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Pharmaceutical topical gel containing proanthocyanidin polymers-rich fraction from *Stryphnodendron adstringens*

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The stem bark of *Stryphnodendron adstringens*, popularly known in Brazil as "barbatimão", has many biological activities, including antifungal activity. Considering the increasing interest of using "barbatimão" extract in the treatment of vaginal candidiasis, the aim of this study was to propose a pharmaceutical topical gel containing a proanthocyanidin polymers-rich fraction for use as a pharmacological agent in vaginal gels, as well as to evaluate the validation parameters for the determination of phenolic compounds. UV/Vis spectrophotometry was used as a quantitative method for quality control of topical gel and a proanthocyanidin polymers-rich fraction was used in this work. The proposed gel seems suitable for use in vaginal infections, and the analytical method was linear, specific, precise, accurate, reproducible and robust. This methodology complies with analytical application demands and it is easily performed in work routine.

Key words: Barbatimão, polyphenols, *Stryphnodendron adstringens*, UV/Vis spectrophotometry, vaginal gel, validation method.

INTRODUCTION

The genus *Stryphnodendron* belongs to the *Leguminosae* family, which has about 48 species, all rich in tannin and native to the savannah in tropical and subtropical climates. Among them is *Stryphnodendron adstringens* (Mart.) Coville. The content of tannins in the bark of *S. adstringens* reaches a minimum of 8% according to the Brazilian Pharmacopeia (Albuquerque et al., 2007; ANVISA, 2010).

The stem bark of *S. adstringens*, popularly known in Brazil as "barbatimão", is employed in popular culture as an anti-inflammatory, antibacterial and antiulcer treatment (Agra et al., 2008; Albuquerque et al., 2007). The use of *S. adstringens* is well documented in the literature as demonstrating anti-inflammatory, antibacterial, healing, antiviral, antiulcer, antitrypanosomal, antileishmanial and antifungal activities (Audi et al., 2004; Felipe et al., 2006;

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Glehn and Rodrigues, 2012; Hernandes et al., 2010; Herzog-soares and Alves, 2002; Ishida et al., 2009; Lanchoti Fiori et al., 2013; Luize et al., 2005; Morey et al., 2016; Pinto et al., 2015). In addition, in Brazil there is a commercialized pharmaceutical ointment named Fitoscar® containing *S. adstringens* extract for wound-healing. Phytochemical studies performed with *S. adstringens* showed that it is composed of proanthocyanidin polymers, mainly several flavan-3-ols derivatives, such as prodelphinidins and prorobinetinidins (Lopes et al., 2008; Mello et al., 1999, 1996).

Additionally, other species from the genus *Stryphnodendron* have shown promise for the treatment of some pathologies. *S. polyphyllum* showed potential molluscicidal activity (Bezerra et al., 2002), *S. obovatum* showed potential antileishmanial activity (Ribeiro et al., 2015) and both showed good activity for wound healing, antibacterial and antioxidant potential (Lopes et al., 2005). *S. rotundifolium* has been ethnopharmacologically very important and used for inflammatory and infectious diseases, gastroprotection and pain complaints (Oliveira et al., 2014); its laboratorial evaluation has shown several activities for the extract of its leaves, such as antiulcer (Awaad et al., 2013; Lopes et al., 2008), extract of bark showed a leishmanicidal and trypanocidal activity and gastroprotection (Oliveira et al., 2018; Vandesmet et al., 2017). The effect of plants from the genus *Stryphnodendron* may be related with some substances of the class of tannins.

Vulvovaginal candidiasis is one of the most frequent mycoses seen in daily practice in gynecology. This fungus affects approximately 70% of women and ranks second among the causes of vaginitis. Despite its clinical importance, no ideal medication for this disease exists. Various imidazole derivatives have achieved higher cure rates than polyenics (85-90%), but with similar side effects. Besides, reports have shown the incident of strains with decreased sensitivity or resistant to some antifungal agents (Fidel, 2007; Odds et al., 2003; Ostrosky-Zeichner, 2008; Sanglard, 2016).

Considering the increasing interest in vaginal candidiasis treatment, we propose a pharmaceutical topical gel for vaginal use containing a proanthocyanidin polymers-rich fraction from *S. adstringens* (F2). Previous reports demonstrated the antifungal activity of F2 against planktonic cells (in suspension) from vaginal isolates of *Candida* spp., and F2 also altered some virulence factors of *C. albicans* as well as led to alterations in budding and cell wall morphology (Ishida et al., 2006). Treatment with F2 reduced biofilm metabolic activity (in sessile and in dispersed cells) during biofilm formation, and in mature biofilms it reduced biofilm biomass during biofilm formation and led to the appearance of dumbbell-shaped blastoconidia and blastoconidia clusters in biofilms (Freitas et al., 2018; Luiz et al., 2015). In addition, toxicological studies using rodents also reported low side effects after oral treatment with F2 (Costa et al., 2013, 2010).

Several methods were already shown for quantitative analyses of polyphenols in *S. adstringes* and its herbal preparations (ANVISA, 2010; Isler et al., 2010; Nascimento et al., 2013). Currently, there is a tendency to prefer HPLC methods for quality control, probably assuming that they are reproducible, can identify and quantify single substances and provide a suitable chromatographic fingerprint. But, the current HPLC methods for herbal drugs with polyphenols are limited to detection of, besides gallic acid, only monomers, such as gallocatechin, epigallocathechin, and epigallocatechin gallate, or dimers whose identification and quantity are difficult due to the absence of reference substances. The UV/Vis spectrophotometric method by colorimetric reaction using Folin-Ciocalteu reagent is a classical method used to determine the content of polyphenols in herbal drugs, including for *S. adstringens* (ANVISA, 2010; Schofield et al., 2001). This method is not limited to some substances but measures the concentration, which is directly proportional to the total phenolic hydroxyl groups, therewith including polymers of high molecular weight (Schofield et al., 2001). Chemical characterization of F2 and its subfractions by mass spectrometry ES-MS and ^{13}C NMR spectroscopy showed the presence of proanthocyanidin polymers (a hexameric compound), composed of prodelphinidin and prorobinetinidin units and gallic acid residues, with an average molecular weight of 2,114 (Ishida et al., 2009, 2006).

In this context, the aim of this study was to validate a method of quality control for quantitative determination of phenolic compounds in a proanthocyanidin polymers-rich fraction (F2) from the stem bark of *S. adstringens*, as well as in a topical vaginal gel (TG), by spectrophotometric method in the ultraviolet region.

MATERIALS AND METHODS

Plant material

Stem bark was collected in São Jerônimo da Serra, Paraná, Brazil (S23° 43′ 7.8″, W50° 45′ 23.5″; altitude 926 m), in March 2008. A voucher herbarium specimen was deposited under number HUM 14321 at the Universidade Estadual de Maringá, and was identified by Prof. Dr. Cássia Mônica Sakuragui, Universidade Federal do Rio de Janeiro. Stem bark was dried at room temperature and then pulverized (Tigre ASN-5).

Preparation of extracts

The crude extract was obtained by turbo-extraction (Skymsen) of 1,000 g of bark with 70% acetone in water for 15 min and temperature under 40°C. The organic solvent was eliminated using a rotavapor under reduced pressure, and the residue was lyophilized to yield a crude extract (F1; 300 g). Next, the F1 (50 g) was suspended in water (500 mL) and partitioned with ethyl acetate (500 mL; 1:1) to obtain a proanthocyanidin polymers-rich fraction (lyophilized water fraction; F2; 35 g).

Preparation of pharmaceutical topical gel and quality control

The composition of the TG is given in Table 1. The gel was prepared by dispersing the gel-forming material in sterile distilled water. Methylparaben was added as a preservative, and sodium carbonate was added as a neutralizer with gentle agitation to avoid the inclusion of air, until the gel acquired the intended consistency and transparency. Afterwards, F2 was incorporated at 0.2% with mixing until the desired homogenate was obtained. The pH value was measured. The TG was transferred into polyethylene tubes to a total amount of 50 g under laminar flow conditions.

Centrifugation test

A sample of 5 g of TG was submitted to centrifugation under the following conditions: 25 ± 2°C, 3,000 rpm and 30 min. Afterwards, the sample was immediately evaluated for the presence of any instability signals.

Preliminary stability study

Tubes containing TG were stored in climate chambers under the following conditions: 30°C/75% relative humidity (RH) and 40°C/75% RH for 3 months. We evaluated possible changes regarding the organoleptic characteristics, pH value, total polyphenols content and antifungal potential.

Antifungal potential by determination of minimum inhibitory concentration (MIC)

Strains of *Candida albicans* (ATCC 10230) were provided by the Oswaldo Cruz Institute (Rio de Janeiro–RJ, Brazil) and stored in water suspension at room temperature. MIC determination was performed as described in document M27-A3. Briefly, serial dilutions of TG in RPMI 1640 medium without sodium bicarbonate (Sigma Chemical Co., MO, USA) buffered with 0.165 M MOPS (Sigma Chemical Co., MO, USA) were made in 96-well microtiter trays, to obtain equivalent concentrations of F2 from 10 to 5,000 µg/mL. A suspension of *C. albicans* of 1-5 × 10^6 cfu/mL was prepared, diluted 1:1000 and 100 µL was dispensed into each well containing 100 µL of medium to obtain a final concentration of 0.5-2.5 × 10^3 cfu/mL. The microtiter trays were incubated at 35°C for 48 h in a humidity chamber. The MIC values were considered to be the lowest concentration that visibly inhibited Candida spp. growth. The F2 was used as positive control and was prepared under the same conditions as TG. The experiment was performed in triplicate in different days.

Total polyphenols content of the proanthocyanidin polymers-rich fraction of S. adstringens (F2) and topical gel containing this fraction (TG)

All dilution operations were protected from light.

Stock solution: 15 mg of F2 was diluted with 25 mL of water in a volumetric flask; 5 mL of this solution was transferred to another 25 mL volumetric flask and diluted with water (F2 stock solution); 1.5 g of TG was diluted with 25 mL of water in a volumetric flask (TG stock solution).

Total polyphenols (TP) solution: The stock solution of F2 or TG (2 mL) was transferred to a 25 mL volumetric flask and mixed with 1 mL of 2 N Folin-Ciocalteu reagent plus 10 mL of water and volumetrically diluted to 25 mL with a 10.75% w/v solution of anhydrous sodium carbonate. After 30 min, the absorbance was measured at 760 nm using water as the compensation liquid and a quartz cell (1 cm path length) in a UV/Vis spectrophotometer (Shimadzu PC-1650).

***Calibration curve*:** In three replicates, 50.0 mg of pyrogallol was dissolved in water immediately before use and diluted to 25 mL. Aliquots of 1.0, 1.75, 2.5, 3.25 or 4.0 mL were diluted to 25 mL in water, and aliquots of 5 mL from these solutions were diluted to 25 mL (stock solution). Each stock solution (2 mL) was prepared according to the procedure described above for TP solution and the absorbance was measured under the same conditions. The final concentrations were 1.28, 2.24, 3.20, 4.16 and 5.12 µg/mL of pyrogallol and the specific absorptivity of pyrogallol was determined from the linear equation curve (concentration vs absorbance).

The percentage of total polyphenols expressed as pyrogallol in F2 and TG was calculated, respectively, by the equations:

$$TP_{F2} = \frac{1562.5.A}{A^{1\%}.m}$$

$$TP_{TG} = \frac{312.5.A}{A^{1\%}.m}$$

Where A = absorbance of the sample for TP solution; $A^{1\%}$ = specific absorptivity of pyrogallol; m = mass of the sample examined, in grams; 1562.5 and 312.5 = dilution factors for the samples F2 and TG, respectively.

Analytical method validation

For validation of the analytical method, the guidelines established by the International Conference on the Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH, 2005) and by Brazilian regulation RE n◦ 899/2003 of the National Health Surveillance Agency (ANVISA, 2003) were employed.

Linearity

To establish linearity of the proposed method, five stock solutions were prepared in three replicates from 5.0, 10.0, 15.0, 20.0 or 25.0 mg of F2. These stock solutions were used to prepare the TP solutions at the concentrations: 3.2, 6.4, 9.6 and 12.8 µg/mL, and their absorbance was measured. TG was prepared with five different concentrations of F2: 0.1, 0.15, 0.2, 0.3 and 0.35%. Then, 1.5 g of each gel, in three replicates, was used to prepare the stock solutions and TP solution for determining the absorbance.

Specificity

Specificity was determined by adding 1 mL of pyrogallol solution (1.0 mg/mL) to each stock solution of F2 described in the linearity test or 1 mL of pyrogallol solution (0.2 mg/mL) to each stock solution of TG described in the linearity test. The method is considered specific if the slopes of the linear equation in tests for linearity and specificity are equal or very similar. Additionally, TG prepared in three replicate samples with placebo (gel base) to confirm no interference of other compounds from formulation in absorbance at 760 nm.

Limits of detection and quantification

The limits of detection (LOD) and quantification (LOQ) were

Table 1. Composition of topical gel.

Gel base	
Ingredient	**Quantity**
Carbopol-940®	1.0%
Methylparaben	0.2%
Sodium carbonate	q.s.
Purified water	100 g

determined from the curves of linearity of F2 and TG. The LOD was established by using the expression 3σ/S and LOQ by expression 10σ/S, where σ is the standard deviation of the response and S is the slope of the linear equation.

Precision

Precision was evaluated on two levels: repeatability (intra-day) and intermediate (inter-day). The repeatability was assessed using six samples of 15.0 mg of F2 or 1.5 g of 0.2% TG, and intermediate level was considered as variation between days of at least 2 days, in six replicates. A coefficient of variation over 10% and a significant difference between days were considered unacceptable for the complex matrix.

Accuracy

The accuracy of the method was established based on the recovery tests and different concentration levels were evaluated: lower concentration (LC), intermediate concentration (IC) and higher concentration (HC). There was addition of 1, 2 or 3 mL of pyrogallol solution (1.0 mg/mL) to the F2 stock solution, in three replicates, or addition of 1, 2 or 3 mL of F2 aqueous solution (0.72 mg/mL) to TG stock solution, in three replicates. Theoretical absorbance was calculated for the sum of the absorbance from samples in the repeatability test and expected absorbance of pyrogallol by calibration curve in each level or expected absorbance of F2 by linearity curve. The measured value was compared with the theoretical value. The accuracy was assessed as the recovery percentage and the method was considered accurate if the recovery percentages were between 85 and 115%.

Robustness

Robustness was demonstrated for F2 by analyzing the influence of natural light (operations without light protection), and changing of anhydrous sodium carbonate solutions to 7.50% and 14.06% (w/v); it was evaluated in three replicates. For TG, the absorbance of samples were evaluated at different times (25 and 35 min) and wavelengths (755 and 765 nm), in three replicates.

Statistical analysis

Data were analyzed with Statistica® 8.0 program (Copyright StatSoft, Inc., 1984-2007) by one-way analysis of variance (ANOVA) followed by Tukey's test, as well as by T-test for two groups, considering $P < 0.05$ as significant. The data were expressed as mean ± standard deviation [relative standard deviations (%)]. Linear correlation tests and residual analyses were performed by simple linear regression, considering R^2 equal or higher than 0.99, and the residual sum of squares was evaluated.

RESULTS AND DISCUSSION

Among the several pharmaceutical forms of topical application for the vaginal canal, topical gel is promising because it can adhere to the vaginal surface for a reasonable time and releases drug faster than cream or ointment if there is no interaction between drug and polymers. Besides, hydrogels, such as gels from Carbopol, showed special advances due to their elastic consistency, which reduces the friction between the pharmaceutical form and physiological tissue (Johal et al., 2016).

The physical stability of gels indicates the absence of chemical integration between the polymer and drugs. The centrifugation test is considered as a screen test for the development of gels, since instability signals indicate increased particle mobility and the need of a new formulation (Dantas et al., 2016). According to our evaluation, TG remained translucent and without precipitates or lumps after centrifugation. Other polymers (sodium carboxymethyl cellulose (Na CMC), hydroxyethyl cellulose – Natrosol and hydroxypropylmethylcellulose – (HPMC) were tested for formation of gel and incorporation of F2. Natrosol and HPMC were incompatible, showing precipitates/lumps. The gel from Na CMC showed viable characteristics and could be a potential polymer for this formulation. Both Carbopol 940 and Na CMC are anionic polymers and there is the probability that there might be repulsion between these polymers and the proanthocyanidins, which have many hydroxyl groups that reduce the possibility of involving the active substance by this formulation (Tatavarti et al., 2004).

The preliminary stability study is important in development phases to anticipate possible problems with stability, therewith saving time in an advanced phase as opposed to industrial development. The organoleptic characteristics assist in evaluating the acceptance parameters of the product by consumers, as well as permit realization of physical stability problems such as lumps, precipitation and turbidity (Chang et al., 2013). According to our observation, TG showed no change in

physical parameters and maintained homogeneity in both storage conditions. However, there was a slight darkening and odor change at 40 °C/75% RH, as well as reduced consistency and shine. Besides, the pH value was 6 at the initial time and was constant after storage in both conditions. The female genital tract can show pH from slightly basic to moderately acidic, and it is important that a topical formulation maintain its pH near physiological values to prevent discomfort in patients. Besides, the change of pH in a pharmaceutical formulation can show some degradation reactions, compounds interactions, microbial contamination, viscosity alteration and others (Cook and Brown, 2018). The TP content in TG was smaller than 5% during the storage time in both conditions. These data comply with the one acceptable for assays in herbal preparations, but special attention should be given to this parameter in future studies. Other factors, besides the formulation, could affect the stability of chemical marker, for example the package material and its isolation. Finally, the MIC of TG was equivalent to 31.25 µg/mL of F2; the same value was determined for positive control (pure F2) and was constant during the study. The determination of microbiological potential in an antimicrobial preparation shows that the chosen formula will not negatively affect the efficacy of pharmaceutical agents by compounds interactions or use of wrong preserving agents.

The data on pharmacotechnical development and from the preliminary stability study showed the formulation was suitable for using the proposed gel in treatment of vaginal infections.

The analytical conditions for UV/Vis spectrophotometric method were evaluated as described by Blainski et al. (2013) and Bueno et al. (2012), and were confirmed with the use of pyrogallol, for 30 min and 760 nm, respectively; reference substance, time reaction and wavelength, as described in material and methods.

The calibration equation was $y = 0.141x + 0.0044$ ($n = 5$, $R^2 = 0.996$) for pyrogallol. Based on statistical analysis of results of the calibration curve of pyrogallol, the points fall near the line, showing a normal distribution for the samples and observing that the residues are distributed randomly around the mean zero. The method satisfies the conditions statistics, showing that the linear model does not show error for lack of fit (sum of pure error bigger than error for lack of fit). Furthermore, variance analysis showed that the regression is significant and lower values of parameters like standard error (SE) of slope and intercept indicated high precision of the proposed methods. Goodness of fit of the regression equations was supported by high regression coefficient values and lower calculated *F*-values (Table 2).

Spectrophotometric methods for natural products are normally performed using the linearity test for the sample in order to posteriorly analyze the specificity of the method, as well as the LOD and LOQ. In this case, the specificity was evaluated by the absence of matrix effects, considering that natural products are complex matrices in which it is not possible to obtain a pure analyte. The absence of matrix effects is verified if the curves of linearity and specificity are parallel, in other words, if both curves show the same or very similar slopes (Blainski et al., 2013; Bueno et al., 2012; Ribani et al., 2004). The data obtained in the linearity and specificity tests for F2 and TG appear in Table 2 and are represented in Figure 1. The linear regression analysis of all four curves showed satisfactory results for the statistical conditions and were similar to the discussion of the calibration curve. The specificity of the method for F2 and TG was confirmed because the slopes of linear equation were, respectively, equal (= 0.046, for linearity and specificity) and very similar (= 1.846 for linearity; 1.821 for specificity; difference smaller than 1.4%).

Additionally, the determination of TP in TG verified there was no interference from formulation compositions. The placebo absorbance [0.0250 ± 0.0006 (2.28%)] was below the limit of detection, probably due to noise of equipment.

Based on the data from linear regression of the linearity test for F2, the LOD and LOQ were 0.702 and 2.341 µg/mL in TP solution, respectively. These concentrations are equivalent to the absorbance of 0.075 and 0.150 uA, respectively. In the same way, the LOD and LOQ for TG were 0.02 and 0.06% of F2 in TG, respectively. These concentrations are equivalent to the absorbance of 0.076 and 0.147 uA, respectively. It is important to consider that the experimental determinations may be affected by several factors, such as equipment noise, human manipulation and laboratorial conditions. Besides, according to the law of Lambert-Beer there is no proportionality between concentration and absorbance after a certain concentration (Vogel, 2002). Then, despite the LOQ results it is recommended that the range between 0.2 and 0.8 uA be used in spectrophotometric analyses.

The repeatability and intermediate precision for F2 shows 0.494 ± 0.018 [3.6%] and 0.500 ± 0.011 [2.1%], respectively, and there was no significant difference between them ($t_{1,10} = -0.74$, $P = 0.26$). For the TG, the repeatability and intermediate precision shows 0.440 ± 0.010 [2.3%] and 0.464 ± 0.007 [1.6%], respectively, and also no significant difference between them ($t_{1,10} = -5.28$, $P = 0.47$). Thus, the proposed methods for F2 and TG have precision for determination of TP.

The results for the accuracy test (Table 3) showed a total recovery of 98.4 and 85.8% for F2 and TG, respectively, with all levels (LC, IC and HC) between 85 to 115%. These results indicate that the method of TP determination has good accuracy for F2 and acceptable accuracy for TG.

For robustness test, the method was insensitive to tested changes for F2 and TG. For F2, there was no significant difference by changing of anhydrous sodium carbonate solution (7.5% and 14.06%, respectively,

Table 2. Statistical data for the regression equations of the calibration curve for pyrogallol, linearity test and specificity test for F2 and TG.

Regression analysis	Calibration curve of pyrogallol		Linearity test of F2		Linearity test of TG		Specificity test of F2		Specificity test of TG	
Slope (SE)	0.1407 (0.0023)		0.0457 (0.0010)		1.8460 (0.0426)		0.0455 (0.0009)		1.8208 (0.0285)	
Intercept (SE)	0.0044 (0.0078)		0.0428 (0.0107)		0.0451 (0.0102)		0.1471 (0.0094)		0.0915 (0.0068)	
Regression coefficient (R^2)	0.9964		0.9932		0.9926		0.9948		0.9966	
Calculated F-value (critical F-value)	3.30 (3.71)		0.29 (3.71)		0.28 (3.71)		0.29 (3.71)		0.29 (3.71)	
Sum of pure error	0.0990		0.0020		0.0005		0.0013		0.0003	
Lack of fit error	0.0097		0.0021		0.0003		0.0018		0.0010	
Analysis of variance	$F_{1,13}$ = 3901.2, P < 0.001		$F_{1,13}$ = 2053.0, P < 0.001		$F_{1,13}$ = 1878.2, P < 0.001		$F_{1,13}$ = 2669.5, P < 0.001		$F_{1,13}$ = 12,265.7, P < 0.001	
95% CL slope	0.1359; 0.1456		0.0435; 0.0479		1.7540; 1.9381		0.0436; 0.0474		1.7592; 1.8823	
95% CL intercept	-0.0125; 0.0213		0.0197; 0.0659		0.0231; 0.0670		0.1268; 0.1673		0.0768; 0.1062	
	SS	**MS**	**SS**	**MS**	**SS**	**MS**	**SS**	**MS**	**SS**	**MS**
Regression	0.5476	0.5476	0.6409	0.6409	0.4396	0.4396	0.6371	0.6371	0.4277	0.4277
Residual	0.0018	0.0001	0.0040	0.0003	0.0030	0.0002	0.0031	0.0002	0.0014	0.0001
Total	0.5494		0.6450		0.4426		0.6402		0.4290	

SE, Standard error; SS, Sum of squares; MS, Mean square; CL, Confidence limits.

Table 3. Accuracy results determined for recovery test in F2 and TG.

Parameter	F2			TG		
	Theory absorbance (uA)	Obtainedsbsorbance (uA) ($\bar{x} \pm dp$) [CV%]	Recovery (%)	Theory absorbance (uA)	ObtainedAbsorbance (uA) ($\bar{x} \pm dp$) [CV%]	Recovery (%)
LC	0.588	0.593 ± 0.020 [2.9]	100.9	0.586	0.503 ± 0.007 [1.3]	85.8
IC	0.679	0.673 ± 0.010 [1.7]	99.1	0.689	0.596 ± 0.009 [1.6]	86.5
HC	0.769	0.733 ± 0.020 [3.1]	95.3	0.793	0.676 ± 0.004 [0.5]	85.2
Total recovery			***98.4***			***85.8***

$\bar{x}$, Mean; sd, Standard deviation; RSD, Relative standard deviations; LC, Lower concentration; IC, Intermediate concentration; HC, Higher concentration.

0.510 ± 0.003 [0.57%] and 0.498 ± 0.003 [0.65%]), as well as by dilution operations without light protection (0.513 ± 0.001 [1.95%]) by statistical analysis ($F_{3,11}$ = 2.8, P = 0.09). For TG, there was no significant difference in the amount of absorbance at different times (25 and 35 min, respectively, 0.440 ± 0.01 [2.28%] and 0.440 ± 0.01 [2.19%]), or different wavelengths (755 and 765 nm, respectively, 0.422 ± 0.010 [2.83%] and

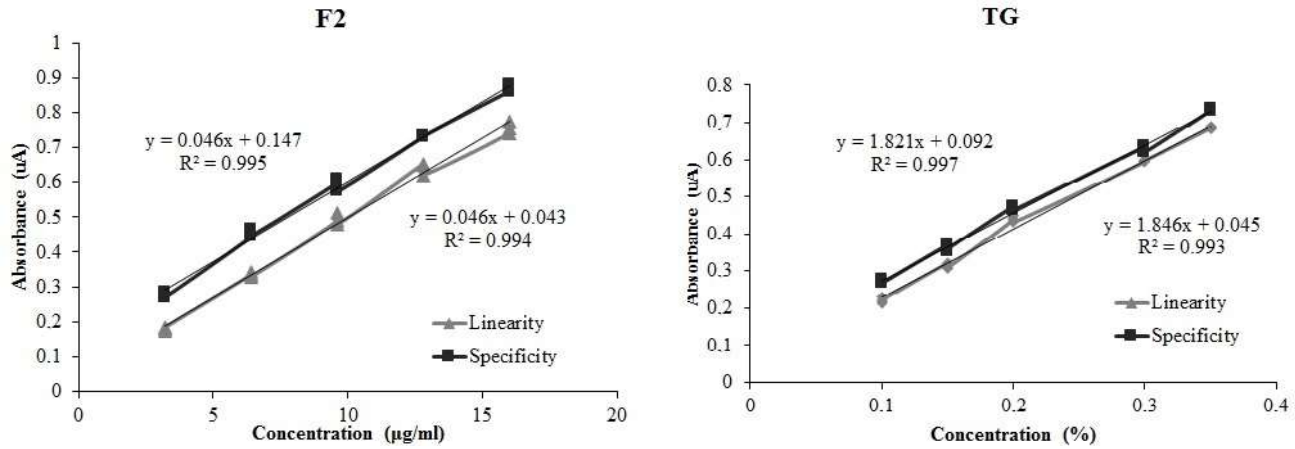

Figure 1. Curve of specificity test compared with curve of linearity test for absorbance of F2 and TG obtained by reaction with Folin-Ciocalteu reagent. (Reproduction size: full page width, 27 cm).

0.424 ± 0.010 [2.91%]) by statistical analysis (F3,8 = 2.5, P = 0.14). This demonstrated the robustness of the method under the evaluated conditions.

Considering the proposed methods for F2 and TG, we could determine the content of TP relative to pyrogallol in both samples. The specific absorptivity is the absorbance of a substance in solution at 1% (w/v; 10,000 µg/mL). The specific absorptivity of pyrogallol was calculated using the linear equation (y = 0.141x + 0.0044); a value of 1,407.3 was obtained. Therewith, the TP content is 36.600% in F2 and 0.067% in TG, which complies with the expected concentration for a TG with 0.2% of F2.

Conclusion

The UV/Vis spectrophotometric method described was successfully validated as an appropriate approach for the determination of total polyphenols relative to pyrogallol in a proanthocyanidin polymers-rich fraction of *S. adstringens* (F2), as well as in topical gel (TG). Furthermore, the proposed TG is an appropriate formulation and may be considered as a potential pharmaceutical agent in the treatment of vaginal candidiasis.

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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