



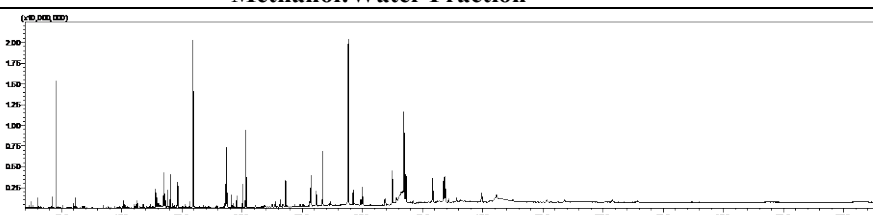
Supplementary Materials: A New Approach to Atopic Dermatitis Control with Low-Concentration Propolis-Loaded Cold Cream

Bianca Aparecida Martin, Camila Nunes Lemos, Luciana Facco Dalmolin, Caroline Arruda, Íris Sperchi Camilo Brait, Maurilio de Souza Cazarim, Estael Luzia Coelho da Cruz-Cazarim, Paula Carolina Pires Bueno, Maurílio Polizello Júnior, Leonardo Régis Leira Pereira, Renata Nahas Cardili and Renata Fonseca Vianna Lopez

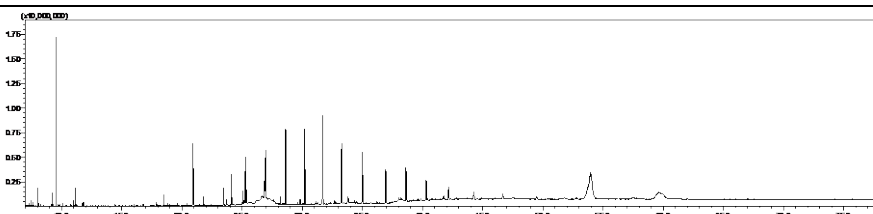
(a)

Methanol:Water Fraction

Raw Besswax



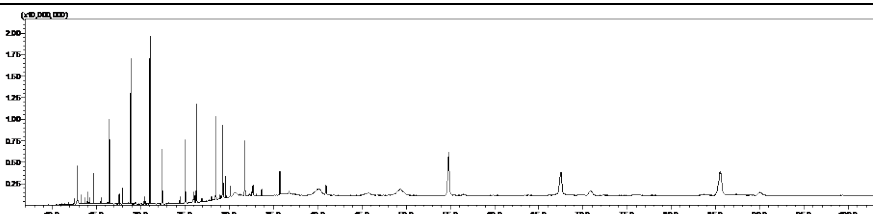
Purified Besswax



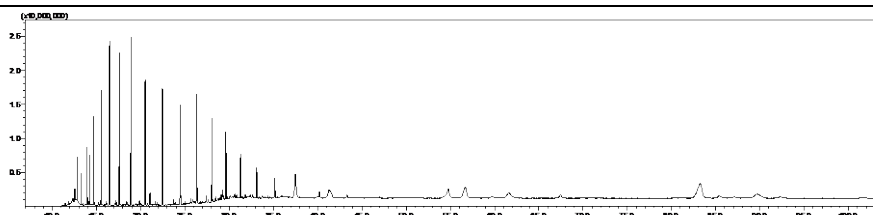
(b)

Hexane Fraction

Raw Besswax



Purified Besswax



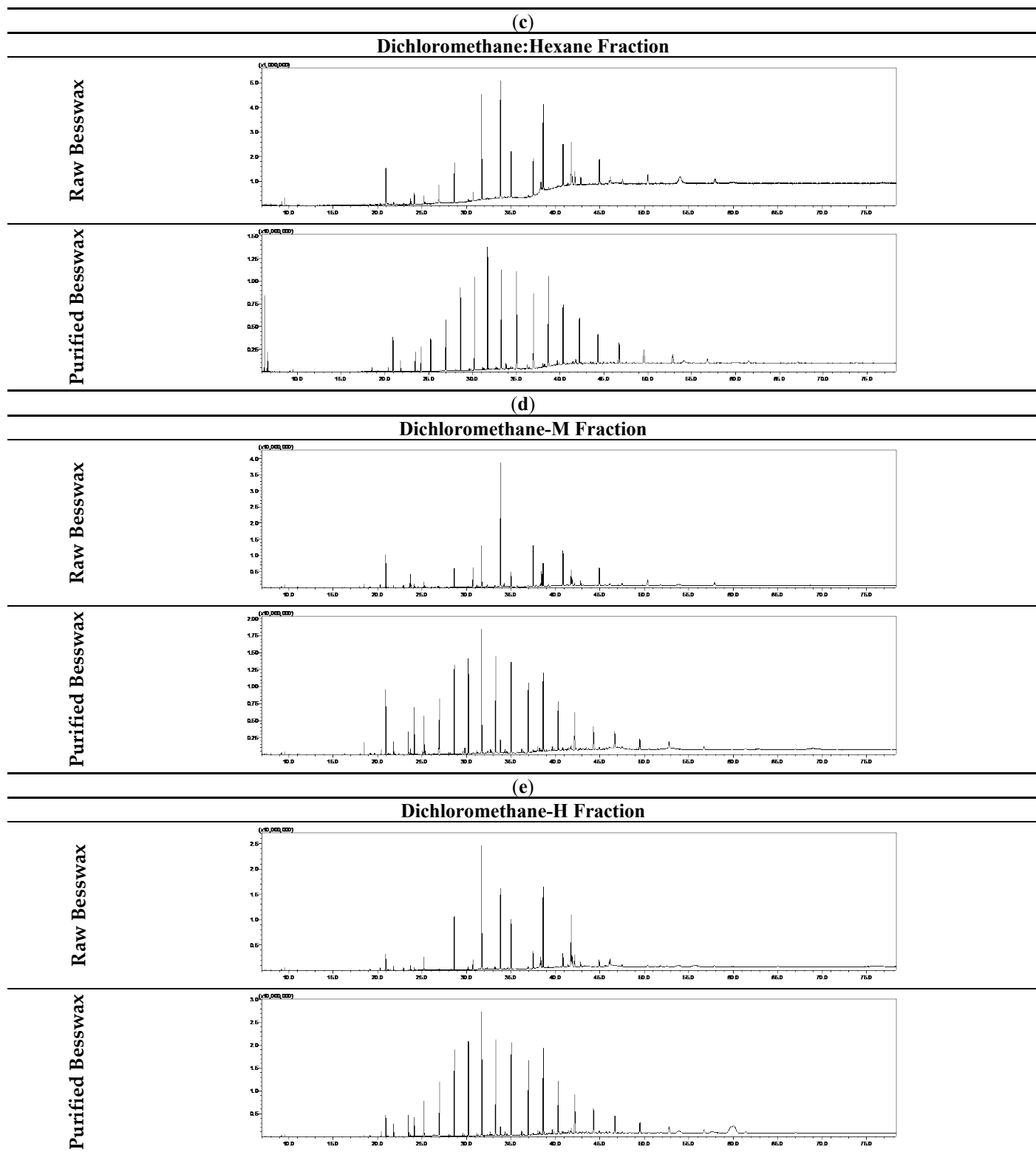


Figure S1. Chromatograms of samples of raw and purified beeswax in the different fractions studied, obtained by GC/MS. Chromatograms of fractions (a) methanol, (b) hexane, (c) dichloromethane:hexane, (d) CH₂Cl₂-MeOH and (e) CH₂Cl₂-Hex. Chromatographic conditions: Helium gas pressure of 187.1 kPa, linear viscosity 31.9 cm/s and column flow 1.5 mL/min. Ion source temperature 250°C and mass range 40 to 700 *m/z* every 0.3 s. Database: NIST11, NIST11-S, WILEY7, NIST08 and FFNSC3.1.

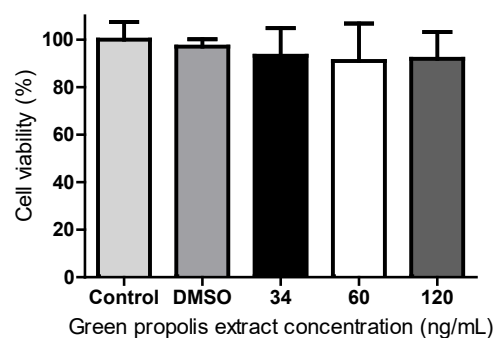


Figure S2. Percentage of viable cells (AMJ-2) after treatments with different concentrations of green propolis extract (ANOVA with Tukey's post hoc test, $p > 0.05$).

Table S1. Substances in raw and purified beeswax. Analyses were carried out by GC/MS and annotation was based on the similarity of theoretical spectra found in the NIST11, NIST11-S, WILEY7, NIST08 and FFNSC3.1 databases.

N°	Components *	Samples						Fraction (Extraction)				
		Raw Beeswax	Purified Beeswax	RT	Database	SI	Metabolic Class	Methanol:Water	Dichloromethane M	Dichloromethane H	Hexane	Dichloromethane:Hexane
				(min)								
1	(Z) 3-Phenyl-2-propenoic acid ou Cinnamic acid (1TMS) *	x	-	15.16	NIST11s	95	Phenylpropanoids	x				
2	1,3-Dipalmitin (1TMS)	-	x	43.72	NIST11s	69	Glycerolipid				x	
3	1,9- Nonanedioic acid ou Azelaic acid (2TMS)	x	x	18.01	NIST11s	92	Fatty acid	x				
4	2-Ethylbutyric acid, nonadecyl ester	-	x	41.31	NIST08	79	Fatty acid				x	
5	4-Hydroxy-3,5,5-trimethyl-4-[3-oxo-1-butenyl]-2-cyclohexen-1-one	x	-	18.10	NIST11s	93	Ketone	x				
6	4-Hydroxy-3-methoxy benzoic acid ou Vanillic acid (2TMS)	x	x	17.57	WILEY7	85	Benzene and substituted derivatives	x				
7	4-Hydroxy-3-methoxy cinnamic acid ou Ferulic acid (1TMS) *	x	-	21.62	NIST08	83	Phenylpropanoids	x				
8	4-methoxy-benzeneacetic acid (1TMS)	x	-	15.04	NIST11	86	Benzene and substituted derivatives	x				
9	4-Methoxy-benzoic acid (1TMS)	x	x	14.81	NIST11	92	Benzene and substituted derivatives	x				
10	9,12-Octadecadienoic acid (Z,Z) (1TMS)	x	-	23.60	WILEY7	96	Fatty acid	x	x	x		

11	9,12-Octadecadienoic acid, ethyl ester	x	-	13.71	WILEY7	82	Fatty acid							x
12	9-Octadecadienoic acid, ethyl ester	x	-	22.91	NIST11	94	Fatty acid	x						
13	9-Octadecenoic acid (1TMS)	x	-	14.05	WILEY7	96	Fatty acid							x
14	9-Octadecenoic acid, ethyl ester	x	-	22.92	NIST11	95	Fatty acid					x		x
15	beta.-L-Mannofuranose, 6-deoxy-1,2,3,5-tetrakis-O-(1TMS)-?	x	-	18.21	WILEY7	88	Sugar	x						
16	Butanedioic acid (2TMS)	-	x	11.81	WILEY7	95	Carboxylic acid and derivatives	x						
17	D-(-)-Tagatofuranose, pentakis, (1TMS) ether (isomer 1)?	x	-	17.88	NIST11	91	Sugar	x						
18	D-(+)-Talofuranose, pentakis, (1TMS) ether (isomer 1)?	x	-	18.17	NIST11	87	Sugar	x						
19	Decanedioic acid ou Sebacic acid (2TMS)	-	x	18.96	NIST08	92	Fatty acid	x						
20	Docosane	-	x	23.45	FFNSC1.3	97	Hydrocarbon	x	x	x	x	x	x	
21	Docosanoic acid (1TMS)	x	-	30.71	NIST11s	95	Fatty acid	x	x	x				x
22	Dodecanoic acid (1TMS)	x	-	16.28	WILEY7	94	Fatty acid	x						
23	Dotriacontanoic acid (1TMS)	x	-	50.36	NIST08	86	Fatty acid		x	x	x	x	x	
24	Dotriacontanol (1TMS)	x	-	47.50	NIST11	95	Fatty acid		x	x	x	x	x	
25	D-Psicofuranose, pentakis, (1TMS) ether (isomer 2)?	x	-	17.80	NIST11	91	Sugar	x						
26	Eicosane	x	x	20.38	FFNSC1.3	97	Hydrocarbon	x	x	x	x	x	x	
27	Glucopyranose, pentakis-O (1TMS)?	x	-	19.24	NIST11	94	Sugar	x						
28	Glyceric acid (3TMS)	x	-	11.94	NIST11s	90	Sugar	x						
29	Hecacosyl hexadecanoate	x	-	54.00	NIST11	88	Fatty acid							x
30	Hentriacontane	x	x	38.61	FFNSC1.3	97	Hydrocarbon		x					
31	Heptacosane	x	x	31.70	FFNSC1.3	97	Hydrocarbon		x					
32	Heptadecanoic acid (1TMS)	-	x	22.39	NIST11s	92	Fatty acid		x					
33	Heptanoic acid, docosyl ester	-	x	87.15	WILEY7	69	Fatty acid						x	
34	Heptatriacontane	-	x	52.80	NIST08	96	Fatty acid		x	x				x
35	Hexacosane	x	x	30.20	NIST11	97	Hydrocarbon						x	
36	Hexacosanoic acid (1TMS)	x	x	37.48	NIST08	96	Fatty acid		x	x	x	x	x	
37	Hexacosanol (1TMS) ether	x	-	35.71	NIST11	84	Fatty acid		x					
38	Hexadecanoic acid (1TMS)	x	x	20.66	WILEY7	96	Fatty acid	x						
39	Hexadecanoic acid, eicosyl ester	-	x	92.17	NIST08	79	Fatty acid						x	
40	Hexadecanoic acid, ethyl ester	x	-	20.29	WILEY7	96	Fatty acid		x	x				
41	Hexadecanoic acid, ethyl ester, ethyl palmitate	x	-	20.28	WILEY7	95	Fatty acid	x						
42	Hexadecanoic acid, octacosyl ester	x	-	85.48	WILEY7	89	Fatty acid						x	
43	Hexadecanoic acid, propyl ester (2TMS)	x	x	29.80	NIST11s	93	Fatty acid	x						
44	Hexadecenoic acid (1TMS)	x	x	20.67	NIST11	92	Fatty acid	x	x					

45	Hexatriacontane	-	x	49.50	NIST08	97	Hydrocarbon	x	x	x	x	x
46	Hexatriacontanoic acid (1TMS)	x	-	68.59	NIST08	78	Hydrocarbon		x			
47	Hydrocinnamic acid ou benzenepropanoic acid (1TMS)	x	x	13.44	NIST11	93	Phenylpropanoids	x				
48	Hydroxycinnamic acid ou p- Coumaric acid (1TMS) *	x	-	19.66	WILEY7	87	Phenylpropanoids	x				
49	Monopalmitin (1TMS) ether	-	x	32.75	NIST11s	93	Glycerolipid	x				
50	Nonacosane ou n-Nonacosane	x	x	35.35	FFNSC1.3	97	Hydrocarbon	x	x	x		x
51	Nonadecane ou n-Nonadecane	x	-	19.17	WILEY7	97	Hydrocarbon		x	x	x	x
52	Nonatriacontane	-	x	61.40	WILEY7	97	Hydrocarbon		x			x
53	Octacosane	x	x	33.27	FFNSC1.3	97	Fatty acid		x			
54	Octacosanoic acid (1TMS)	x	x	40.80	NIST08	89	Fatty acid	x	x			x
55	Octacosanol (1TMS)	x	-	39.19	NIST11s	97	Fatty acid		x		x	x
56	Octadecanoic acid ou stearic acid (1TMS)	x	x	24.12	NIST11s	95	Fatty acid	x	x	x		x
57	Octadecenoic acid (1TMS)	x	x	23.69	WILEY7	96	Fatty acid	x	x			x
58	Octadecenoic acid, ethyl ester	x	-	22.90	NIST11	94	Fatty acid	x				
59	Octatriacontane	-	x	56.70	NIST08	96	Fatty acid		x	x		x
60	Pentacosane	x	x	28.61	FFNSC1.3	97	Hydrocarbon	x	x	x		x
61	Pentatriacontane	-	x	46.66	NIST11s	92	Hydrocarbon	x	x	x		x
62	p-Hydroxybenzoic acid (1TMS)	x	x	16.02	NIST11s	90	Benzene and substituted derivatives	x				
63	Ricinoleic acid (1TMS)	x	x	26.87	WILEY7	93	Fatty acid	x	x	x		x
64	Tetracontane	-	x	67.00	FFNSC1.3	83	Hydrocarbon		x			x
65	Tetracontanoic acid (1TMS)	x	-	40.94	NIST08	85	Fatty acid				x	
66	Tetracosan-1-ol (1TMS) ether	x	-	32.31	NIST08	81	Fatty acid		x	x		
67	Tetracosane	x	x	26.95	WILEY7	97	Hydrocarbon	x	x	x		x
68	Tetracosanoic acid (1TMS)	x	x	33.80	NIST11	95	Fatty acid	x	x	x		x
69	Tetracosanoic acid, ethyl ester	x	-	33.18	FFNSC1.3	93	Fatty acid		x			
70	Tetracosanol (1TMS) ether	x	-	32.32	NIST08	81	Hydrocarbon		x			
71	Tetradecanoato, ethyl	x	-	33.17	FFNSC1.3	94	Hydrocarbon			x		
72	Tetradecanoic acid ou myristic acid (1TMS)	x	x	18.50	WILEY7	96	Fatty acid	x	x	x		x
73	Tetratriacontane	-	x	44.25	NIST11	97	Hydrocarbon	x	x	x		x
74	Triacotane	x	x	36.95	FFNSC1.3	97	Hydrocarbon	x		x		x
75	Triacotanoic acid (1TMS)	x	-	44.91	WILEY7	86	Fatty acid	x	x	x		x
76	Triacotanol (1TMS) ether	x	-	42.82	NIST11	89	Fatty acid		x	x		x
77	Tricosane ou n-Tricosane	x	x	25.21	WILEY7	97	Hydrocarbon	x	x	x		x
78	Tritriacontadiene	x	-	41.39	NIST11	94	Hydrocarbon			x		

* Shadow compounds are aromatic substances found in green propolis. TMS: Substances identified in the databases as silylated derivatives. RT: Average retention time considering all values found in the different fractions. SI: Similarity index. NOTE¹: The classification of annotated substances was carried out according to the Classyfire database. NOTE²: The nomenclature provided by the database records was maintained in the breakdown of compounds.

Table S2. Apparent viscosity (30 s) and area of hysteresis of the creams as a function of storage time at room temperature.

Time (day)	CBlank		CPropolis	
	Viscosity (Pa.s)	Area of hysteresis	Viscosity (Pa.s)	Area of hysteresis
0	2.9 ± 0.2 *	14,959 ± 1171 *	1.7 ± 0.5	11,783 ± 309
15	3.0 ± 0.1	15,653 ± 909	2.9 ± 0.2	14,296 ± 3319
30	3.2 ± 0.5	12,166 ± 452	3.2 ± 0.1	11,689 ± 1586
45	3.7 ± 1.1	11,220 ± 1214	2.3 ± 0.3	13,595 ± 2301
60	3.7 ± 0.2	12,400 ± 2586	3.5 ± 0.7 **	14,379 ± 3152

Values expressed as mean ± SD. *t*-test for the same times of different samples and ANOVA, with Tukey's post hoc test for all times of the same sample ($p < 0.01$) ($n = 3$). * Statistical difference between the T0 of the two samples. ** Statistical difference of the same sample in relation to the T0.

Table S3. Confounding variables taken into account in the distribution of groups.


Population	Control Group ($n = 8$)	Frequency (%)	Intervention Group ($n = 8$)	Frequency (%)	<i>p</i> -value
MTX dose increase	1	12.5	0	0	0.608 *
Introduction of oral corticosteroids	0	0	1	12.5	
Introduction of oral antibiotics and topical corticosteroids	1	12.5	0	0	
MTX withdrawal	0	0	1	12.5	
Cyclosporine dose decrease	0	0	1	12.5	
Oral corticosteroids withdrawal	0	0	1	12.5	

MTX= methotrexate. * Probability of significance (*p*-value) calculated by Fisher's exact test.

Table S4. Allergic comorbidities presented by patients included in the study.


Comorbidities	Control Group ($n = 8$)	Frequency (%)	Intervention Group ($n = 8$)	Frequency (%)
Rhinit	3	37.5	3	37.5
Asthma	2	25	0	0
Asthma and Rhinit	0	0	3	37.5

Figure S3. Sociodemographic and clinical data.



ATOPIC DERMATITIS RESEARCH – FCFRP/USP

SOCIODEMOGRAPHIC AND CLINICAL DATA



Name: _____ Telephone: _____

Registration N°: _____ Address: _____

Date of 1st interview: ____/____/____ Date of 2nd interview: ____/____/____ Date of 3rd interview: ____/____/____

SOCIODEMOGRAPHIC DATA

1. Gender: ☐ M ☐ F 2. Pregnant or Lactating? ☐ No ☐ Yes 3. Age: _____

4. Birth: ____/____/____ 5. Marital Status: ☐ Single ☐ Married ☐ Widower ☐ Divorced

Others: _____

6. Education Level: ☐ Illiterate ☐ Elementary school ☐ Incomplete elementary school ☐ Middle school

☐ Higher education ☐ Postgraduate

7. Profession: _____

8. Professional situation: ☐ Employed ☐ Unemployed ☐ Student ☐ Retiree

9. Companion: ☐ No ☐ Yes

Who? _____ Telephone: _____

CLINICAL DATA

10. First medical diagnosis of atopic dermatitis: _____

11. Number of dermatologist appointments per year: _____

12. Do you have contact dermatitis? ☐ No ☐ Yes To which material? _____

12.1 Avoid contact? ☐ No ☐ Yes To which material? _____

13. Perform environmental prophylaxis? ☐ No ☐ Yes

14. Do you have dogs? ☐ No ☐ Yes

15. Do you have cats? ☐ No ☐ Yes

16. Do you use sofa cover? ☐ No ☐ Yes 17. Do you have rugs at home? ☐ No ☐ Yes

18. Are you allergic to any drug? ☐ No ☐ Yes Which one? _____

19. Are you allergic to propolis (substance present in bee honey)? ☐ No ☐ Yes ☐ I do not know

20. Are you allergic to cocoa butter and/or cocoa? ☐ No ☐ Yes ☐ I do not know

ATOPIC DERMATITIS RESEARCH – FCFRP/USP

21. The use of cosmetic was recommended by the doctor?(moisturizing cream): ☐ No ☐ Yes Which? _____

22. Do you use cosmetics as a complement to the treatment of atopic dermatitis? ☐ No ☐ Yes Which? _____

23. What is the bath time? _____ 23.1 Do you use bath sponge? ☐ No ☐ Yes

23.2 Do you use warm water? ☐ No ☐ Yes

24. Have you ever had any previous surgery? ☐ No ☐ I do not know ☐ Yes, Which? _____

☐ Tonsillectomy ☐ Adenectomy Others: _____

25. Do you have allergic diseases? ☐ No ☐ I do not know ☐ Yes, Which? _____

☐ Allergic Rhinitis ☐ Asthma ☐ Allergic conjunctivitis Others: _____

26. Do you have other diagnosed diseases? ☐ No ☐ Yes, Which? _____

☐ Liver Diseases ☐ Depression ☐ Neurological Diseases

☐ Anxiety ☐ Kidney Diseases ☐ Cardiovascular Diseases

☐ Metabolic Diseases ☐ Osteo-articular Diseases Others: _____

27. Is there another family member with atopic dermatitis? ☐ No ☐ Yes Who? _____

28. What is the period of the year/weather ☐ Summer ☐ Winter ☐ Rain ☐ Indifferent Others _____ when the atopic dermatitis gets worse?

29. How do you consider your illness? ☐ Mild ☐ Moderate ☐ Severe

30. Do you understand how to use the cream? ☐ Yes ☐ No

DRUG THERAPY

Drug (Oral)	How many times/days and what time	Continuous use (yes or no)	Treatment time
Drug (Topical)	How many times/days and what time	Continuous use (yes or no)	Treatment time

ATOPIC DERMATITIS RESEARCH – FCFRP/USP

Drug (Injectable)	How many times/days and what time	Continuous use (yes or no)	Treatment time
Drug (Sublingual)	How many times/days and what time	Continuous use (yes or no)	Treatment time
Drug (Nasal)	How many times/days and what time	Continuous use (yes or no)	Treatment time

INJURIES REGIONS

31. Presence of injuries 0 month: Presence of injuries 2 month:

HEALTH BEHAVIORS

32. Do you consume alcoholic drink? ☐ No ☐ Yes Quantity per day: _____

33. Do you smoke? ☐ No ☐ Ex Smoker ☐ Yes Quantity per day: _____

34. Do you exercise? ☐ No ☐ Yes Frequency: _____

DATA ON FORMULATION ADHESION – AFTER 1 MONTH OF TREATMENT

35. Have you ever stopped using the cream? ☐ No ☐ Yes How many days: _____ Why? _____

36. Do the cream cause any discomfort? ☐ No ☐ Yes Which: _____

37. Regarding the cream, what are the complaints below:

☐ Inefficient treatment ☐ Treatment interferes with daily activities ☐ Fear of side effects

☐ Instructions are unclear ☐ Cream has unpleasant smell ☐ Cream caused itching

☐ Cream leaves stains on clothes or surface

☐ Cream changed appearance ☐ Cream left the skin dry

☐ Cream caused burning ☐ Cream makes the skin oily

☐ Cream is difficult to spread ☐ Cream is hard to get out of the bottle

Others: _____

ATOPIC DERMATITIS RESEARCH – FCFRP/USP

DATA ON FORMULATION ADHESION – AFTER 2 MONTH OF TREATMENT

38. Have you ever stopped using the cream? ☐ No ☐ Yes How many days: _____ Why? _____
39. Do the cream cause any discomfort? ☐ No ☐ Yes Which: _____
40. Did you use any other moisturizer during the study period? ☐ No ☐ Yes Why? _____
41. Is there any cream left in the bottle? ☐ No ☐ Yes 41.1 How many bottles? _____
42. Regarding the cream, what are the complaints below:
- ☐ Inefficient treatment ☐ Treatment interferes with daily activities ☐ Fear of side effects
- ☐ Instructions are unclear ☐ Cream has unpleasant smell ☐ Cream caused itching
- ☐ Cream leaves stains on clothes or surface
- ☐ Cream changed appearance ☐ Cream left the skin dry
- ☐ Cream caused burning ☐ Cream makes the skin oily
- ☐ Cream is difficult to spread ☐ Cream is hard to get out of the bottle
- Others: _____

LABORATORY EXAMS

43. IgE Values: _____
44. Prick test: ☐ Accomplished ☐ Unaccomplished
- To which elements? _____
45. Patch test: ☐ Accomplished ☐ Unaccomplished
- To which elements and number of crosses? _____