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Quantitative Assessment of Total Aerobic Mesophiles in Apitoxin, Royal Jelly, Propolis, Honey, and Bee Pollen-Based Products Through an Automated Growth-Based System

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Abstract

Bee-derived products such as apitoxin, royal jelly, propolis, bee pollen, and honey are increasingly being used as part of cosmetic products, because all of them contain a large number of bioactive compounds with antioxidant, anti-inflammatory, anti-microbial, and regenerative properties, which enable them to be used for therapeutic purposes. The aim of this investigation was to assess the performance of an automated growth-based system in order to make a quantitative examination of the total of aerobic mesophiles in bee-derived personal care products using NF-TVC vials that contain a nutrient-based medium with dextrose as the carbon source. According to USP general chapter <1223>, pivotal validation criteria such as linearity, equivalence of results, operative range, precision, accuracy, ruggedness, limit of quantification, and limit of detection have demonstrated that the automated system can be used for reliable mesophile quantitative assessment. Moreover, the actual research demonstrated that polysorbates efficiently block the anti-microbiological potential of bioactive compounds such as phenols, flavonoids, enzymes, peptides, and fatty acids, which naturally occur in apitoxin, royal jelly, propolis, bee pollen, and honey, allowing an efficient microorganism recovery from the bee-made products tested. Therefore, this groundbreaking technology could be applied efficiently within the cosmetic industry to assess the total of aerobic mesophiles in bee-derived products such as capillary treatments, toothpaste, and anti-aging cream, affording several benefits associated with faster product release into the market.

Keywords: apitoxin; royal jelly; propolis; bee pollen; honey; bacteria; yeast; molds; automated growth-based system

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1. Introduction

Natural products such as apitoxin, royal jelly, propolis, bee pollen, and honey are increasingly being used in the cosmetic industry, because the wide spectrum of bioactive compounds such as phenols, flavonoids, enzymes, proteins, and fatty acids have wound-healing properties, high nutritional values, and regenerative potential that enable them to be included in several personal care formulations [1–13]. These biological compounds are active in fighting oxidative stress, boosting collagen production, reducing inflammation, diminishing facial line expression, and inducing capillary cellular regeneration [1–13]. The present investigation aims to assess the performance of an automated growth-based system (AGBS) to carry out a quantitative examination of the aerobic mesophiles from bee-derived personal care products. This cutting-edge technology brings an array of benefits, such as reducing microbiological process time for total aerobic mesophile assessment from 7 days, as with the reference standard method (RSM), to just 2 days, with reliable outcomes, since the AGBS meets all the requirements outlined in general chapter <1223>, which are suitability of the method, linearity, equivalence of results, operative range, precision, accuracy, robustness, specificity, limit of detection, and limit of quantification [13–19]. Previously, we have proven the good performance of the AGBS as a quantitative method using as a pharmaceutical matrix an antacid oral suspension targeting objectionable pathogens like the *Burkorderia cepacia* complex, yeasts, and molds [13,14]. However, its performance using bee-made products such as apitoxin-royal jelly-based anti-aging creams, propolis-honey-based toothpaste, and bee pollen, apitoxin, and royal jelly-based creams have been unexplored for quantitative total aerobic mesophile assessment. Regarding the antimicrobial properties of honey, apitoxin, royal jelly, bee pollen, and propolis, which prevent microbiological harvesting, it is interesting to test polysorbate as a chemical neutralizer of these bioactive compounds [1–13]. Therefore, tests of the suitability of the method were carried out for all the bee-derived personal care products under study by means of chemical neutralization. Mesophile examination was performed by testing *Staphylococcus aures*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Aspergillus brasiliensis* as suitable representatives of mesophiles.

Regarding this matter, personal care products are not required to be completely aseptic, but a rapid and accurate microbiological assessment is required to examine the microbiological product's quality in order to guarantee that the product complies with microbiological specifications. Therefore, in microbiological specification terms, cosmetic products with bioregulator activity must have a total viable aerobic microorganism count $<10^2$ CFU/mL or g., and harmful pathogens such as *S. aureus*, *P. aeruginosa*, and *E. coli* must be undetectable in 1 mL or g [16].

To assess the AGBS performance, the suitability of the method with apitoxin-royal jelly-based anti-aging creams, propolis-honey-based toothpaste, and bee pollen and apitoxin-royal jelly-based creams was tested and was shown to provide chemical neutralization of the all of these antimicrobials agents. Once the suitability of the method was proven, calibration curves were constructed by effecting a simultaneous recovery of the microorganisms by means of the RSM and AGBS for each bee-made product using *S. aures*, *E. coli*, *P. aeruginosa*, *C. albicans*, and *A. brasiliensis* as representatives of all aerobic mesophiles' viable count in order to demonstrate compliance with essential validation criteria such as linearity, equivalence of results, operative range, precision, accuracy, ruggedness, limit of detection, and limit of quantification [13].

The AGBS is based on microbial metabolism using a nutrient-based liquid medium with dextrose as the carbon source (NF-TVC vials). NF-TVC vials are divided into two zones, growing and reading. Microorganisms are allowed to grow in the growing zone (liquid-enriched medium), which permits the growth of a broad spectrum of aerobic microorganisms including bacteria, yeasts, and molds. In this way, mesophiles grow in the NF-TVC vial yielding carbon dioxide, which diffuses through a gas-permeable layer from the growth medium toward the reading zone, which is a soft agar plug containing a dye indicator of the Soleris NF-TVC vial. Only gases can enter the reading zone; microorganisms, medium, and particulates are blocked. When carbon dioxide reaches the reading zone of the vial, it will trigger a color change from green (absence of microorganisms) to yellow (presence of microorganism) [13–19]. This colorimetric change is detected and recorded by

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the equipment's software and corresponds to the detection time (DT, h), indicative of a positive test result [13–19].

2. Materials and Methods

(a) Reagents

The bee-made products chosen to carry out the present investigation, as well as their antimicrobial agents, are summarized in Table 1, as described in the literature. For each bee-made product, three different lots were chosen to carry out microorganism recovery for both the AGBS and the RSM. The strains *A. brasiliensis* (Cat. No. ATCC 16404), *C. albicans* (Cat. No. ATCC 10231), *P. aeruginosa* (Cat. No. ATCC 9027), *E. coli* (Cat. No. ATCC 8739), and *S. aureus* (Cat. No. ATCC 6538) were studied. Test microorganisms were from frozen stocks using culture maintenance techniques to ensure that viable microorganisms used for inoculation were not more than five passages removed from the master seed-lot. The reagents, namely tryptic soy broth, TSB (Scharlab, code 02-200); sabouraud dextrose agar, SDA (Neogen, cat. No. NCM0008); tryptic soy agar, TSA (Scharlab, code 02-200); and Tween® 80 (Scharlab, code 73625), were used as provided.

Table 1. Antimicrobial activity reported for apitoxin, royal jelly, propolis, bee pollen, and honey.

Bee-made products
1. Apitoxin-royal jelly based anti-aging cream. Antimicrobial agents: Melittin, 10-hydroxy-2-decenoic acid [4,6,23,24].
2. Propolis-honey based toothpaste. Antimicrobial agents: Enzymes, phenols, and flavonoids [3,23,24].
3. Bee pollen, apitoxin, royal jelly-based cream. Antimicrobial agents: Fatty acids, flavonoids, and phenols [1,4,6,23,24]

(b) Automated Growth-Based System

The Soleris® 128 instruments and supplies were acquired from Neogen. This system includes four incubator drawers (128 vial places) with a precise temperature control for each drawer (28.5 ± 0.5 °C) with dedicated software and computer. The design qualification (DQ), installation qualification (IQ), operational qualification (OQ), and performance qualification (PQ) of the system were satisfactorily fulfilled by both user and supplier. NF-TVC vials containing a nutrient-based medium with dextrose as the carbon source were used to quantify the *S. aures*, *E. coli*, *P. aeruginosa*, *C. albicans*, and *A. brasiliensis* as representatives of the total aerobic viable count.

(c) Inoculum Standardization

S. aures, *E. coli*, and *P. aeruginosa* were reactivated from frozen stocks in TSA and incubated for 48 h at 30–35 °C. *C. albicans* and *A. brasiliensis* were reactivated from frozen stocks in SDA and incubated for 48 hours at 20–25 °C. After the growth time was completed, isolated colonies were taken and resuspended in 0.9% saline solution until they reached a McFarland standard of 2 (equivalent to $1.0x10^8$ CFU/mL).

(d) Suitability of the Method

The bee-derived product's preservatives (phenols, flavonoids, enzymes, fatty acids, melittin-peptide, and other antimicrobial agents) were neutralized using polysorbate 80 to obtain a successful microbiological determination by means of RSM and AGBS, in order to set up an equivalence between the methods and generate a calibration curve for apitoxin-royal jelly-based anti-aging creams, propolis-honey-based toothpaste, and bee pollen, apitoxin, and royal jelly-based creams for

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the validation. Thus, carrying out independent assays, 1 mL of inoculum dilution from each microorganism (*S. aures*, *E. coli*, *P. aeruginosa*, *C. albicans* and *A. brasiliensis*) was added to a Schott bottle and mixed with 90 mL of tryptic soy broth in the presence of the selected neutralizing agent (1 mL /L of Tween® 80 in TSB). Then 10 g or 10 mL of the corresponding bee-derived product was added and vigorously shaken to ensure homogenization of the sample.

(f) Calibration Curve

Once proven the efficacy of the neutralizing polysorbate on bioactive compounds of apitoxin, royal jelly, propolis, bee pollen, and honey-based products, calibration curves were built by testing the 5 strains. From this, a serial dilution was performed. From each dilution (D1–D5), 1 mL was placed directly into each NF-TVC vial, and they were incubated for 48 hours at 28.5 °C in the Soleris® equipment. The NF-TVC vials were inoculated in duplicate. Simultaneously, TSA plates were also inoculated in duplicate with 1 mL of each dilution by means of the spread plate technique. The plates were incubated at 30 °C for 7 days. This series of experiments was performed using at least three different lots for each bee-derived product. An uninoculated NF-TVC vial was used as a negative control, and an inoculated vial with 1 mL of the dilution (count of 10–100 CFU/mL) was used as a positive control for each strain tested. Each detection time (DT) recorded by the Soleris® system within the incubation period (48 h), also confirmed by color change, was an indication of the presence of microorganisms. Data generated by both the AGBS (detection times, DT) and RSM (colony forming unit, CFU) were plotted in Soleris@ software to generate calibration curves by plotting DTs relative to the corresponding log CFU values.

(g) Linearity and Equivalence of Results

The plotted values in the calibration curve were fit to a least-squares regression, and the coefficient of determination (R^2) was calculated. The linearity was determined using the chi-square (x^2) goodness-of-fit test model in order to evaluate the relationship between the CFUs and the DT data obtained from the RSM and the AGBS, respectively. In this way, for each bee-product tested, 5 calibration curves were constructed, corresponding to the *S. aures*, *E. coli*, *P. aeruginosa*, *C. albicans*, and *A. brasiliensis* strains tested.

(h) Accuracy

The coefficient of correlation (CC) obtained from the calibration curves was a measure of accuracy, according to USP general chapter <1223>. Additionally, DT values and their log 10 equivalent CFU counterparts were statistically analyzed using the Pearson goodness-of-fit test based on a Poisson distribution. In this way, DT yields with AGBS were automatically converted into CFU by means of calibration curves constructed for each microorganism tested. Thus using the Pearson goodness-of-fit test, it was possible to predict CFU from DT with a 95% confidence interval.

(i) Limit of Detection and Limit of Quantification

The limit of detection (LOD) and limit of quantification (LOQ) were determined from the calibration curves based on the fewest number of recoverable microorganisms (<10 CFU). The LOD and LOQ for both methodologies (RSM and AGBS) were calculated using the following equations: LOD=3.3*SD/m and LOQ=10*SD/m, where SD is the standard deviation and m is the slope of the linear regression obtained for each calibration curve, as stated in the International Council of Harmonization (Q2) R2 and USP guidelines [21].

(j) Precision

S. aures, *E. coli*, *P. aeruginosa, C. albicans,* and *A. brasiliensis* were used to determine the suitability of the method with apitoxin-royal jelly-based anti-aging creams, propolis honey-based toothpastes, and bee pollen, apitoxin, and royal jelly-based creams using three different lots for each bee-derived

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product tested. Serial dilutions were carried out, and the microorganisms were recovered simultaneously via RSM and AGBS. Dilutions that recovered microorganisms in the 10 to 300 CFU range via the RSM and AGBS were analyzed in order to determine the standard deviation (SD) and coefficient of variation (CV).

3. Results

3.1. Suitability of the Method (Antimicrobial Neutralization)

Antimicrobial activity has been widely described in apitoxin, honey, royal jelly, propolis, and bee pollen because of the existence of bioactive compounds with antimicrobial activity, which prevent microbial harvesting [1–13] (Table 1). Hence it is important to consider that suppression of antimicrobial activity needs to be done in all the bee-made products in order to successfully recover microorganisms and guarantee the product's microbiological quality assessment. Therefore, polysorbate 80 was used to neutralize the antimicrobial activity of the bee-derived products (Table 1). As outlined in USP <61>, polysorbate is able to neutralize quaternary ammonium compounds (QACs), iodine, and parabens [23]. In the current investigation, it was shown that polysorbate 80 can neutralize bioactive preservative compounds such as flavonoids, phenols, enzymes, royal jelly major proteins, melittin, and fatty acid such as 10 HDA, permitting a suitable microorganism recovery, as observed for the S. aures, E. coli, P. aeruginosa, C. albicans and A. brasiliensis calibration curves (Table 1).

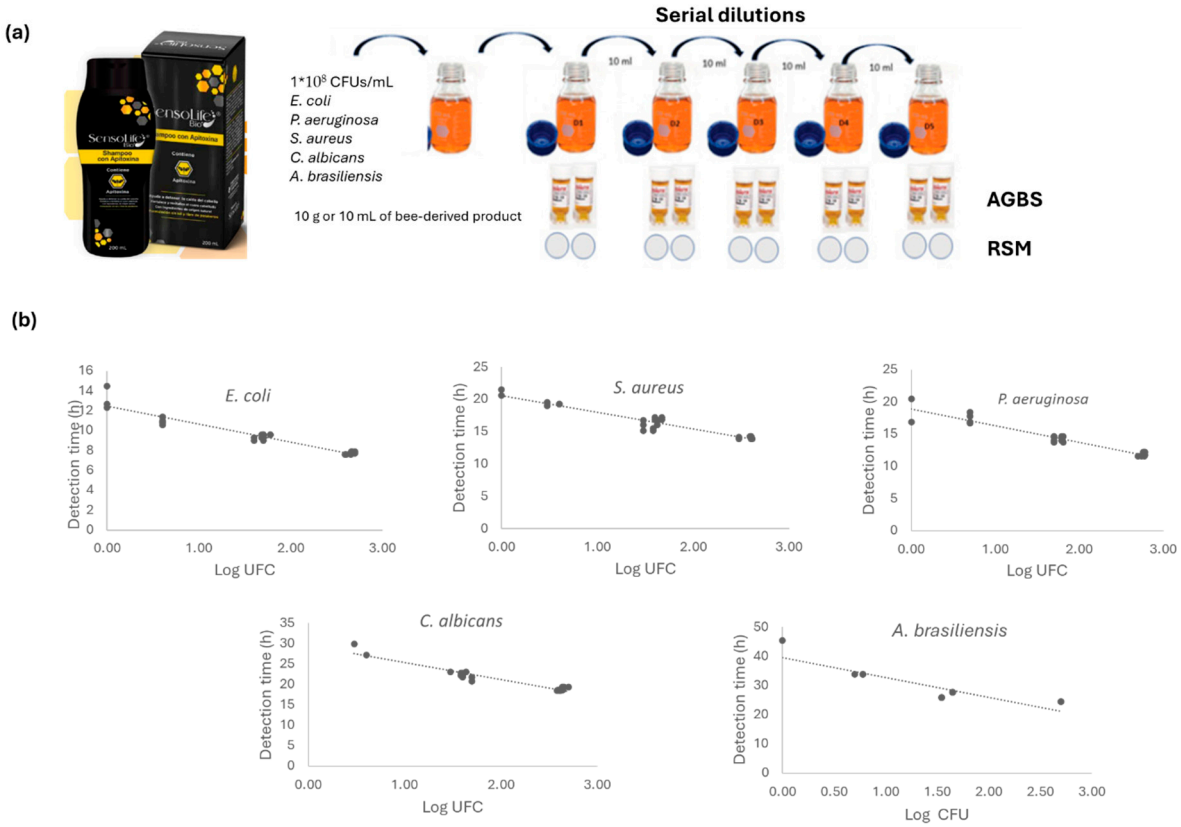

Figure 1. (a) Experimental design used for the validation testing of the AGBS. Construction of correlation curves. The suitability of the method test was carried out for each bee-derived product. From this, a serial dilution was performed. From each dilution (D1–D5), 1mL was placed directly into each NF-TVC vial, and then incubated for 48 h at 28.5 °C in the Soleris equipment. Simultaneously, agar plates were also inoculated in duplicate with 1mL of each dilution by means of the pour-plate technique. The plates were incubated at 25 °C for 7 days for yeast and molds recovery and at 30 °C for 5 days for mesophiles recovery. Data generated by both the alternative

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and conventional method were plotted to generate calibration curves by plotting DTs relative to the corresponding log CFU values. For each bee-product tested, 5 calibration curves were constructed, corresponding to the S. aures, E. coli, P. aeruginosa, C. albicans, and A. brasiliensis.

3.2. Linearity, Operative Range, and Equivalence of Results

Considering that the RSM and AGBS yield quantitative data with different units (CFU vs. DT), an equivalence of results correlation needs to be done through building calibration curves. Thus calibration curves for all bee-made products were derived by plotting DT values with their respective equivalents in log CFU. Linear regression analysis yielded the relationship between DTs and log CFU values, as shown in Table 2. The linearity observed in all bee-derived products was consistent with USP <1223> ($R^2 \geq 0.9025$), and linearity for all bee-made products tested ranged from 1 CFU to around 1000 CFU (Table 2).

Table 2. Linearity data obtained for the calibration curves constructed for S. aures, E. coli, P. aeruginosa, C. albicans, and A. brasiliensis of each bee-made product.

Bee-made product	Microorganisms	Linear Regression	R^2	x^2 square test ($P \leq 0.05$)	Upper Range of Quantification
Apitoxin-royal jelly based anti-aging creams	*S. aureus*	y = -2.3189x + 20.952	0.9225	P ≤ 0.05	3.9 x 10^2
	E. coli	y = -1.7331x + 13.046	0.9485	P ≤ 0.05	4.0 x 10^2
	P. aeruginosa	y = -2.6517x + 17.735	0.9343	P ≤ 0.05	2.9 x 10^2
	C. albicans	y = -6.1878x + 37.049	0.9319	P ≤ 0.05	3.0 x 10^2
	A. brasiliensis	y = -0.1605x + 7.5561	0.9692	P ≤ 0.05	3.5 x 10^3
Propolis-honey based toothpaste	*S. aureus*	y = -2.7201x + 18.76	0.9136	P ≤ 0.05	2.8 x 10^2
	E. coli	y = -1.7514x + 11.9	0.9174	P ≤ 0.05	2.9 x 10^2
	P. aeruginosa	y = -2.89x + 17.68	0.9106	P ≤ 0.05	3.0 x 10^2
	C. albicans	y = -3.4748x + 25.611	0.9281	P ≤ 0.05	2.0 x 10^2
	A. brasiliensis	y = -0.0771x + 4.2792	0.9421	P ≤ 0.05	4.0 x 10^2
Bee pollen, apitoxin, royal jelly-based cream (capillary treatments)	*S. aureus*	y = -2.5676x + 20.618	0.9241	P ≤ 0.05	4.1 x 10^2
	E. coli	y = -1.8052x + 12.46	0.9166	P ≤ 0.05	5.0 x 10^2
	P. aeruginosa	y = -2.5797x + 18.917	0.9204	P ≤ 0.05	6.0 x 10^2

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C. albicans	y = -3.6917x + 28.444	0.9206	P ≤ 0.05	5.0×10^2
A. brasiliensis	y = -5.8226x + 41.169	0.9107	P ≤ 0.05	1.0×10^3

3.3. Accuracy

The Pearson goodness-of-fit test was chosen to assess the comparison of the results obtained by AGBS and those by RSM for each bee-made product for all the strains tested. As shown in Table 3 , DT values can be calibrated in CFU values according to the Pearson goodness-of-fit test, P ≥ 0.05, (Table 3).

Table 3. Accuracy. Goodness-of-fit test (Pearson) and correlation coefficient obtained for all the bee-derived products.

Bee - made product	Strains used to build calibration curves	Goodness-of-Fit Tests	Coefficient of Correlation
Apitoxin-royal jelly based anti-aging creams	*S. aureus*	P ≥ 0.05	0.9500
	E. coli	P ≥ 0.05	0.9700
	P. aeruginosa	P ≥ 0.05	0.9700
	C. albicans	P ≥ 0.05	0.9700
	A. brasiliensis	P ≥ 0.05	0.9692
Propolis-honey based toothpaste	*S. aureus*	P ≥ 0.05	0.9600
	E. coli	P ≥ 0.05	0.9600
	P. aeruginosa	P ≥ 0.05	0.9500
	C. albicans	P ≥ 0.05	0.9600
	A. brasiliensis	P ≥ 0.05	0.9700
Bee pollen, apitoxin, royal jelly-based cream (capillary treatments)	*S. aureus*	P ≥ 0.05	0.9613
	E. coli	P ≥ 0.05	0.9574
	P. aeruginosa	P ≥ 0.05	0.9594
	C. albicans	P ≥ 0.05	0.9595
	A. brasiliensis	P ≥ 0.05	0.9543

3.4. Limit of Detection and Limit of Quantification

The LOD and LOQ were calculated using the standard deviation of data obtained for the fewest number of recoverable microorganisms (<10 CFUs) and the slope of the corresponding calibration curves constructed for S. aures, E. coli, P. aeruginosa C. albicans, and A. brasiliensis. As is shown in Table 4, the LOD and the LOQ of the AGBS for all bee-made products tested were < 10 CFU for all the strains tested, showing that it is not inferior in terms of sensibility compared to the RSM (< 10 CFUs).

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Table 4. Limit of Detection (LOD) and Quantification (LOQ) for RSM and AGBS at lowest microbial threshold before total decay of response (SD: standard deviation).

Bee-made products	Strains used to build calibration curves	RSM, CFU/Sample			AGBS, CFU/Sample		
		LOD	LOQ	SD	LOD	LOQ	SD
Apitoxin-royal jelly based anti-aging creams	*S. aureus*	1	3	1	2	6	1
	E. coli	1	3	1	3	10	2
	P. aeruginosa	1	1	1	1	4	1
	C. albicans	1	1	1	1	1	1
	A. brasiliensis	5	14	5	5	16	5
Propolis-honey based toothpaste	*S. aureus*	0	0	0	1	1	1
	E. coli	1	3	1	4	11	2
	P. aeruginosa	1	2	1	3	8	2
	C. albicans	1	2	1	1	1	1
	A. brasiliensis	1	1	1	1	1	1
Bee pollen, apitoxin, royal jelly-based cream (capillary treatments)	*S. aureus*	2	5	1	3	8	2
	E. coli	3	8	2	7	22	4
	P. aeruginosa	3	8	2	3	11	3
	C. albicans	1	2	1	1	3	1
	A. brasiliensis	4	13	4	8	24	8

3.5. Intermediate Precision and Ruggedness

The precision was measured based on a dilution that recovered CFUs within a range of 10–300 using AGBS and its equivalent in RSM for all the strains tested (S. aures, E. coli, P. aeruginosa C. albicans, and A. brasiliensis). As shown in Table 5, for all bee-made products tested, the coefficient of variation (CV) was below USP specification, <15% (30–300 CFUs).

For this experiment, the ruggedness was interpreted as intermediate precision, a type of intra-laboratory precision involving the effect of different lots on the test result variability, as well as on the repeatability (CV <15%).

Table 5. Precision of alternative and traditional methods for each bee-made product.

Bee-made product	Strains used to build calibration curves	Mean DT	Standard Deviation	Coefficient of variation	Mean CFU	Standard Deviation	Coefficient of variation
Apitoxin-royal jelly based anti-	*S. aureus*	17.51	0.26	1.51	30.33	5.31	17.51
	E. coli	10.4	0.18	1.73	33.17	8.66	26.12
	P. aeruginosa	19.97	3.22	16.15	21.67	6.34	29.27
	C. albicans	28.22	1.50	5.33	24.00	5.48	22.82
	A. brasiliensis	38.1	0.00	0.00	45.00	7.94	17.63

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aging creams							
Propolis -honey based toothpa ste	*S. aureus*	15.54	0.28	1.82	18.80	4.80	25.5
	E. coli	9.53	0.21	2.25	24.83	5.13	20.6
	P. aeruginosa	13.33	0.68	5.15	27.83	5.98	21.4
	C. albicans	20.8	0.2	0.96	21.30	2.16	10.15
	A. brasiliensis	39.8	0.0	0.0	31.66	2.8	9.12
Bee pollen, apitoxin , royal jelly-based shampo o	*S. aureus*	16.35	0.77	4.71	38.55	6.25	16.21
	E. coli	9.40	0.23	2.48	49.67	6.08	12.24
	P. aeruginosa	14.22	0.41	2.92	56.13	6.69	11.91
	C. albicans	22.42	0.80	3.61	39.86	6.01	15.08
	A. brasiliensis	27.70	0.00	0.00	110	10.00	9.09

4. Discussion and Conclusions

We have shown that polysorbate 80 can be used as a suitable chemical neutralizer of bioactive compounds with well-known antimicrobial potential, such as melittin, phenols, flavonoids, enzymes, and fatty acids such 10 HDA. This is a notable finding, since chemical neutralization is pivotal in routine pharmaceutical analyses to assess the microbiological quality through a total aerobic mesophile enumeration. Therefore, thanks to the suitability of the method, an optimal microorganism's recovery was possible from the three different bee-made matrices tested by means of AGBS and RSM. Hence, using *S. aures*, *E. coli*, *P. aeruginosa C. albicans,* and *A. brasiliensis* as suitable representatives of all aerobic mesophiles, it was possible to prove essential validation criteria such as linearity, operative range, equivalence of results, precision, accuracy, ruggedness, limit of detection, and limit of quantification.

Statistical analysis of the x^2 goodness-of-fit test demonstrated that there exists a strong relationship between threshold bioburden (dilutions) in NF-TVC vials and DTs. This occurs because carbon dioxide is a direct measure of the microbial burden in the NF-TVC, so a high concentration of aerobic mesophiles in AGBS yields low DT values, while at low bioburden this automated system yields high DT values.

Using the Pearson goodness-of-fit test, the AGBS was able to predict the CFUs from the DT values with a 95% confidence interval in the calibration curves constructed for S. aures, E. coli, P. aeruginosa, C. albicans, and A. brasiliensis ($P \geq 0.05$, Table 3). Therefore, AGBS is able to predict an equivalent CFU result from a DT through the calibration curve. Each tested product should have its own calibration curve, since the active chemical and biological principle of the bee-made produtc may have a strong impact on the kinetic of the microorganism's recovery. Even though AGBS yielded quantitative results in DT, those results will be converted automatically into equivalent CFU, meaning that the AGBS would have the same specification as described by the RSM (CFU/mL).

The use of this broth-based technique (with dextrose as the carbon source) for routine analyses of bee-made products such as apitoxin-royal jelly-based anti-aging creams, propolis-honey-based toothpaste, and bee pollen, apitoxin, and royal jelly-based creams enables reducing the microbiological assessment from 7 days, as is usual with the RSM, to just 2 days. This fact was clearly demonstrated by means of the calibration curves obtained for each bee-made product, in which the AGBS was able to detect 1 CFU for total viable counts in a maximum incubation time of 48 h, while the RSM was able to detect 1 CFU within a maximum time of 7 days of incubation. The

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implementation of this ground-breaking method is supported by USP guidelines, since it provides benefits such as being less labor intensive, reducing company warehousing costs, improving efficiency in inventory control, increasing the ability to respond more quickly to adverse microbiological results, and allowing faster product release into the market, as well as being more sensitive than the RSM.

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