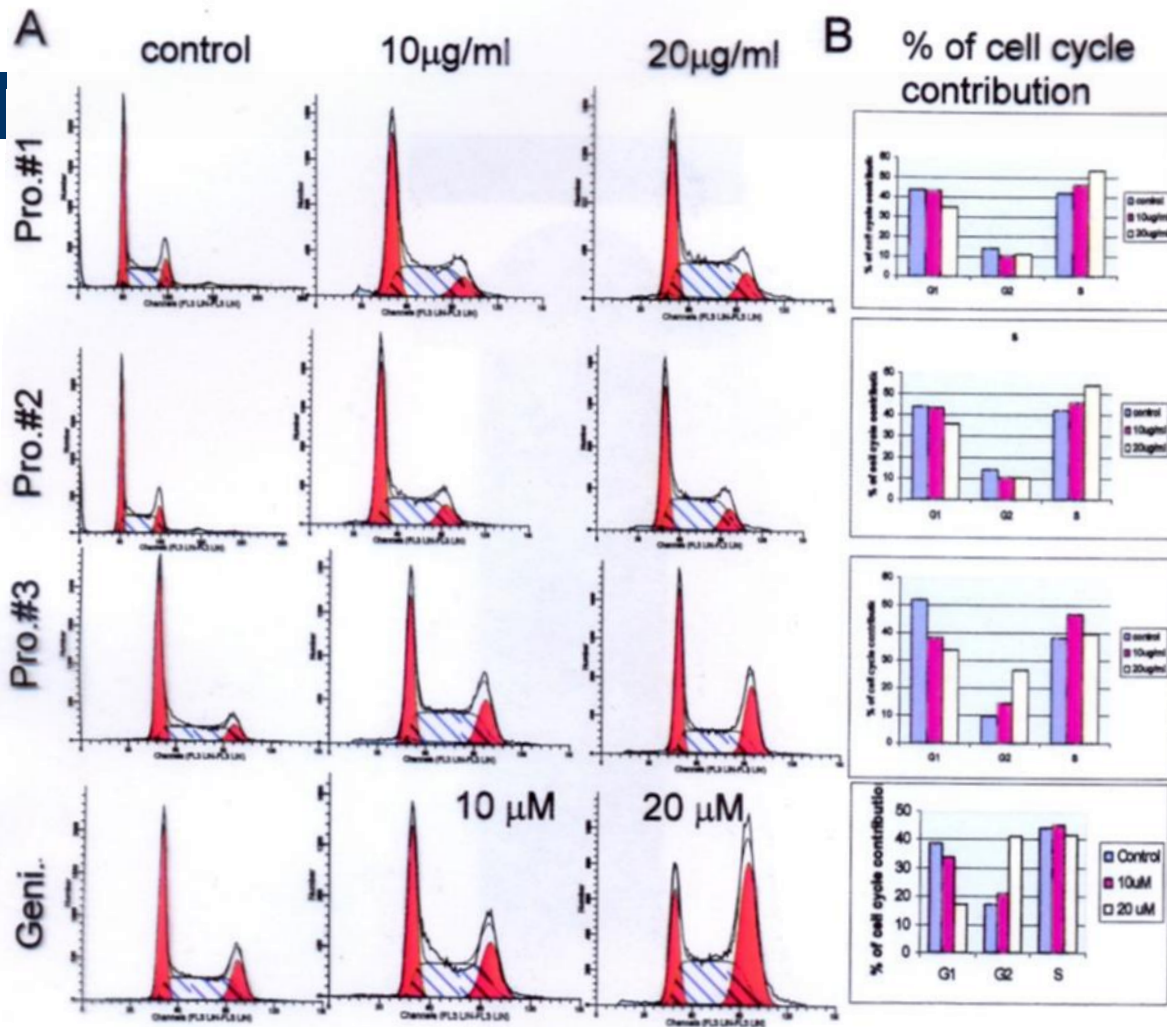
The 50% growth inhibition of normal human prostate epithelial (PrEC) and primary tumor-derived human prostate cancer (RC-58T/h/SA#4) cells and by propolis #1 (botanical origin of propolis group 12), #2 (Green Propolis) and #3 (Poplar propolis)

	PrEC, (ug/ml)	RC58T/h/SA#4, (ug/ml)
Propolis #1	7.5	5.5
Propolis #2	8.75	5
Propolis #3	5.5	3

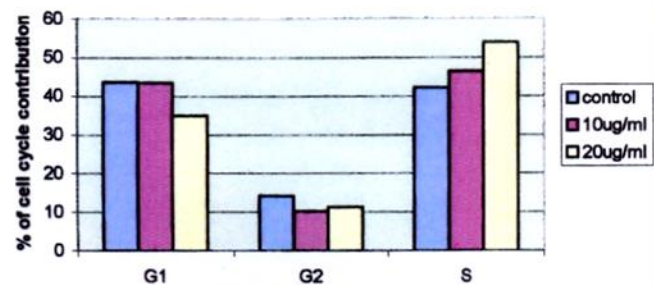
PrEC = Primary human prostate epithelial cells

RC-58T/h/SA#4 = Primary malignant tumor-derived human prostate cancer cell line

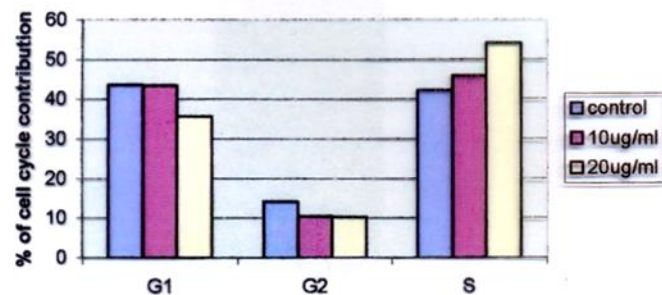
Effect of propolis on cycle progression in RC-58/h/AS#4) cells in KGM medium



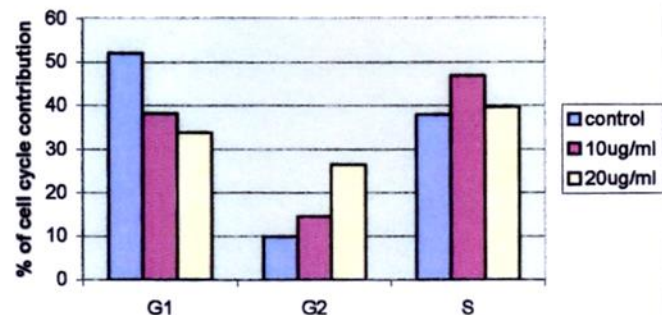
S arrest in Pro#1 treated RC58T/h/SA#4 cells



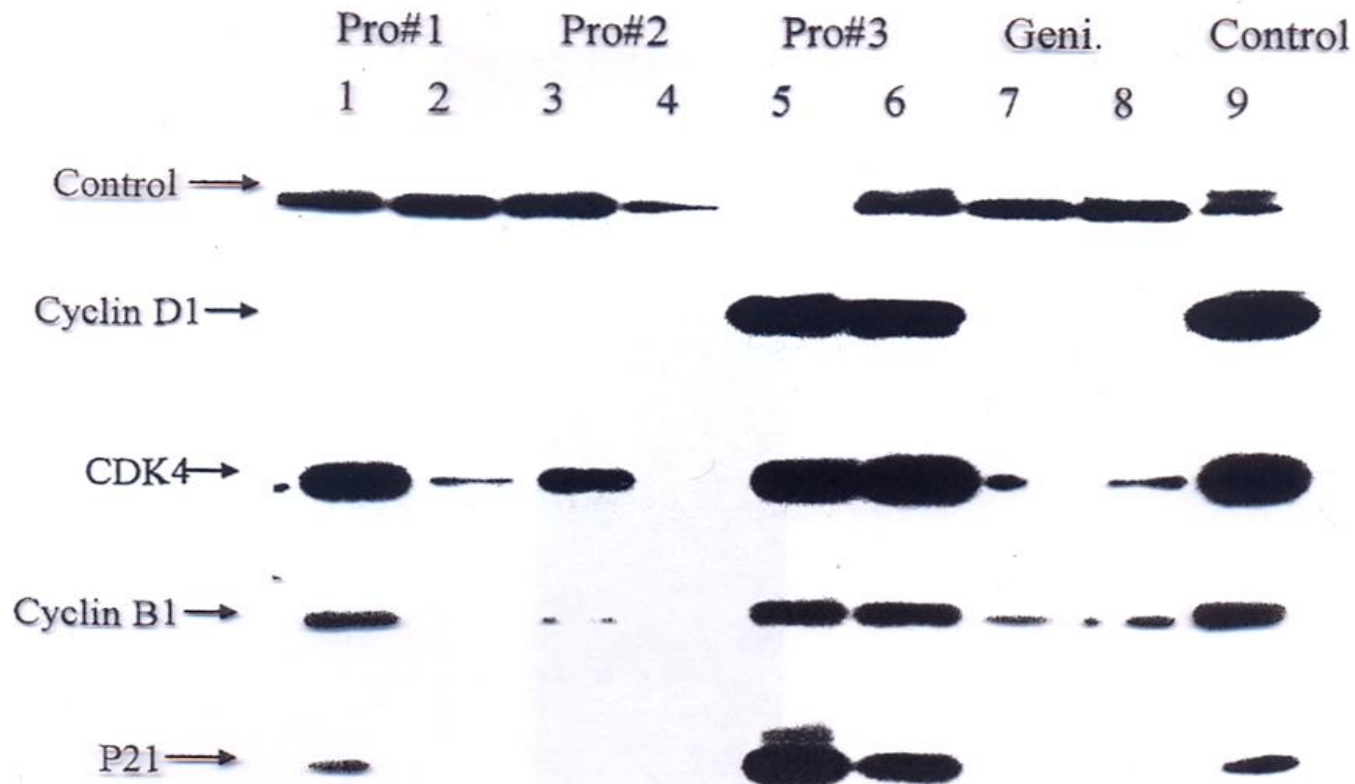
S arrest in PRO#2 treated RC58T/h/SA#4 cells



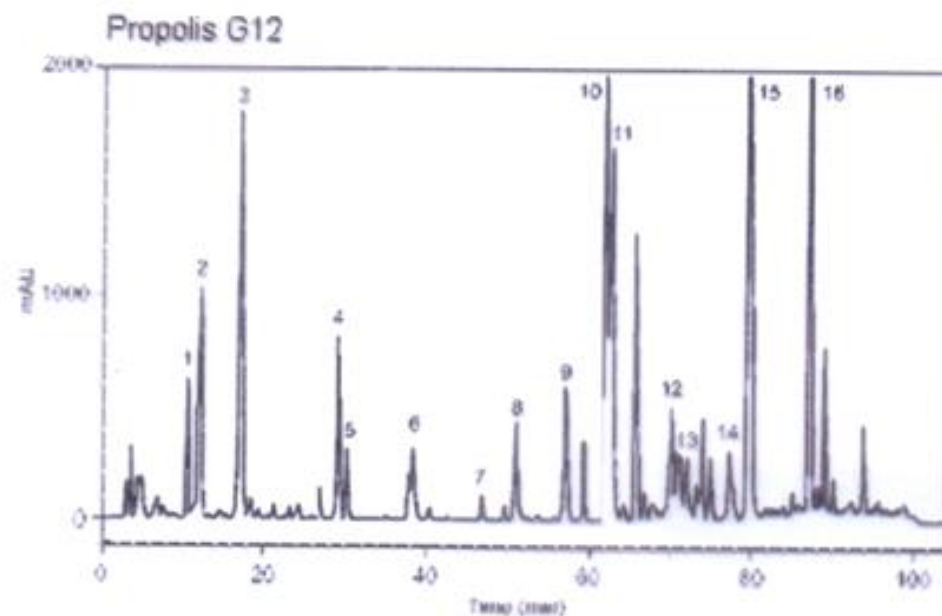
G2 arrest in Pro#3 treated RC58T/h/SA#4 cells



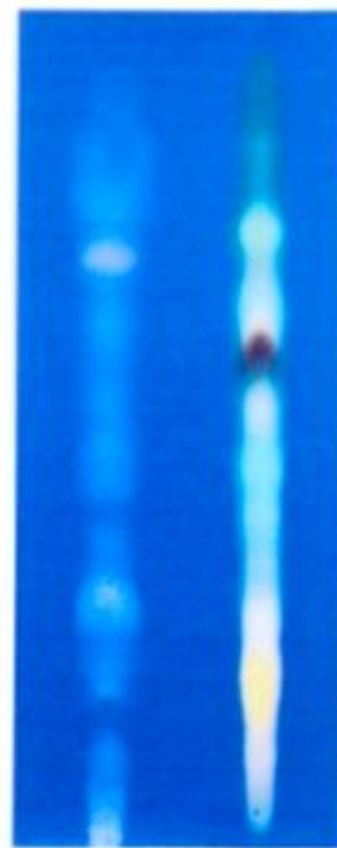
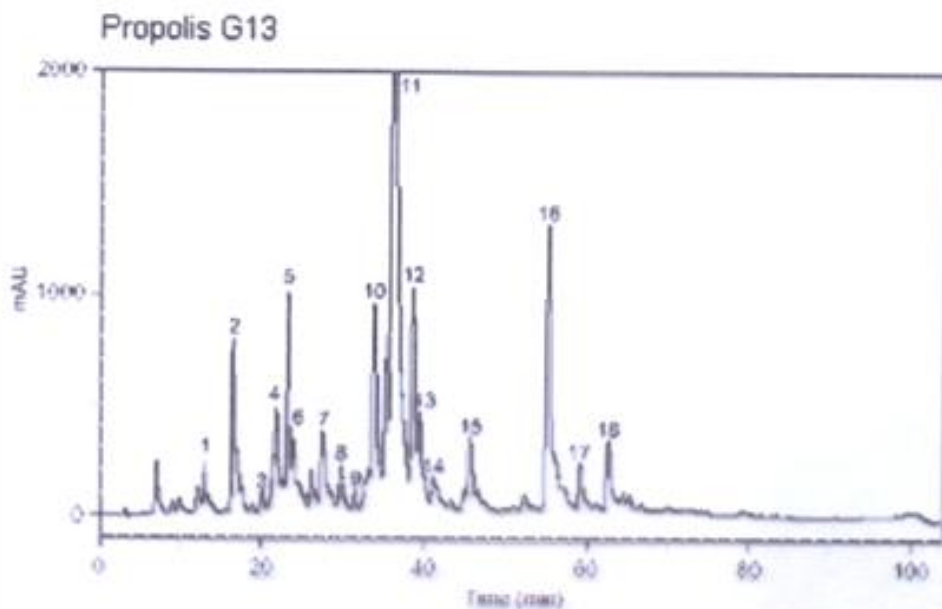
Propolis regulates cell cycle related protein expression in RC58T/h/SA#4 cells.



RPHPLC chromatograms of ethanolic extracts of propolis G12 & G13



RPHPTLC of ethanolic extracts of propolis G12 & G13



G12

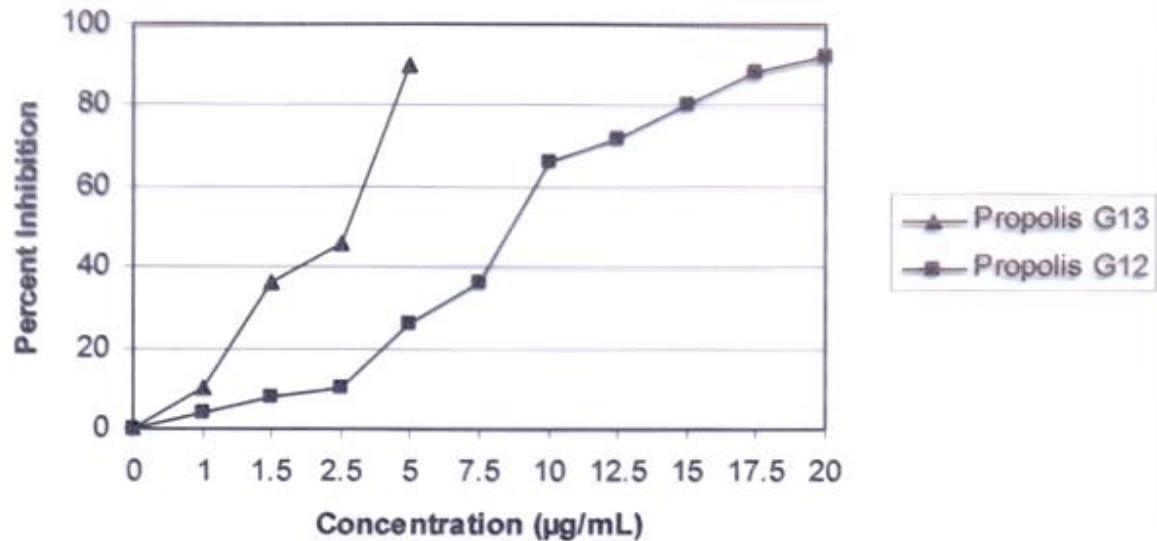
G13

Flavonoids and other chemical constituents of própolis G12 & G13 were determined by RPHPLC (mg/g)

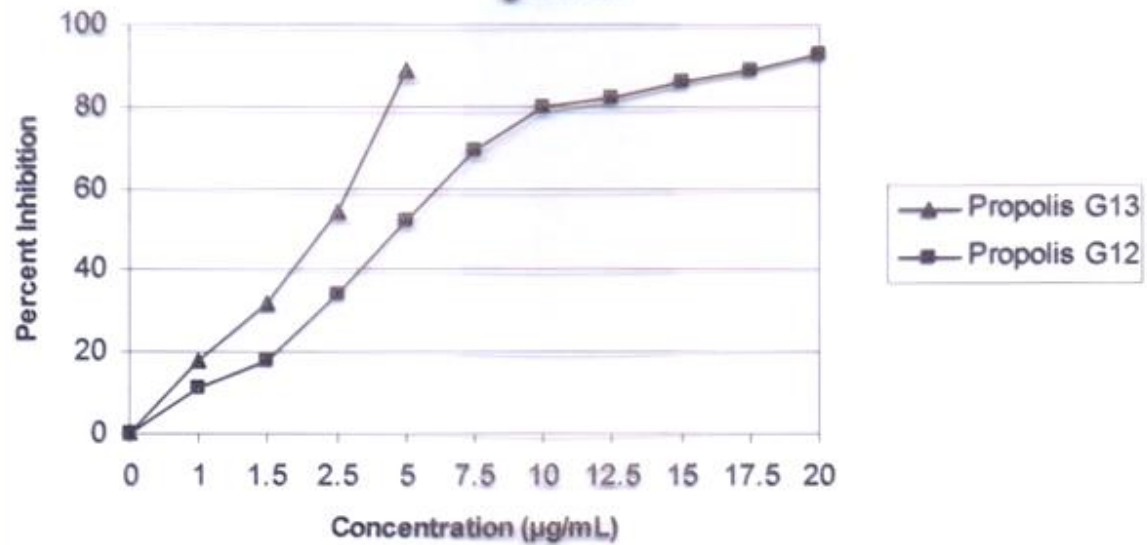
Própolis G12			Própolis G13		
Peak no.	Compound	Quantity in mg/g of propolis	Peak no.	Compound	Quantity in mg/g of propolis
1	Coumaric acid	10.7	1	Rutin	1.3
2	Ferulic acid	2.4	2	Liquiritigenin	7.1
3	Λ 245 nm ^a	+	3	Daidzein	4.3
4	Cinnamic acid	2.6	4	Pinobanksin	6.0
5	Pinobanksin	1.7	5	Λ 251, 292 nm ^a	+
6	Kaempferol	1.3	6	Quercetin	1.9
7	Isosakuranetin	4.9	7	Luteolin	2.1
8	Chrysin	1.9	8	Λ 241, 272, 281 nm ^a	+
9	Acacetin	6.7	9	Dalbergin	0.9
10	Kaempferide	12.6	10	Isoliquiritigenin	12.1
11	Λ 244 nm ^a	+	11	Formononetin	19.5
12	Λ 230 nm ^a	+	12	Λ 235, 263 nm ^a	+
13	Λ 245 nm ^a	+	13	Pinocembrin	7.1
14	Λ 228, 246 nm ^a	+	14	Pinobanksin 3-acetato	2.6
15	Artepillin C	38.6	15	Biochanin A	1.5
16	Λ 223, 276 nm ^a	+	16	Λ 238, 260, 269 nm ^a	+
			17	Λ 233, 249, 329 nm ^a	+
			18	Λ 233, 256 nm ^a	+

Effects of propolis samples (G12 & G13) on cells' growth

a - Effect of propolis samples on PrEC cells' growth



b - Effect of propolis samples on RC-58T/h/SA#4 cells' growth



The fifty percent (50%) growth inhibition (GI50) of normal human prostate epithelial cells (PrEC) and primary tumor-derived human prostate cancer cells (RC-58T/h/SA#4) by propolis G12 and G13.

Cells/própolis	PrEC	RC-58T/h/SA#4
Propolis G12	8.75	5.00
Própolis G13	3.25	2.75